INVESTIGATING MECHANISMS OF SUCCESS: NUCLEAR MIGRATION IN THE RICE BLAST FUNGUS AND THE SELF-ADVOCACY OF STEM UNDERGRADUATES WITH ADHD AND LEARNING DISABILITIES

by

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(Under the Direction of Chang-Hyun Khang and Julie Dangremond Stanton)

ABSTRACT

My dissertation is comprised of research from two distinct fields: fungal cellular biology and discipline-based education research (DBER). The fungal cellular biology portion of my dissertation focuses on mechanisms of nuclear migration during rice cell invasion and proliferation by the blast fungus, *Magnaporthe oryzae*. Specifically, I characterized the involvement of the mitotic spindle in mediating nuclear migration at three different stages of rice blast infection: nuclear migration through the germ tube of developing appressoria, nuclear migration through the narrow penetration peg, and nuclear migration through the narrow invasive hyphal peg. The structure of the nuclear envelope is also described during nuclear migration through the germ tube of developing appressoria. Conserved kinesin motor proteins, MoKin5 and MoKin14, were identified, and their function in mediating nuclear migration through the penetration peg was analyzed using an overexpression approach. These studies provide fundamental knowledge about the cellular biology of the rice blast fungus during the early stages of rice cell invasion and colonization that can serve as a basis for future research. My DBER studies focus on the self-advocacy experiences of students with ADHD and specific learning disabilities (SLD) in

undergraduate STEM courses. Research interviews with 25 STEM majors with ADHD and SLD were conducted. From an in-depth qualitative analysis, a revised conceptual model of self-advocacy emerged. This revised self-advocacy model is tailored to STEM undergraduates with ADHD and SLD. We utilized this revised self-advocacy model to conduct an additional analysis of the interview data. In this study, the factors that influence self-advocacy were identified. We proposed a model to understand how these factors interact to support or hinder self-advocacy within undergraduate STEM courses. Our revised model of self-advocacy provides implications for both future research and teaching.

INDEX WORDS: *Magnaporthe oryzae*, *Pyricularia oryzae*, intermediate mitosis, extreme nuclear migration, inner nuclear membrane, core nucleoporins, kinesin-5, kinesin-14, self-advocacy, students with disabilities, undergraduate STEM, discipline-based education research

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CHAPTER 1

INTRODUCTION

The introduction chapter of my dissertation includes three sections. The first section describes my journey to graduate school and discusses my motivations for pursuing a Ph.D. The second section provides a summary of the overall structure of my dissertation. The final section summarizes additional teaching, mentoring, and university service opportunities I pursued in graduate school.

My journey to graduate school

As an undergraduate STEM major, I felt equally interested in my microbiology courses as I did in my secondary education courses. This dual interest led me to become an undergraduate teaching assistant in the general microbiology labs at the University of Wyoming (UW). In this role, my passion for both microbiology and education was fueled. At the end of my undergraduate career, I participated in a scholarship of teaching and learning project in the microbiology program at UW. Studying how students learn science and engage in scientific practices was thrilling; I loved that it required both my microbiology content knowledge and the skills I developed as a preservice teacher. This experience was an epiphany for me. I learned that there are researchers within college science, technology, engineering, and mathematics (STEM) departments who solely conduct STEM education research. I became intrigued by the possibility of conducting discipline-based education research (DBER). DBER is an interdisciplinary field in which researchers employ education research methods to study phenomena unique to their own content discipline of expertise. Although I was interested

in conducting DBER, no one I knew had pursued this type of doctoral training. At that time, I decided to pursue other career interests.

One career interest I pursued before applying to graduate school was in postsecondary disability services. In this role, I managed the accommodated exam testing schedule for a university disability resource center, and I served as the accommodation coordinator for a caseload of students with disabilities. My experience working with students with disabilities, fellow coordinators, and instructors from across the campus was eye-opening. I noticed that many of the first-year students I met would begin their college careers as STEM majors, but after the first semester many students changed their major to a non-STEM discipline. As a coordinator, I was concerned about the negative accommodation experiences these students shared with me after-the-fact. I wondered, why do some students not communicate with me during the semester when they experience accommodation issues? As a lover of science, this pattern also troubled me. Why were so many students leaving their STEM majors? I turned to the literature and found very little information about the experiences of students with disabilities in undergraduate STEM courses. This lack of published research combined with the experiences of my students, and my own personal experiences working with STEM instructors motivated me to apply to graduate school. I felt called to develop my own STEM content knowledge, and conduct research that may help to improve the conditions of students in undergraduate STEM courses. I also hoped that earning a Ph.D. in a STEM field, as opposed to earning a Ph.D. in an education field, would make me a more credible source to other STEM Ph.Ds in the future.

Feeling motivated to earn a Ph.D. in a STEM field, I decided to apply to graduate school. I learned that the University of Georgia (UGA) offered a unique program that fit both my science and education research interests. At UGA, graduate students can be dually trained in bench science and in STEM education research. I felt I could

successfully earn a Ph.D. in a STEM field at UGA because I would develop knowledge and research expertise in two fields of deep personal interest. This type of dual training would also help me obtain my ultimate career goal, becoming a faculty member in a college STEM department. I applied and joined the labs of Dr. Chang-Hyun Khang and Dr. Julie Dangremond Stanton. In the Khang lab I conducted bench science research, and in the Stanton lab I conducted STEM education research.

The structure of my dissertation

The structure of my dissertation reflects my dual training in bench science and in STEM education research. The bench science portion of my dissertation focuses on a central research question. How does the rice blast fungus, *Magnaporthe oryzae,* successfully position its nuclei during the early stages of rice infection? The rice blast fungus is one of the world's most destructive fungal pathogens to cereal crops, including rice, wheat, barley, and finger millet. Despite the destructive potential of the blast fungus, little is known about the cellular biology of the fungus as it infects host plants, such as rice. Chapter 2 of my dissertation provides a review of the relevant literature for my bench project. In my dissertation, I used genetic and cellular biology approaches to investigate the mechanisms of nuclear migration during three distinct stages of rice blast infection. Specifically, I examined the dynamics of nuclear envelope proteins, microtubules, and motor proteins within the fungus at different rice infection stages. Chapter 3 describes the state of the nuclear envelope and the contribution of the mitotic spindle in mediating nuclear migration through the germ tube of developing appressoria. Appressoria are specialized infection structures that help the fungus puncture through rice leaf cuticles in order to invade host plants. Chapter 4 demonstrates that the mitotic spindle becomes strikingly angled to mediate nuclear migration through the narrow invasive hyphal peg. Invasive hyphal pegs are fungal infection structures that span plasmodesmata connecting the first-invaded rice cell to neighboring cells. Chapter 5

constitutes the crux of my bench research. I investigate mechanisms of extreme nuclear migration through the narrow penetration peg by genetically perturbing the mitotic spindle. The penetration peg is another unique and narrow fungal infection structure that serves as a conduit from the appressorium located on top of the rice leaf to a hypha developing within the first-invaded rice cell. Together these studies establish that during rice blast infection, *M. oryzae* syncs nuclear movement to mitotic division. The major findings and implications of this research are included within the first half of Chapter 8.

The STEM education research component of my dissertation examines the self-advocacy experiences of STEM undergraduates with disabilities. Students with disabilities are underrepresented in STEM majors. Self-advocacy is related to accessing and using academic accommodations, such as extended time exams or notetaking services. Academic success of college students with disabilities is linked to self-advocacy. Yet our understanding of how college students practice self-advocacy in their everyday lives is not well characterized. This lack of knowledge regarding self-advocacy is especially true for different groups of students with disabilities, and within academic disciplines, such as STEM.

In my dissertation, I conducted semi-structured interviews with 25 STEM majors with attention-deficit/hyperactivity disorder (ADHD) and/or specific learning disabilities (SLD) to answer two research questions: (1) How do STEM majors with ADHD and/or SLD practice self-advocacy? and (2) What factors influence the self-advocacy of STEM majors with ADHD and/or SLD? Data were analyzed by a diverse team of researchers including at least one or more researchers who was, or were, a STEM major with ADHD and/or SLD. To address the first research question, we utilized an existing conceptual model of self-advocacy and conducted content analysis. Chapter 6 of my dissertation includes the results of this analysis, which permitted development of our refined model of self-advocacy for STEM majors with ADHD and/or SLD. In Chapter 7 of my dissertation,

we use our model of self-advocacy to define the factors that influence the self-advocacy of STEM majors with ADHD and/or SLD. A discussion of the major findings and implications of my STEM education research is presented within the second half of Chapter 8.

Besides the self-advocacy study, I also published a *CourseSource* article with the mentorship of Dr. Stanton. This was a project I began during my six-week rotation in her lab at the start of graduate school. *CourseSource* articles are peer-reviewed lesson plans for college biology instructors. Each lesson is aligned to the relevant professional society learning goals, explains the rationale for the lesson, provides detailed directions for instructors to complete the learning activity, and offers suggestions for assessment of student learning. The *CourseSource* lesson addresses five learning goals for undergraduate education established by the American Society of Cell Biology. The lesson activity, designed by Dr. Stanton, is used by multiple faculty in an upper-division Cell Biology course at UGA. In this lesson, students develop cell biology knowledge by examining the data from two seminal research papers that describe the discovery of mitochondrial pyruvate carrier proteins. The resulting *CourseSource* article is included as Appendix A.

Conducting both bench and STEM education research as part of my dissertation provided me a myriad of training opportunities to develop my research expertise. For example, I became skilled in experimental design, confocal microscopy, microscopy image analysis, qualitative interviewing, qualitative analysis, and communicating in oral and written formats to diverse audiences. I developed grantsmanship skills through the mentorship of my advisors and by taking a course in grant writing. I was awarded a National Science Foundation Graduate Research Fellowship for the STEM education research proposal I submitted. Later, I was selected for an ARCS Foundation

Scholarship based on my split dissertation research. Besides research, I also had the opportunity to teach, mentor, and engage in university service during my Ph.D.

Additional training experiences in graduate school

Teaching was one of the highlights of my doctoral training. I taught BIOL 1107 and PBIO 1210 labs for a total of three semesters. Teaching these labs reinforced my long-term career goal of becoming a faculty member in the future, and my interest in conducting STEM education research. I also had the opportunity to mentor graduate students, visiting research scholars, and undergraduate students across my bench and STEM education research projects. At first, mentoring seemed very intimidating to me, but then I realized it is essentially teaching someone how to conduct research. While this realization is not profound, it helped me establish my mentoring style. I now see my role as a mentor as fostering learning experiences for new researchers, supporting the development of their own research skills, and providing access to supportive social networks. My mentees, in turn, taught me many valuable lessons. Their perspectives and questions helped me think more deeply about the research projects, and how to best design research experiences for students. I learned from mentoring that having clear expectations and hypotheses is essential because it makes troubleshooting much easier.

During graduate school, I became involved in three university organizations. The Mycology Graduate Student Organization (MGSO), the Plant Biology Graduate Student Association (PBGSA), and the Scientists Engaged in Education Research (SEER) Center. Through my involvement in these organizations, I met colleagues and had the opportunity to engage in professional development, university service, and community outreach. In MGSO, I helped secure funding and design a research symposium that was funded in part by the Genetics Society of America. I also helped design and run a table at STEMZone that focused on fungi. STEMZone is a community outreach event held at

a UGA football game each year. In PBGSA, I served as the Teaching and Community Outreach Chair. In this role, I designed a workshop about submitting a *CourseSource* manuscript and I developed online modules with teaching resources when UGA transitioned to rapid online learning as a result of the COVID-19 pandemic. My involvement with the SEER Center supported development of my leadership skills as the Graduate Chair on the Executive Community. I attended workshops and journal clubs that supported my development as a discipline-based education researcher.

Completing a dissertation consisting of two distinct research projects challenged me. It taught me how to establish realistic goals, manage my time, communicate effectively, and to prioritize. I was fortunate to travel across the globe to share my research findings and to network with colleagues. Conducting a split dissertation advanced my love of both science and education. I hope that the research I produced during my Ph.D. serves a purpose, either to the rice blast community or to STEM students with disabilities. Looking to the future, I plan to continually seek opportunities to develop my knowledge of science and education. I strive to be an ambassador of both fungal cellular biology and STEM education research. I hope to use my diverse expertise to inform and to inspire others to continually question what is known, how we know it, and perhaps most importantly, what questions remain.

CHAPTER 2

A NUCLEAR CONTORTIONIST: THE MITOTIC MIGRATION OF *MAGNAPORTHE ORYZAE* NUCLEI DURING PLANT INFECTION[1]

[1] Pfeifer, M. A., & Khang, C. H. (2018). A nuclear contortionist: The mitotic migration of *Magnaporthe oryzae* nuclei during plant infection. *Mycology*, *9*(3), 202-210. Reprinted here with permission of the publisher.

Abstract

Magnaporthe oryzae is a filamentous fungus, which causes significant destruction to cereal crops worldwide. To infect plant cells the fungus develops specialized constricted structures such as the penetration peg and the invasive hyphal peg. Live-cell imaging of *M. oryzae* during plant infection reveals that nuclear migration occurs during intermediate mitosis, in which the nuclear envelope neither completely disassembles nor remains entirely intact. Remarkably, in *M. oryzae,* mitotic nuclei show incredible malleability while undergoing confined migration through the constricted penetration and invasive hyphal pegs. Here we review early events in plant infection, discuss intermediate mitosis, and summarize current knowledge of intermediate mitotic nuclear migration in *M. oryzae.*

Introduction

Magnaporthe oryzae, also known as the rice blast fungus, is a filamentous hemibiotrophic plant pathogen. It is capable of mass destruction to valuable plant crops such as rice and wheat, as well as barley, finger millet, and foxtail millet (Gladieux et al. 2018). In fact, each year *M. oryzae* causes an estimated $66 billion in economic damage to rice crops, destroying enough food to have fed 60 million people (Pennisi 2010). In the field, *M. oryzae* is developing increased resistance to commonly used fungicides (Ribas e Ribas et al. 2016) and, recently, wheat blast emerged in Bangladesh (Islam et al. 2016; Malaker et al. 2016). Understanding cellular processes unique to *M. oryzae* is an important first-step in the development of novel and effective methods to control the deadly plant pathogen and ensure global food security.

Proper positioning of the nucleus within eukaryotic cells is vital and relies upon successful nuclear migration into incipient cells (Morris 2000). A full gamut of mitotic forms is possible in eukaryotes, ranging from completely closed to completely open (Heath 1980; Boettcher and Barral 2013). In fungi, nuclear migration into incipient cells occurs before, during, or after mitosis (Gladfelter and Berman 2009). Recent studies reveal that *M. oryzae* undergoes mitosis that is not completely closed or open and that the mitotic nucleus becomes highly deformed while migrating through narrow structures that arise during plant infection (Jones, Jenkinson et al. 2016; Jenkinson et al. 2017).

In this review, we highlight the nuclear dynamics of *M. oryzae* during plant infection with a focus on mitosis and mitotic nuclear migration. We contextualize these cellular processes by discussing the early events in rice blast infection and the range of mitotic programs documented in fungi. We provide an outlook on what mechanisms of nuclear migration likely exist in *M. oryzae* and discuss whether similar cellular processes are present in other plant pathogens.

Early Events in Rice Blast Infection

Rice blast infection begins when an asexual three-celled conidium attaches to the surface of a rice plant (Hamer et al. 1988). A polarized germ tube develops, and the fungus forms a melanized dome-shaped cell called an appressorium in the presence of the appropriate extracellular physical and chemical signals, for instance hydrophobicity of the leaf surface (Veneault-Fourrey et al. 2006; Ryder and Talbot 2015). The most apical nucleus of the conidium undergoes mitosis, and one nucleus migrates to the incipient appressorium, followed by autophagy of the conidium (Veneault-Fourrey et al. 2006; Saunders, Aves, et al. 2010). It remains unknown if extracellular cues trigger mitosis during appressorium development. Accumulation of turgor pressure in the appressorium in coordination with septin-dependent cytoskeletal rearrangements at the appressorial pore leads to formation of the penetration peg (Howard et al. 1991; Dagdas et al. 2012). The penetration peg is a specialized hypha that physically breaches the leaf cuticle, allowing the fungus to enter rice cells approximately 24 hours post inoculation (Kankanala et al. 2007). Several S-phase checkpoints have been identified, which regulate appressorium development and formation of the penetration peg (Saunders, Aves, et al. 2010; Osés-Ruiz et al. 2017). Once inside the first-invaded rice cell, the penetration peg gives rise to the primary hypha (Kankanala et al. 2007). The apical tip of the primary hypha switches from filamentous to depolarized growth causing the apical tip to swell (Shipman et al. 2017). Tip expansion of the primary hypha appears to serve as a size threshold which triggers mitosis in the single nucleus located in the appressorium (Shipman et al. 2017). This nucleus begins mitosis inside the appressorium and undergoes a long-distance migration during presumed anaphase B to its eventual position in the swollen tip of the primary hypha (Jenkinson et al. 2017; Shipman et al. 2017). Subsequently, septation occurs, and the first cell of the bulbous invasive hyphae (IH) is formed (Shipman et al. 2017). Incongruent descriptions of the exact location of

mitosis at this infection stage exist in the literature. Two previous reports describe the appressorial nucleus migrating from the appressorium into the primary hypha and then undergoing mitosis, rendering the appressorium anucleate for a time (Veneault-Fourrey et al. 2006; Fernandez et al. 2014). However, later work reports mitosis as most commonly occurring within the appressorium (Jenkinson et al. 2017; Osés-Ruiz et al. 2017; Shipman et al. 2017). The reason for this discrepancy remains unknown.

M. oryzae secretes effector proteins during plant infection. Effectors function to dampen plant immune responses and change the metabolism of the plant (Giraldo et al. 2013). The biotrophic interfacial complex (BIC) is a plant-derived membrane-rich structure that lies outside the fungal cytoplasm and is the site at which cytoplasmic effectors accumulate during plant infection (Khang et al. 2010; Giraldo et al. 2013). The BIC first appears at the tip of the primary hypha but then is repositioned to the side of the first bulbous cell (Khang et al. 2010; Shipman et al. 2017). Cytoplasmic effectors are secreted to the BIC via nonconventional secretion and eventually enter plant cells, while apoplastic effectors undergo conventional secretion and remain contained within the extra-invasive hyphal membrane (Giraldo et al. 2013).

To invade neighboring plant cells, IH appear to seek out pit fields and undergo a morphological shift from polarized to isotropic growth controlled by the Pmk1 mitogen-activated protein kinase (Kankanala et al. 2007; Sakulkoo et al. 2018). IH then form highly-constricted IH pegs to cross plant cell walls (Kankanala et al. 2007). The first-invaded rice cells are alive but die when the fungus enters adjacent cells (Kankanala et al. 2007; Jones, Kim, et al. 2016) Colonization of the first-invaded plant cell takes approximately 8-12 hours while in subsequently-invaded cells, the fungus only develops for 2-3 hours (Kankanala et al. 2007; Jones, Kim, et al. 2016). One possible explanation for the difference in colonization time between first-invaded and subsequently-invaded cells is that effectors move cell-to-cell likely through plasmodesmata and prime

neighboring cells for fungal invasion (Kankanala et al. 2007; Khang et al. 2010). Within 4-5 days of initial infection, blast lesions become apparent on the plant tissue (Sakulkoo et al. 2018).

The mitotic spectrum in fungi

A fundamental property of eukaryotic life is the division of replicated genetic information to new daughter cells through mitosis. Typically, when we think of mitosis, we recall cellular events characteristic of mitosis in plant and animal cells that use completely open mitosis where the nuclear envelope (NE) disassembles during prophase. However, multiple forms of mitosis exist across the eukaryotic domain (Arnone et al. 2013; Sazer et al. 2014; Makarova and Oliferenko 2016). In fungi, it is often assumed that all species rely on completely closed mitosis, yet, closer study of mitotic nuclear dynamics reveal a spectrum of mitotic programs in fungi (Heath 1980; De Souza and Osmani 2007).

Mitosis is categorized based on the state of the NE during nuclear division (Arone et al. 2013; Sazer et al. 2014; Makarova and Oliferenko 2016). In completely closed mitosis, the NE remains entirely intact throughout nuclear division. Spindle pole bodies, embedded within the NE, serve as microtubule-organizing centers and permit the spindle to form within the nucleus (Arnone et al. 2013). The spindle elongates in coordination with NE expansion, and eventually the condensed chromosomes are separated. Conversely, in completely open mitosis, all components of the NE undergo systematic dismantling during prophase (Arnone et al. 2013). This regulated disassembly of the NE grants the spindle, which is nucleated in the cytoplasm, access to chromosomes which are then separated. The NE is reformed at the end of mitosis, enclosing two daughter nuclei.

Historically, classification of closed or open mitosis was determined by observing the state of the NE using transmission electron microscopy throughout all phases of

mitosis in classical model organisms, such as the closed mitosis of *Saccharomyces cerevisiae* (Sazer et al. 2014). As studies of NE dynamics during mitosis expanded beyond model organisms, it became obvious that classifying mitosis as either completely closed or completely open is not always clear, especially in fungi (Heath 1980). The NE of some species persists throughout mitosis but is not fully intact, thus exhibiting characteristics of both a closed and open mitosis (Heath 1980; Sazer et al. 2014). Mitosis that is not completely closed nor completely open is called intermediate mitosis (De Souza and Osmani 2007; Arnone et al. 2013). Intermediate mitosis involves changes to the structure and integrity of the NE, which leads to a significant loss of compartmentalization between the nucleus and the cytoplasm. Several terms exist to describe forms of intermediate mitosis, for instance, semi-open in *Aspergillus nidulans* (Lin 2015), modified-open in *Ustilago maydis* (Straube et al. 2005), and partially-open as a collective term for all forms of intermediate mitosis (Arnone et al. 2013; Sazer et al. 2014). Generally, mechanisms of intermediate mitosis in fungi can be grouped into two broad categories: tearing of the NE or altering the composition of a largely-intact NE to enhance permeability. Fungi such as *U. maydis* and *Schizosaccharomyces japonicus* experience NE tearing during mitosis (Straube et al. 2005; Aoki et al. 2011; Yam et al. 2011). In *A. nidulans*, a subset of proteins found within nuclear pore complexes (NPCs) disperse into the cytoplasm during mitosis (De Souza et al. 2004; Osmani et al. 2006).

Intermediate mitosis in *Magnaporthe oryzae*

Mitosis in *M. oryzae* bears a hallmark of intermediate mitosis, the dramatic loss of compartmentalization between the nucleus and the cytoplasm. During mitosis, cytoplasmic ZsGreen signal equalizes between the cytoplasm and the nucleus (Bourett et al. 2002), and the import of tubulin into the nucleus increases dramatically after mitotic onset (Czymmek et al. 2005). Following the dynamics of green fluorescent protein fused with nuclear localization signal (GFP-NLS) during plant infection provides further

evidence of intermediate mitosis (Jones, Jenkinson, et al. 2016; Jenkinson et al. 2017). GFP-NLS signal remains within the nucleus during interphase in the appressorium and invasive hyphae. However, at the start of mitosis, GFP-NLS signal becomes cytoplasmic with subsequent re-importation of GFP-NLS once mitosis is complete (Figure 2.1). Together, these reports show that mitosis in *M. oryzae* is not completely closed where the NE would be expected to function as an intact barrier to prevent mixing of nuclear and cytoplasmic proteins. Possible explanations for this observed loss of compartmentalization include: (1) the NE of *M. oryzae* tears or (2) the NE persists but loses integrity during mitosis. Differentiating between these two possibilities requires following the dynamics of the NE throughout mitosis.

Staining the outer nuclear membrane using the lipophilic dye 3,3'-dihexyloxacarbocyanine iodide (DiOC6) showed that the outer nuclear membrane remains intact during mitosis in the germ tube at the appressorium development stage of infection (Saunders, Dagdas, et al. 2010). Additional studies of the NE at the same infection stage reveal that the core nucleoporin (Nup), Nup84, localizes to the polar edges of the dividing nucleus (Pfeifer and Khang, unpublished data). The persistence of Nup84 at the NE throughout mitosis confirms that portions of the NE do remain intact during nuclear division. Additionally, the polar localization of Nup84 in *M. oryzae* is similar to other fungi known to use intermediate mitosis, including *S. japonicus* (Aoki et al. 2011; Yam et al. 2011) and *A. nidulans* (Osmani et al. 2006). While these results confirm distinct components of the NE remain intact during mitosis, the details of the mechanism responsible for intermediate mitosis remain to be discovered in *M. oryzae*.

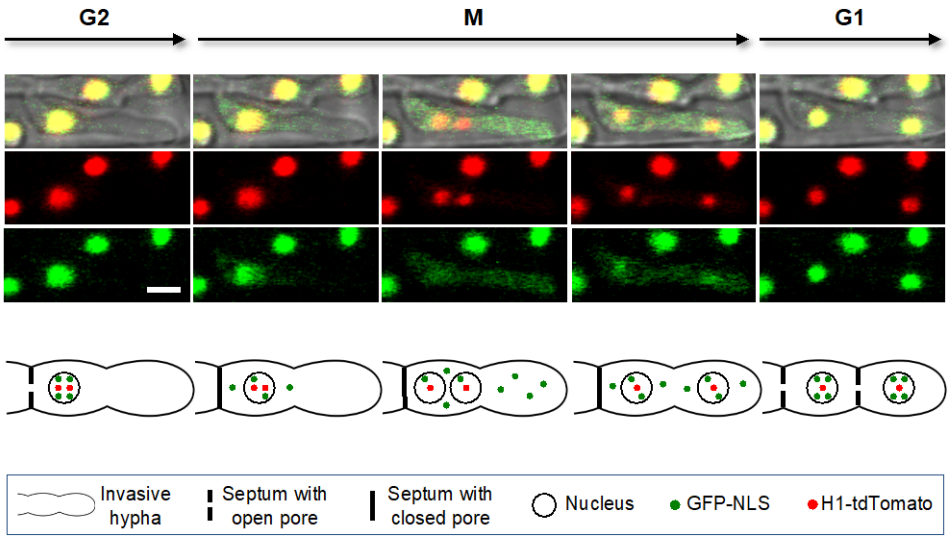

Figure 2.1. Time-lapse confocal fluorescence images and schematic diagram of intermediate mitosis in an invasive hypha of *M. oryzae* within the first-invaded rice cell. This strain expresses histone H1-tdTomato and GFP-NLS. The top panel shows five sequential fluorescence pattern stages in both merged bright-field and fluorescence (top), red fluorescence alone (middle), and green fluorescence alone (bottom). The interphase nucleus in G2 appears yellow due to colocalization of H1-tdTomato (red) and GFP-NLS (green) within the nucleus. H1-tdTomato remains associated with DNA throughout the cell cycle. During the early stages of mitosis (M), GFP-NLS spills into the cytoplasm, indicating a change to the integrity of the nuclear envelope. GFP-NLS is contained within the dividing cell by presumed closure of septal pores. Following mitosis, GFP-NLS is reimported back into the nucleus and the nucleus again becomes yellow during interphase (G1). The bottom panel presents a schematic summary of these cellular events. Bar = 5 μm. This figure is modified from Jones, Jenkinson, et al (2016).

Interestingly, *M. oryzae* shares many mitotic similarities with *A. nidulans*, a model organism with well-studied mitotic dynamics. The NE of both species remains intact throughout mitosis (Robinow and Caten 1969; Saunders, Dagdas, et al. 2010) while the permeability of the NE increases during mitosis (Suelmann et al. 1997; Bourett et al. 2002; Ovechkina et al. 2003; De Souza et al. 2004; Czymmek et al. 2005; Osmani et al. 2006; Jones, Jenkinson, et al. 2016; Shipman et al. 2017). Furthermore, *A. nidulans* and *M. oryzae* show loss of compartmentalization between the nucleus and cytoplasm with a coordinated closure of septal pores during mitosis (Ovechkina et al. 2003; De Souza et al. 2004; Osmani et al. 2006; Shen et al. 2014; Jones, Jenkinson, et al. 2016). In *M. oryzae*, cytoplasmic GFP-NLS remains contained within the dividing cell during mitosis, which suggests cell-cycle regulation of septal pores (Jones, Jenkinson, et al. 2016). A similar case is observed in *A. nidulans* where the NIMA kinase coordinates septal pore opening and closing to be out of sync with the dispersal of Nups from NPCs during prophase (Shen et al. 2014). This regulated closure of septal pores likely prevents diffusion of mitotic kinases throughout neighboring cells thereby preventing precocious mitoses which could be detrimental to the fungus (Shen et al. 2014). Future study of the NIMA homolog will reveal if similar regulation of septal pores exists in *M. oryzae*.

Although mounting evidence strongly suggests that *M. oryzae* uses a form of intermediate mitosis, details about the process are lacking. Data describing the dynamics of all components of the NE throughout mitosis is needed to fully characterize the form of intermediate mitosis used by *M. oryzae*. To date, only the localization of the outer nuclear membrane and Nup84 has been studied during mitosis at one stage of rice blast infection, appressorium development. An important direction for future research is to visualize the inner nuclear membrane using a combination of confocal and transmission electron microscopy throughout mitosis to fully describe whether this component of the NE remains intact during mitosis in *M. oryzae*. Given the mitotic

similarities to *A. nidulans*, we hypothesize that a key feature of mitosis in *M. oryzae* is dispersal of peripheral Nups during prophase while other NE components (the outer and inner nuclear membranes along with core Nups) remain intact during mitosis. Demonstrating that core Nups remain associated with the NE during mitosis while peripheral Nups localize to the cytoplasm will implicate a mechanism akin to *A. nidulans* (Osmani et al. 2006).

Although we hypothesize *M. oryzae* shares key mitotic features with *A. nidulans*, it is important to note that nuclear distribution differs between the two species. *M. oryzae* is typically a mononuclear species, with only one nucleus per cell. *A. nidulans*, however, has multiple nuclei within one common cytoplasm. Since *A. nidulans* is syncytial, the persistence of the NE may help prevent spindle microtubules from interacting with the chromosomes of a nearby dividing nucleus in an adulterous manner (De Souza and Osmani 2007). Why then does *M. oryzae* use intermediate mitosis? The variety of mitotic programs present in fungi suggests that each type of mitosis conveys advantages and disadvantages to the organism (Boettcher and Barral 2013). We speculate that intermediate mitosis in *M. oryzae* confers a to-be-determined advantage, perhaps to permit more efficient plant infection. However, research in this area is very much in its nascence. Future work in *M. oryzae* may better address what advantages intermediate mitosis brings to fungi during plant infection.

Nuclear constrictions during plant infection

M. oryzae's IH are highly plastic as they grow inside plant cells. For instance, during cell-to-cell movement as IH cross from the first-invaded cell to adjacent cells via the narrow IH peg, IH constrict from an initial average diameter of 5 μm to 0.5μm (Kankanala et al. 2007; Sakulkoo et al. 2018). Live-cell imaging of cell-to-cell movement revealed that hyphae developing in the newly invaded neighbor cell must grow significantly before becoming nucleated (Kankanala et al. 2007). These results

suggested that nucleation of the incipient fungal hypha depends upon mitosis and the successful delivery of the daughter nucleus across the IH peg. Until recently, the details of this process remained enigmatic, largely due to the technical challenges of capturing rapid nuclear migration through the IH peg using time-lapse imaging.

An elegant combination of GFP-NLS and histone H1-tdTomato fluorescent reporter proteins was utilized to study nuclear dynamics during plant infection. GFP-NLS spill from the nucleus into the cytoplasm indicates entrance into prophase and that the nucleus will divide within a few minutes, while histone H1-tdTomato remains associated with DNA throughout the cell cycle (Jones, Jenkinson, et al. 2016; Jenkinson et al. 2017). This combination of fluorescent proteins provided crucial temporal and spatial cues to time-lapse image nuclear migration events inside the rice plant without causing phototoxicity to dividing fungal cells (Jones, Jenkinson, et al. 2016; Jenkinson et al. 2017). Using GFP-NLS as an indicator for mitosis demonstrates that nuclear migration occurs during intermediate mitosis. That is, GFP-NLS disperses from the nucleus before migration occurs and is not fully reimported back until after nuclear migration. (Jones, Jenkinson, et al. 2016; Jenkinson et al. 2017).

These studies further revealed several important findings about nuclear migration during plant infection (Figure 2.2 and full videos available on youtube.com by searching Khang Lab at UGA Rice Blast). Firstly, the nucleus adopts an extremely constricted morphology as it migrates through the IH peg. One nucleus (interphase diameter of ~ 2 μm) was observed to stretch to over 5 μm in length while moving through the IH peg (Jones, Jenkinson, et al. 2016). After squeezing through the IH peg, the nucleus assumed its typical spherical shape and continued to migrate a total distance of 16.9 μm from the site of chromosome separation to the now nucleated fungal hyphal cell located in the second-invaded rice cell (Jones, Jenkinson, et al. 2016).

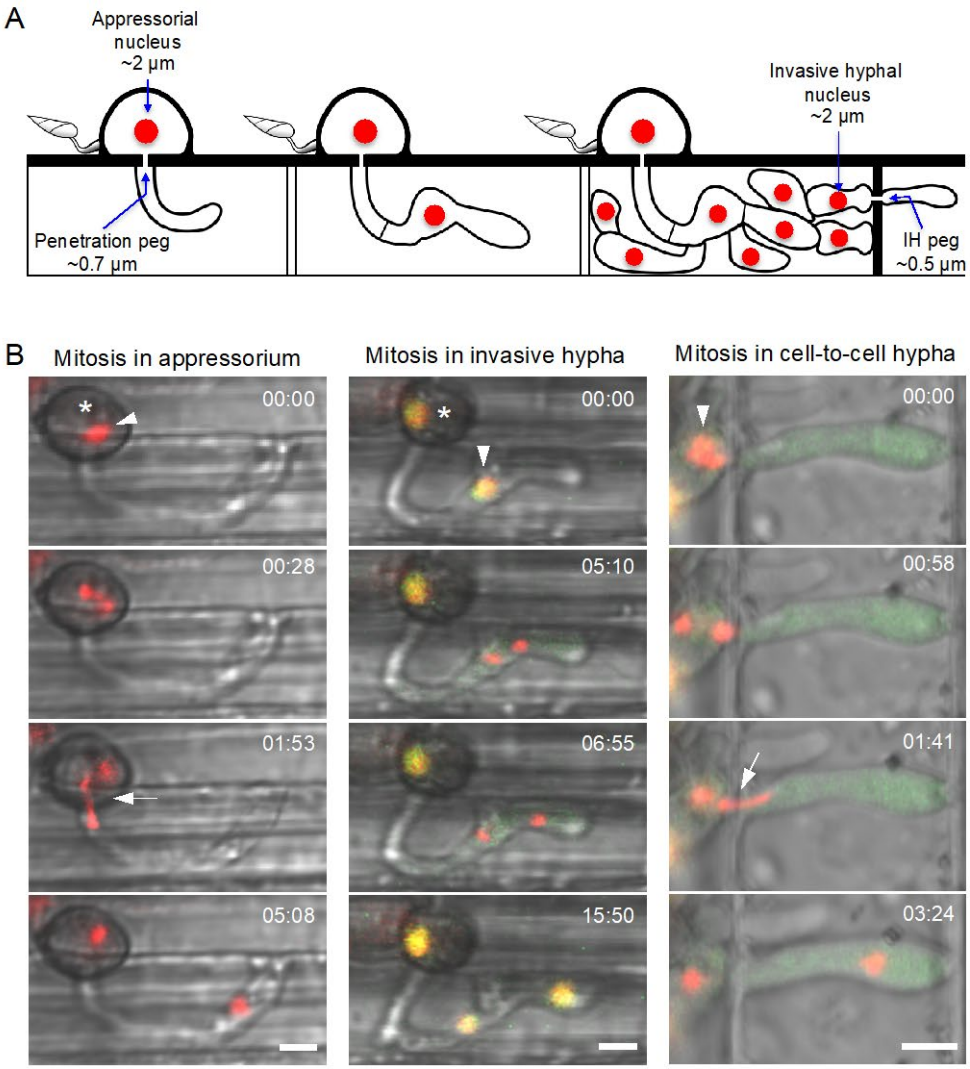

Figure 2.2. Mitotic migration of *M. oryzae* nuclei during early rice blast infection. Legend continues on next page.

Figure 2.2. (continued) Mitotic migration of *M. oryzae* nuclei during early rice blast infection. (A) Schematic diagram summarizing key cellular structures and mononuclear positioning during plant invasion. The nucleus in the appressorium (interphase diameter of ~2 μm) must traverse the constricted penetration peg (diameter of ~0.7 μm) for final receipt in the incipient primary hypha. Once inside the first-invaded cell, the primary hypha becomes bulbous to form invasive hyphae (IH). To move into adjacent rice cells, IH seek out pit fields and develop a constricted IH peg (diameter of ~0.5 μm). (B) A time-lapse series of nuclear dynamics at three distinct stages of early rice blast infection. Asterisks denote the appressorium, arrowheads label a nucleus about to undergo mitotic nuclear migration, and arrows highlight extreme nuclear morphology during confined nuclear migration through peg structures. (Left; merge of bright-field and H1-tdTomato) Mitosis begins in the appressorium, and the daughter nucleus becomes highly constricted and elongated during confined mitotic nuclear migration through the penetration peg. The original nucleus remains located in the appressorium throughout this event. GFP-NLS dynamics (data not shown) confirms nuclear migration occurs during intermediate mitosis at this infection stage (Jenkinson et al., 2017). (Middle panel; merge of bright-field, GFP-NLS and H1-tdTomato) During mitosis in the invasive hypha, the interphase nucleus appears yellow due to colocalization of H1-tdTomato and GFP-NLS in the nucleus. After onset of mitosis, GFP-NLS disperses into the cytoplasm, and the nucleus undergoes an unconfined nuclear migration. Following receipt of the nucleus into the new invasive hypha cell, mitosis ends, and GFP-NLS is fully reimported back into the nucleus. (Right panel; merge of bright-field, GFP-NLS and H1-tdTomato) Here, confined nuclear migration through the IH peg occurs. In early mitosis, the sister chromatids separate and during presumed anaphase B, a single daughter nucleus undergoes confined nuclear migration through the constricted IH peg. The daughter nucleus again becomes spherical and continues to migrate to the tip of the IH in the second-invaded cell prior to GFP-NLS reimport into the nucleus. Times are shown in minutes:seconds. Bars = 5 μm. This figure is modified from Jones, Jenkinson et al (2016) and Jenkinson et al (2017).

This remarkable nuclear morphology was also observed at an earlier stage of plant infection, when the appressorial nucleus moved through the penetration peg (diameter of .7μm) during mitosis (Jenkinson et al. 2017; Shipman et al. 2017). Here, the appressorial nucleus became highly constricted and elongated with a maximum length of 13 μm reported (Jenkinson et al. 2017). At both stages of infection, the migrating nucleus appeared to be tethered to the mother nucleus (Jones, Jenkinson, et al. 2016; Jenkinson et al. 2017). Notably, only the migrating nucleus could enter and cross the penetration or IH peg. Together these observations suggest that *M. oryzae* possesses a cellular mechanism responsible for the confined migration of the mitotic nucleus through narrow pegs arising during plant infection.

The ability of *M. oryzae*'s nuclei to withstand such extreme constrictions during plant infection is certainly captivating. Fungal nuclei are known to be flexible, largely attributed to the fact that fungi lack true lamin proteins (Steinberg et al. 2012; Ciska and Moreno Díaz de la Espina 2014). The nuclei of fungi such as *U. maydis* (Straube et al. 2005)*, Neurospora crassa* (Roca et al. 2010), and *Candida albicans* (Finley and Berman 2005) all display some degree of elongation during migration. However, to our knowledge, the morphology adopted by the nuclei of *M. oryzae* during confined migration through the penetration or IH peg is the most drastic morphology to be reported.

Nuclear migration during plant infection

Nuclear migration in many fungi requires the coordination of microtubules, the motor protein cytoplasmic dynein, and additional microtubule-associated proteins; for extensive reviews of this topic see Gladfelter (2009), Roberts and Gladfelter (2016), and Xiang (2017). For example, in *S. cerevisiae*, astral microtubules are nucleated at the spindle pole body and rely on dynamic instability to search the cell cortex of the bud to locate Num1, a cortical dynein-interacting protein (Carminati and Stearns 1997; Heil-

Chapdelaine et al. 2000; Farkasovsky and Küntzel 2001). Once the plus-ends of astral microtubules bind to Num1, they slide against the cell cortex in a dynein-mediated manner which moves the spindle and eventually the daughter nucleus into the newly-formed bud (Heil-Chapdelaine et al. 2000). Functional homologs of Num1 are also required for proper nuclear migration in multinucleate fungi, including *A. nidulans* (Veith et al. 2005) and *Ashbya gossypii* (Grava et al. 2011). In *M. oryzae,* a Num1 homolog, MoAND1, has been characterized (Jeon et al. 2014). Without MoAND1, nuclear positioning in vegetative hyphae and conidia is affected (Jeon et al. 2014). Importantly, ΔMoand1 shows a reduced ability to initially invade rice cells, suggesting that a cortical dynein anchor is necessary for the fungus to penetrate plants.

A recent study of a class myosin-II motor, Momyo2, in *M. oryzae* showed that disruption of Momyo2 resulted in aberration in nuclear distribution (Guo et al. 2017). Like the ΔMoAND1, the ΔMomyo2 strain shows reduced ability to penetrate into plants (Guo et al. 2017). In other fungi, class-II myosins in cooperation with actin play important roles in cytokinesis and septation (Takeshita 2016). Future research investigating the functions of microtubules and actin along with associated motor proteins will yield valuable information about *M. oryzae*'s mechanism of nuclear migration during plant infection.

Outlook and Conclusion

We now know that *M. oryzae* nuclei become extremely constricted while migrating through the confined channels of the penetration or IH peg and that nuclear migration occurs during intermediate mitosis. Does the NE regularly rupture during mitotic migration? Is NE rupture more frequent as the nucleus migrates through the pegs? What are the motor and accessory proteins needed for successful nuclear migration during plant infection? The answers to these intriguing questions remain to be discovered.

Interestingly, other fungi also form confined structures during plant infection. For example, *Colletotrichum* spp. form an appressorium-derived penetration peg to initially enter their host cells (Mendgen et al. 1996; Nesher et al. 2008; De Silva et al. 2017). *Fusarium graminearum* forms intracellular hyphae that exhibit apparent constriction during cell-to-cell movement (Jansen et al. 2005). Although it is currently unknown whether *F. graminearum* uses intermediate mitotic nuclear migrations during infection, some other *Fusarium* spp., such as *F. oxysporum* and *F. verticillioides,* show signs of intermediate mitosis (Bourett et al. 2002; De Souza and Osmani 2007). Future work in filamentous fungi including *M. oryzae* will provide evidence needed to draw broader conclusions regarding conserved mechanisms of mitotic nuclear migration during plant infection.

As technology advances to allow studies of cellular processes at single-cell resolution, an important aim will be to discover how intracellular fungal pathogens position nuclei during plant infection. *M. oryzae* represents one of the most significant threats to global food production, and resistance to fungicides such as azoles is increasing (Ribas e Ribas et al. 2016). Investigating nuclear migration in *M. oryzae* during plant infection will likely identify fungal-specific cellular targets to halt nuclear migration and thereby prevent infection progression. Insight into these fascinating cellular mechanisms could aid in the development of new fungicides to control the deadly rice blast fungus and other fungal plant pathogens.

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CHAPTER 3

NUP84 PERSISTS WITHIN THE NUCLEAR ENVELOPE OF THE RICE BLAST FUNGUS, *MAGNAPORTHE ORYZAE*, DURING MITOSIS[1]

[1] Pfeifer, M. A., & Khang, C. H. (2020). Nup84 persists within the nuclear envelope of the rice blast fungus, *Magnaporthe oryzae*, during mitosis. *Fungal Genetics and Biology*, *146*, 103472. Reprinted here with permission of the publisher.

Abstract

The arrangement of the nuclear envelope in the rice blast fungus, *Magnaporthe oryzae*, was previously undetermined. Here, we identified two conserved components of the nuclear envelope, a core nucleoporin, Nup84, and an inner nuclear membrane protein, Src1. Live-cell super-resolution structured illumination microscopy revealed that Nup84-tdTomato and Src1-EGFP colocalized within the nuclear envelope during interphase and that Nup84-tdTomato remained associated with the dividing nucleus. We also found that appressorium development involved a mitotic nuclear migration event through the germ tube.

Introduction

The architecture of the nuclear envelope in the rice blast fungus, *Magnaporthe oryzae*, remains largely uncharacterized. The nuclear envelope is composed of two bilipid membranes: the outer nuclear membrane, which is continuous with the endoplasmic reticulum, and the inner nuclear membrane. The outer and inner nuclear membranes are spanned by multiprotein nuclear pore complexes (NPCs) made of nucleoporins (Nups). A previous study showed that the outer nuclear membrane of the nuclear envelope remains intact during mitosis in *M. oryzae* (Saunders et al., 2010). Yet loss of compartmentalization between the nucleus and the cytoplasm is reported during mitosis in vegetative hyphae, appressoria, and in invasive hyphae of *M. oryzae* during rice infection (Bourett et al., 2002; Czymmek et al., 2005; Jenkinson et al., 2017; Jones et al., 2016). This loss of compartmentalization during mitosis indicates a change to the structure and permeability of the nuclear envelope, and is a hallmark of intermediate mitosis (reviewed in Pfeifer and Khang, 2018). Mitotic programs in eukaryotes are diverse. Nuclear envelopes that remain intact throughout the cell cycle define closed mitosis, while nuclear envelopes that fully disassemble during nuclear division define open mitosis. The nuclear envelope of some fungi displays an intermediate state during mitosis in which compartmentalization between the nucleus and the cytoplasm is lost, although portions of the nuclear envelope remain intact (e.g., Aoki et al., 2013; Liu et al., 2008; Osmani et al., 2006; Yam et al., 2011). We refer to these types of mitotic programs broadly as intermediate mitosis, and note that many terms exist to describe this form of nuclear division within fungi, e.g., semi-open, semi-closed, modified-open, and partially open (reviewed in Pfeifer and Khang, 2018). Here, we investigate previously uncharacterized nuclear envelope components to determine their dynamics during interphase and mitosis in conidia and appressoria towards defining a mechanism of intermediate mitosis in *M. oryzae*.

Results and Discussion

Localizing components of the nuclear envelope in *M. oryzae.* We identified components of the nuclear envelope in *M. oryzae* based on homology to previously characterized nuclear envelope proteins in *Aspergillus nidulans* using BLASTP. Nup84 in *M. oryzae* (MGG_16457) shared 44.2% similarity to *A. nidulans* Nup84 (AN1190), and contained a putative conserved core Nup84/Nup107 domain (pfam04121) (Osmani et al., 2006). Src1 in *M. oryzae* (MGG_04363) shared 55.2% similarity with *A. nidulans* Src1 (AN3910), possessed a N-terminal LEM-like domain (cd12935), and a Man1-Src1p-C-terminal domain (pfam09402) (Liu et al., 2015). *Aspergillus nidulans* undergoes a form of intermediate mitosis, called semi-open mitosis. In *A. nidulans*, Nup84 is a core Nup, and Src1 is an inner nuclear membrane protein, both of which remain associated with the nuclear envelope throughout the cell cycle, while peripheral Nups disperse from the NPCs which allows many soluble nuclear and cytoplasmic proteins to equilibrate across the nuclear envelope (Liu et al., 2015; Osmani et al., 2006).

To investigate the arrangement of the nuclear envelope, we generated *M. oryzae* fluorescent reporter strain CKF3810 that coexpressed Nup84-tdTomato driven by its native promoter, and two GFP constructs to label nuclear contents (histoneH1-EGFP and EGFP-NLS; NLS for Nuclear Localization Signal). We conducted live-cell imaging of CKF3810 conidia with super-resolution structured illumination microscopy (SR-SIM) during interphase and mitosis. During interphase, our results showed Nup84-tdTomato at the nuclear periphery surrounding histone H1-EGFP and EGFP-NLS (Figure 3.1A). This arrangement demonstrated that Nup84-tdTomato localized within the nuclear envelope during interphase, and resembled the localization of Nup84-GFP during interphase in *A. nidulans* (Osmani et al., 2006).

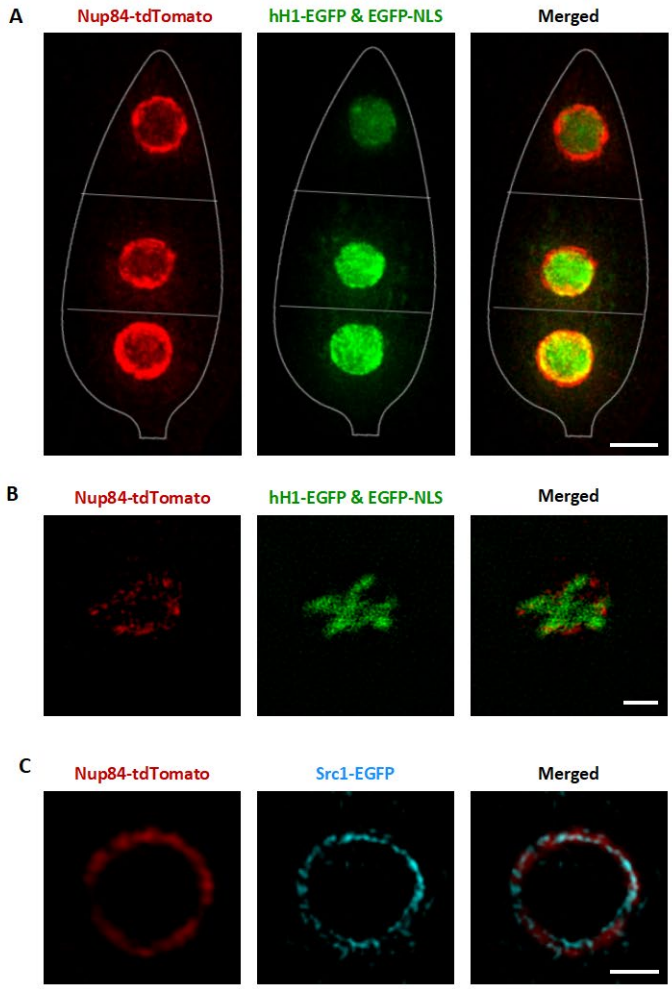

Figure 3.1. Arrangement of two nuclear envelope components in *M. oryzae*. (A) Maximum intensity projection of SR-SIM image of *M. oryzae* strain CKF3810. A conidium of CKF3810 shows three nuclei in interphase. Nup84-tdTomato (left panel, red) localizes within the nuclear envelope. Nuclear contents are labelled with two constructs, histoneH1 (hH1)-EGFP and EGFP-NLS (middle panel, green). Right panel is a merged image. Scale bar = 2 μm. (B) Single z-slice SR-SIM image of CKF3810 nucleus in the early stage of mitosis in the apical cell of the conidium. Nup84-tdTomato (left panel, red) surrounds hH1-EGFP (middle panel, green). A single z-slice image is displayed because it shows hH1-EGFP signal in optimal detail. EGFP-NLS is dispersed from the nucleus (data not shown). Right panel is a merged image. Scale bar = 1 μm. (C) Single z-slice SR-SIM image of CKF3881 nucleus in the apical cell of the conidium in interphase. Nup84-tdTomato (left panel, red) is distributed along the circumference of Src1-EGFP (middle panel, pseudocolored cyan). Right panel is a merged image. Scale bar = 1 μm.

Furthermore, during the early stages of mitosis, we found Nup84-tdTomato signal to surround histoneH1-EGFP when it was arranged in a palmate manner, which indicates condensation of DNA into mitotic sister chromatids, in the apical cell of the conidium (Figure 3.1B). The palmate arrangement of histoneH1-EGFP is consistent with a previous report in *M. oryzae* that showed sister chromatids are condensed in early mitosis, and kinetochores are clustered near the midzone of the spindle before non-synchronous declustering in the later stages of mitosis (Yadav et al., 2019).

We analyzed another fluorescent strain CKF3881 to determine the relative arrangement of nuclear envelope components, and to validate Nup84-tdTomato as a marker of the nuclear envelope in *M. oryzae*. CKF3881 coexpressed Nup84-tdTomato and Src1-EGFP (pseudocolored cyan) both driven by their native promoters. Our SR-SIM live-cell studies of CKF3881 revealed an irregular distribution of Nup84-tdTomato signal near the circumference of Src1-EGFP (a reporter for the inner nuclear membrane), in conidia during interphase (Figure 3.1A and 3.1C). Observing regions of brighter Nup84-tdTomato signal within the nuclear envelope during interphase suggested that NPCs in *M. oryzae* are irregularly distributed, as reported in other fungi (De Souza et al., 2004; Theisen et al., 2008; Winey et al., 1997). Data gleaned from both CKF3810 and CKF3881 established Nup84-tdTomato as a reliable marker of the nuclear envelope in *M. oryzae*.

Nup84-tdTomato remains associated with the dividing nucleus

We examined the dynamics of Nup84-tdTomato during nuclear division, using live-cell SR-SIM as the appressorium developed (Figure 3.2A). Nup84-tdTomato was redistributed towards the polar edges of the dividing nucleus in the germ tube of the developing appressorium. After the daughter nucleus arrived in the appressorium, Nup84-tdTomato adopted a circular localization pattern, consistent with our observations of nuclei in the conidium during interphase.

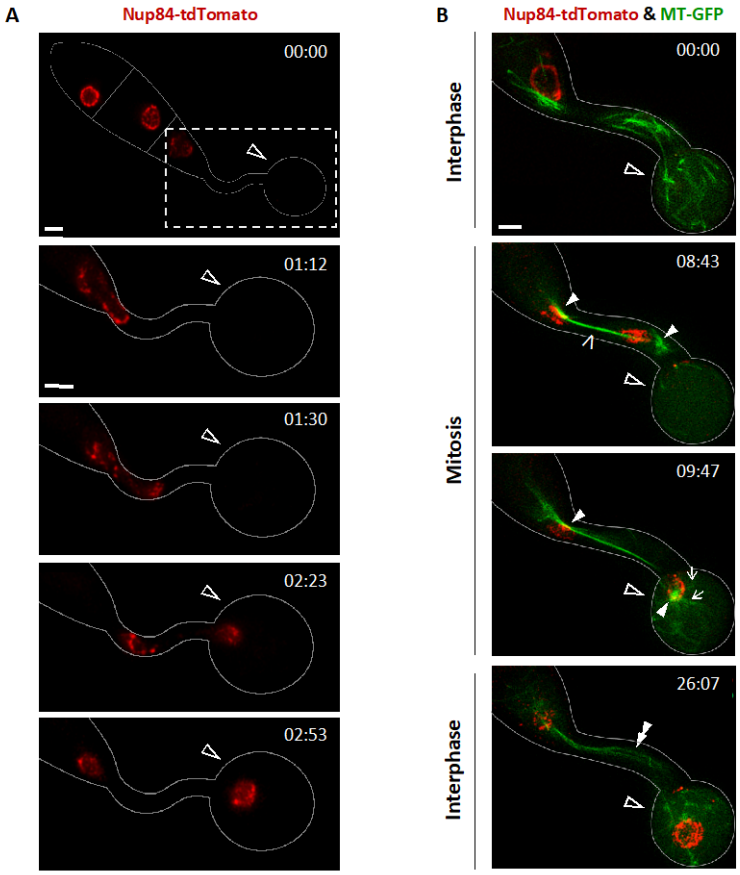

Figure 3.2. Dynamics of Nup84-tdTomato during mitotic nuclear migration through the germ tube of developing appressoria. (A) Time-lapse of nuclear division in the germ tube of a developing appressorium (open arrowheads) in *M. oryzae* strain CKF3810 (red channel only). A series of maximum intensity projections are shown. Nup84-tdTomato appears as clusters within the nuclear envelope of the two basal cells of the conidium, while Nup84-tdTomato begins its dynamic redistribution towards the polar edges of the dividing nucleus in the apical cell (00:00). The dashed-line box represents the region magnified in subsequent panels. Nup84-tdTomato continues to accumulate near the polar edges of the dividing nucleus as the nucleus appears to stretch and expand within the germ tube (01:12 and 01:30). Nup84-tdTomato signal is detected within the developing appressorium (02:23), and Nup84-tdTomato adopts a circular arrangement, indicating mitotic exit and transition into interphase (02:53). Scale bar = 1 µm. (B) Time-lapse of Nup84-tdTomato (red) dynamics in relation to microtubules (MT-GFP; green) in *M. oryzae* strain CKF3870 in the germ tube of a developing appressorium (open arrowheads). A series of maximum intensity projections are shown. Images correspond to Video 1. As noted in Video Legend 1, red and green channels were acquired separately. The red channel was acquired before the green channel causing a channel acquisition-related time lag, likely resulting in Nup84-tdTomato appearing to lag behind MT-GFP. Nup84-tdTomato is in a tear-dropped arrangement in the apical cell of the conidium with cytoplasmic microtubules (MTs) distributed in the conidium, germ tube, and developing appressorium (00:00). Nup84-tdTomato is distributed near a presumed SPB (solid arrowheads) of the mitotic spindle (V at 08:43). The daughter nucleus arrives in the appressorium with Nup84-tdTomato beginning to resume a circular arrangement, astral MTs (arrows) appear to emanate from the presumed SPB (solid arrowhead) (09:47). Nup84-tdTomato is arranged in a fully circular manner, and cytoplasmic MTs are apparent (double arrowhead) (26:07). Scale bar = 2 µm.

Importantly, Nup84-tdTomato did not disperse into the cytoplasm during mitosis. Nup84-tdTomato remained associated with the dividing nucleus (Figure 3.2A, panels 01:12 through 02:53). The observed polar localization of Nup84-tdTomato during mitosis towards the presumed spindle pole bodies (SPBs) was consistent with NPC distribution within the nuclear envelope during mitosis in *S. japonicus* and *A. nidulans* (Aoki et al., 2013; Liu et al., 2008). This polar localization of Nup84-tdTomato near the SPBs suggests Nup84 and other core Nups are associated with mitotic chromatin, as has been demonstrated in *A. nidulans* (Suresh et al., 2017). The localization of Nup84-tdTomato throughout the cell cycle supports the conclusion that Nup84 in *M. oryzae* is a core Nup localized within the nuclear envelope.

Nuclear migration through the germ tube occurs during mitosis. In fungi, nuclear migration can occur at various stages of the cell cycle (reviewed in Gladfelter and Berman, 2009). We investigated the cell cycle timing of nuclear migration through the germ tube of developing appressoria by conducting live-cell SR-SIM of *M. oryzae* strain CKF3870 that coexpressed Nup84-tdTomato and β-tubulin-GFP (MT-GFP; reporter for microtubules) in Figure 3.2B and Video 3.1[2] (n = 8 time-lapse series). Prior to spindle formation an enlarged tear-drop-shaped nuclear envelope was evident (Nup84-tdTomato Figure 3.2B) in the apical cell of the conidium, and cytoplasmic microtubules (MTs) were observed. Mitosis began and the spindle was arranged perpendicularly to the growth axis of the germ tube. At this time, Nup84-tdTomato signal appeared to be heterogeneously distributed across the nuclear envelope with brighter foci of Nup84-tdTomato detected near the presumed SPBs of the dividing nucleus (solid arrowheads in Figure 3.2B). These data indicate the start of Nup84-tdTomato

[2] Video 3.1 is available at https://ars.els-cdn.com/content/image/1-s2.0-S1087184520301638-mmc1.mp4

redistribution and accumulation at the polar edges near the SPBs of the dividing nucleus. As mitosis in *M. oryzae* progressed, the spindle was aligned to the growth axis of the germ tube, and Nup84-tdTomato localized at the polar edges of spindle (Figure 3.2B). These data are consistent with Nup84-tdTomato localization dynamics in another strain CKF3810 (Figure 3.2A). Astral MTs (arrows in Figure 3.2B) appeared to emanate from the presumed SPB (solid arrowheads) of the daughter nucleus as the daughter nucleus journeyed towards the developing appressorium (open arrowhead). Notably, the spindle continued to elongate in the early stages of mitotic nuclear migration. When the daughter nucleus arrived in the appressorium, the spindle spanned the entire length of the germ tube. Once the daughter nucleus was received in the appressorium, the spindle disassembled (double arrowhead). Nup84-tdTomato then adopted a circular arrangement, marking mitotic exit and transition into interphase. Following the dynamics of Nup84-tdTomato in relation to MT-GFP clearly demonstrated that the mitotic spindle delivered a single daughter nucleus to the developing appressorium (09:47 in Figure 3.2B). We interpret this data to unequivocally demonstrate that nuclear migration through the germ tube of the developing appressorium occurs during the later stages of mitosis, namely anaphase B. Moreover, the timing of this nuclear migration event is consistent with previous reports of mitotic nuclear migration in *M. oryzae* during appressorium-mediated plant penetration, invasive hyphal development, and during nuclear migration through the invasive hyphal peg (Jenkinson et al., 2017; Jones et al., 2016; Pfeifer et al., 2019; Shipman et al., 2017; Yadav et al., 2019).

Our data provide insights into nuclear envelope structure and the probable mechanism of intermediate mitosis in *M. oryzae.* Our data show that Nup84 is a core Nup because of its consistent association with the nuclear envelope throughout the cell cycle. In *A. nidulans*, core Nups remain embedded within NPCs in the nuclear envelope, while another subset of Nups, called peripheral Nups, disperse into the cytoplasm

thereby enhancing permeability of the nuclear envelope during mitosis (Osmani et al., 2006). We hypothesize *M. oryzae* uses a similar mechanism. Investigating the dynamics of *M. oryzae* peripheral Nups along with additional inner nuclear membrane proteins during mitosis will determine if our hypothesis is supported. Not only will these studies elucidate the mechanism of intermediate mitosis in *M. oryzae*, but they will reveal the extent of nuclear envelope stretching as the dividing nucleus migrates through the germ tube. Knowledge gained from our current and proposed future studies is important. During rice blast infection, the migrating mitotic nucleus of *M. oryzae* becomes extremely elongated to squeeze through narrow infection structures, such as the penetration and invasive hyphal pegs (Jenkinson et al., 2017; Jones et al., 2016; Pfeifer et al., 2019; Pfeifer and Khang, 2018; Shipman et al., 2017). In higher eukaryotes that undergo similar constricted nuclear migration events, stability of the nuclear envelope along with rapid repair of transient nuclear envelope damage is essential (Bone et al., 2016; Denais et al., 2016; Raab et al., 2016). In these organisms, a nuclear lamina stabilizes the nuclear envelope during constricted nuclear migration events. Yet fungi lack canonical nuclear lamina within their nuclear envelopes (Melcer et al., 2007). Without nuclear lamina, intriguing questions about the biology of *M. oryzae* emerge: (1) Is nuclear envelope stability required for successful nuclear migration through constricted spaces like the germ tube and other infection-associated structures? (2) If nuclear envelope stability is required, what subcellular components mediate nuclear envelope stability in *M. oryzae*? Future studies following fungal markers for nuclear envelope damage and repair will help answer these and other related questions. Such studies may lead to identification of novel fungal targets that could be exploited to develop new strategies to fight rice blast disease.

Methods

Nup84 and Src1 in *M. oryzae* were identified by obtaining *A. nidulans* Nup84 (AN1190) and *A. nidulans* Src1 (AN3910) protein sequences from the Aspergillus Genome Database (http://www.aspgd.org/), and querying the *M. oryzae* 70-15 reference genome using BLASTP. Global protein sequence similarity values were determined using EMBOSS Stretcher (https://www.ebi.ac.uk/Tools/psa/emboss_stretcher/; Madeira et al., 2019). *M. oryzae* fluorescent strains, CKF3810, CKF3881, and CKF3870, were generated by transforming wild-type strain O-137 sequentially with binary vectors using *Agrobacterium*-mediated transformation (Khang et al., 2005). CKF3810 was generated by inserting plasmid pCK1898 into recipient strain CKF3770, containing plasmids pBV229 (hH1-EGFP; Shipman et al., 2017) and pCK1288 (3xEGFP-NLS; Jones et al., 2016). The NLS sequence used was produced by cloning three tandem repeats of the nuclear localization signal from simian virus large T-antigen (Jones et al., 2016). pCK1898 was produced by cloning the native promoter and Nup84 coding sequence at the 5' end of tdTomato into binary vector pCK1806 (Nourseothricin selection marker). CKF3881 was constructed by inserting plasmid pCK1967 into recipient strain CKF3846, containing pCK1898. pCK1967 was produced by cloning the native promoter and Src1 coding sequence at the 5' end of EGFP into the binary vector pBV1 (Hygromycin selection marker). CKF3870 was generated by inserting plasmid pCK1722 into recipient strain CKF3846. pCK1722 was generated by cloning β-tubulin at the 5′ end of *GFP* under control of *Neurospora crassa* ccg-1 promoter from pMF309 (Freitag et al., 2004) into binary vector pBGt (G418 selection).

Conidia were harvested using Miracloth from *M. oryzae* strains cultured on oatmeal agar plates at 24°C under continuous light. Appressoria development was induced by dropping a suspension of 3-4 x 10^4 conidia into a hydrophobic slide chamber (Hamer et al., 1988; Saunders et al., 2010). Conidia were incubated at room temperature for 3 and

4 hours to permit appressoria development. Microscopy was performed on a Zeiss ELYRA S1 (SR-SIM) Super Resolution Microscope using a Plan-Apochromat 63X/1.4 Oil DIC M27 objective. Excitation/emission wavelengths were 488 nm/495–550 nm (GFP), and 561 nm/570–620 nm (tdTomato). Images were acquired with an Andor iXon EM-CCD camera. ZEN 2011 software with a SIM analysis module was used for image acquisition and structured illumination reconstruction. Images were further analyzed using a combination of the Zen software (Blue and Black editions), Adobe Photoshop, and ImageJ (http://imagej.nih.gov/ij/).

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CHAPTER 4

A STRIKINGLY-ANGLED SPINDLE MEDIATES NUCLEAR MIGRATION DURING COLONIZATION OF RICE CELLS INFECTED BY *MAGNAPORTHE ORYZAE*[1]

[1] Pfeifer, M. A., Jones, K., & Khang, C. H. (2019). A strikingly-angled spindle mediates nuclear migration during colonization of rice cells infected by *Magnaporthe oryzae*. *Fungal Genetics and Biology*, *126*, 56-60. Reprinted here with permission of the publisher.

Abstract

To cause rice blast disease, *Magnaporthe oryzae* must properly organize microtubules and position nuclei during colonization of host cells. Live cell confocal imaging of fluorescently-tagged microtubules and nuclei of *M. oryzae* invasive hyphae reveals that microtubules form a cage-like arrangement around nuclei during interphase and that the mitotic spindle forms and mediates nuclear migration while integrity of the nuclear envelope is lost. Our results also unveil a strikingly-angled spindle during nuclear migration through the narrow invasive hyphal peg, suggesting a yet-to-be discovered mechanism of mitotic nuclear migration when invasive hyphae move to adjacent rice cells.

Introduction

Magnaporthe oryzae is a destructive fungal pathogen, causing blast disease in rice and other economically significant plants. To cause disease, *M. oryzae* develops a specialized penetration cell, called the appressorium, which can deliver multiple daughter nuclei to invasive hyphae (IH) growing inside rice cells (Jenkinson et al., 2017). The fungus uses a form of intermediate mitosis to proliferate within the first-invaded rice cell (Jones et al., 2016; Pfeifer and Khang, 2018; Shipman et al., 2017). After colonization of the first-invaded cell, IH begin cell-to-cell movement by scanning the rice cell wall presumably searching for suitable crossing points located in pitfields (Kankanala et al., 2007; Sakulkoo et al., 2018). Once a crossing point is identified, *M. oryzae* develops an extremely constricted structure, called the IH peg (~0.5 μm), which serves as a conduit connecting a mother IH cell to a daughter IH cell located in a newly-invaded rice cell (Kankanala et al., 2007). In our previous studies of IH cell-to-cell movement, a single nucleus divided in the mother IH cell, and one daughter nucleus elongated to successfully cross the narrow IH peg by an unknown mechanism (Jones et al., 2016).

In fungi, microtubules (MTs) play significant roles mediating nuclear migration throughout the cell cycle (Xiang, 2017). In *M. oryzae*, MT arrangement and behavior during vegetative growth and appressorium development are characterized, but little is known about MT dynamics during colonization of host cells (Czymmek et al., 2005; Row et al., 1985; Saunders et al., 2010a; Saunders et al., 2010b; Veneault-Fourrey et al., 2006). Here, we report MT dynamics in IH located in the first-invaded cell and during cell-to-cell movement. We demonstrate that nucleation of incipient IH cells occurs via mitotic nuclear migration, and that spindle choreography is complex during nuclear migration through the IH peg.

Results and Discussion

Interphase arrangements of microtubules during rice infection. We created *M. oryzae* strain CKF3578 by introducing β-tubulin-GFP, labelling MTs (Freitag et al., 2004), into an *M. oryzae* strain coexpressing histone H1 fused to tdTomato (H1-tdTomato) and tdTomato fused to a nuclear localization signal (tdTomato-NLS). H1-tdTomato and tdTomato-NLS colocalize within the nucleus in interphase, but at the start of mitosis tdTomato-NLS disperses from the nucleus into the cytoplasm, as similarly shown with the *M. oryzae* mitotic reporter strain expressing GFP-NLS and H1-tdTomato (Jones et al., 2016). Thus, *M. oryzae* CKF3578 permits study of MTs in the context of the *M. oryzae* cell cycle, with dispersal of tdTomato-NLS from the nucleus serving as an indicator of loss of nuclear envelope integrity (Pfeifer and Khang, 2018). Confocal microscopy of CKF3578 growing inside rice cells revealed that MTs are typically positioned along the growth axis and follow the curvature of IH cells during interphase (Figure 4.1A). In many fungi, such as *Neurospora crassa* and *Saccharomyces cerevisiae*, MTs are nucleated from microtubule-organizing centers (MTOCs) associated with the nuclear envelope called spindle pole bodies (SPBs) during interphase (Kilmartin and Adams, 1984; Roca et al., 2010). In contrast, the cytoplasmic MTs we observe during interphase in *M. oryzae* did not appear to nucleate from a SPB and instead form a cage-like arrangement around the nucleus (Figure 4.1A). This is similar to patterns of SPB-independent MTs observed in the yeast-like growth of *Ustilago maydis* (Straube et al., 2005) and suggests that *M. oryzae* utilizes MTOCs other than SPBs during interphase.

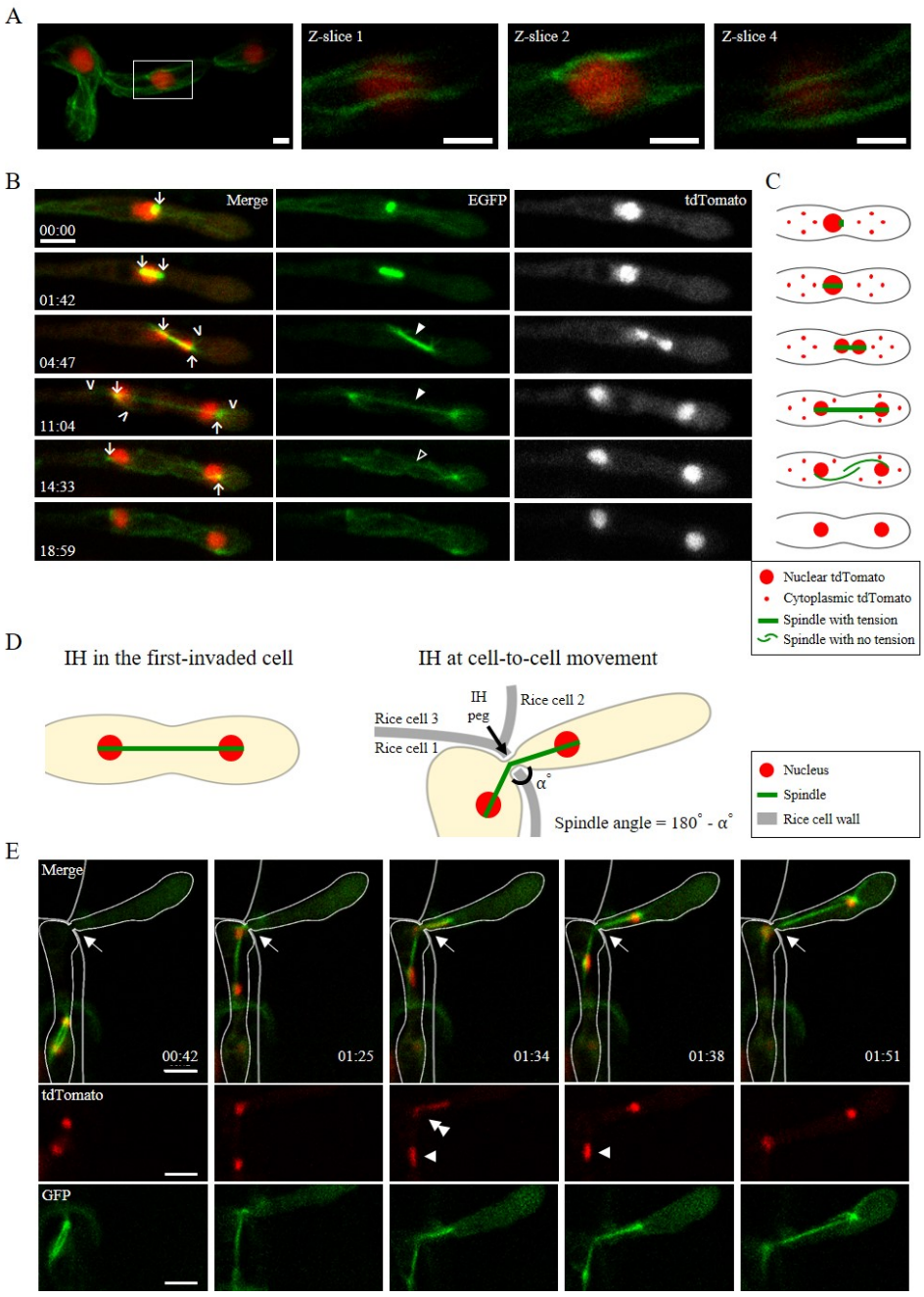

Figure 4.1. Microtubule (MT) and nuclear dynamics of *M. oryzae* strain CKF3578 during rice infection. CKF3578 expresses β-tubulin-GFP (green), H1-tdTomato (red) and tdTomato-NLS (red). Note that H1-tdTomato is associated with DNA throughout the cell cycle whereas tdTomato-NLS is localized in nuclei during interphase but dispersed in the cytoplasm during mitosis. Figure 4.1. legend continues on next page.

Figure 4.1. (continued) A) Confocal images revealing MT arrangement during interphase of *M. oryzae* invasive hyphae (IH) in the first-invaded rice cell. Interphase is indicated by absence of tdTomato-NLS in the cytoplasm. MTs are arranged along the growth axis and follow the curvature of IH. The left panel shows a maximum intensity projection of four z-slices, spanning 4 µm. The white box denotes a cage-like arrangement of MTs around the nucleus and is shown in detail in the second through fourth panels, which are at different focal planes. In relation to z-slice 1, z-slice 2 is 1 µm below, and z-slice 4 is 3 µm below. Bars = 2.5 µm. (B) Time-lapse confocal images revealing MT and nuclear dynamics during mitosis in leading IH in the first-invaded rice cell. The images included are select still images from Video 4.1 shown as merged fluorescence (left), β-tubulin-GFP (middle) and H1-tdTomato/tdTomato-NLS (pseudocolored white; right). In the merged panels, arrows show spindle pole bodies, and arrowheads denote noticeable astral microtubules. In the EGFP panels, solid arrowheads show tension within the spindle (04:47 and 11:04), and loss of spindle tension is shown by an open arrowhead (14:33). Cytoplasmic tdTomato (tdTomato-NLS) is already dispersed from the nucleus at 00:00 and is fully reimported into the nucleus in the bottom panel (18:59). Times are in minutes: seconds. Bar = 5 µm. (C) A schematic representation of tdTomato localization relative to spindle dynamics during mitosis in leading IH in the first-invaded rice cell. (D) A schematic representation of nuclear (red) and spindle (green) positioning in leading IH located in the first-invaded rice cell (left) and during the hyphal movement from Rice cell 1 to Rice cell 2 (right). (E) Time-lapse confocal images revealing spindle and nuclear dynamics during hyphal cell-to-cell movement through the IH peg as represented in Figure 1D right. The images included are select still images from Video 4.2. Video 4.2 still images are shown in the top panel (merge). tdTomato fluorescence (middle) shows nuclear dynamics. tdTomato-NLS signal is faintly observed in the cytoplasm throughout the time-series, indicating mitosis. GFP fluorescence (bottom) indicates the spindle. Arrows indicate the IH peg. Single arrowheads show a slightly elongated nucleus, and a double arrowhead shows a stretched nucleus migrating through the IH peg. As the spindle migrates through the IH peg, it adopts an angle of 66° (01:34). Bars = 5 µm.

Mitotic dynamics during IH growth inside the first-invaded rice cell. Using time-lapse confocal microscopy of *M. oryzae* CKF3578, we determined mitotic MT dynamics in the most apical, or leading, IH cell growing inside the first-invaded rice cell. A representative example is shown in Video 4.1[2], with selected single-frames from Video 4.1 included in Figure 4.1B. We first identified an IH cell in prophase based on the presence of a single nucleus with tdTomato-NLS dispersed in the cytoplasm and a bright focus of β-tubulin-GFP at the edge of the nucleus, presumably indicating duplicated

[2] Video 4.1 is available at https://ars.els-cdn.com/content/image/1-s2.0-S1087184518302287-mmc1.mp4

SPBs (Video 4.1, Figure 4.1B; 00:00). At this stage, interphase cytoplasmic MTs are visible but begin disassembling. We observe SPBs migrate to opposite sides of the nucleus (01:42). As the tdTomato-tagged chromosomes separate, astral MTs appear and form transient associations with the cell cortex, behaving dynamically and increasing in average length (04:47). Oscillatory movements of the spindle (e.g., multiple reversals in migration direction), combined with spindle extension, result in the net movement of the daughter nucleus closer to the incipient IH cell (Video 4.1). Once the daughter nucleus approaches its interphase position in the incipient IH cell, the spindle reaches a maximum length (11:04). At this stage, the spindle appears as a straight-line, which we interpret as tension within the spindle (11:04). As the spindle disassembles, tension in the spindle is lost, and the spindle appears to be unraveled (14:33). Subsequently, interphase cytoplasmic MTs reappear, and tdTomato-NLS is reimported into the nucleus (18:59). Our results indicate that the spindle forms and mediates nuclear migration while compartmentalization between the nucleus and the cytoplasm is lost (Figure 4.1C). This suggests increased permeability of the nuclear envelope during mitosis and supports our earlier hypothesis that *M. oryzae* uses intermediate mitosis during rice infection (Jenkinson et al., 2017; Jones et al., 2016; Pfeifer and Khang, 2018; Shipman et al., 2017). Our results also show that spindles are straightly arranged when IH colonize the first-invaded rice cell (Figure 4.1B and 4.1D left).

During nuclear movement through the IH peg, the spindle adopts an extremely angled morphology. Using the same time-lapse approach as we did in the first-invaded cell, we examined the dynamics of MTs during nuclear migration through

the IH peg (Figure 4.1D right, Video 4.2[3], and selected frames in Figure 4.1E). The example shown in Video 4.2 is representative, and select still images from Video 4.2 are presented in Figure 4.1E. In early anaphase, the interpolar MTs of the spindle appear as bars connecting separated chromosomes (Figure 4.1E, 00:42). Prior to nuclear migration through the IH peg, the spindle is aligned with the growth axis of the mother IH cell in Rice cell 1 (Figure 4.1D right and Figure 4.1E 01:25). During nuclear migration through the IH peg, the nucleus stretches, and the spindle is noticeably angled (Figure 4.1E, 01:34 and 01:38). Once the migrating nucleus crosses the IH peg, it again becomes spherical (01:38). The spindle continues to elongate in an angular-manner (01:38). Once maximum length of the spindle is achieved (01:51), a sudden loss of tension in the spindle occurs, marking the start of spindle disassembly (not shown in Video 4.2). In all observations (n=10), the spindle persists with obvious tension between the two SPBs before, during, and after nuclear migration through the IH peg, indicating that nuclear migration through the IH peg occurs during mitosis. Intriguingly, we also observe the spindle displaying distinct choreography as the nucleus crosses the IH peg (Video 4.2; Figure 4.1E, 01:34 and 01:38). In 80% of nuclear migration events through the IH peg, the spindle becomes strikingly angled, ranging from 30° to 74° (52° mean ± 14°). The spindle in the remaining 20% of events appears less-angled and more curved. Compared to other fungi, e.g., *Schizosaccharoymces pombe* and *Saccharomyces uvarum,* the angled spindle during migration through the IH peg in *M. oryzae* is extreme (Kilmartin and Adams, 1984; Tanaka and Kanbe, 1986). Such angled spindle morphology is likely a function of the unique shape of IH cells at the cell-to-cell

[3] Video 4.2 is available at https://ars.els-cdn.com/content/image/1-s2.0-S1087184518302287-mmc2.mp4

movement stage of rice infection. It is common to observe these IH displaying extensively curved or angled geometries compared to leading IH. Given the link between cell geometry and spindle alignment (Daga and Nurse, 2008), we propose that successfully crossing the IH peg requires special coordination for mitotic nuclear migration.

In sum, our results provide novel insight about mitosis and nuclear migration of *M. oryzae* during rice infection. We show that during interphase in IH cells, MTs form a cage-like arrangement around the nucleus. Prior to spindle formation, compartmentalization between the nucleus and cytoplasm is lost, a hallmark of intermediate mitosis (Pfeifer and Khang, 2018). Spindle dynamics demonstrate that nucleation of incipient IH within the first-invaded cell and at the cell-to-cell movement stage of infection occurs via mitotic nuclear migration. Remarkably, a majority of spindles adopt drastic angles during nuclear migration through the confined IH peg. These observations suggest that mechanisms of spindle positioning and alignment are especially important for proper nuclear migration at the cell-to-cell movement stage of rice infection. Discovering the key molecular players in the underlying mechanism may lead to identification of targets for antifungals in the future, providing a means to block fungal proliferation beyond the first-invaded host cell.

Methods

M. oryzae wild-type strain O-137 was transformed sequentially with two binary vectors pCK1528 and pCK1728 to generate transformant CKF3578 using *Agrobacterium*-mediated transformation (Khang et al., 2005). pCK1528 was produced by cloning NLS (three tandem repeats of the nuclear localization signal from simian virus large T-antigen) at the C terminus of tdTomato under control of the *M. oryzae* ribosomal protein 27 gene (RP27) promoter in binary vector pBGt (G418 selection; Kim et al., 2011). pCK1728 was produced by cloning histone *H1* at the 5' end of tdTomato under control of the RP27 promoter (Shipman et al., 2017) and β-tubulin (Bml) at the 5' end of *GFP* under control of *Neurospora* ccg-1 promoter from pMF309 (Freitag et al., 2004) in binary vector pBHt2 (hygromycin selection; Mullins et al., 2001). Rice variety YT16 was grown and inoculated as previously described (Jones and Khang, 2018).

Confocal microscopy was performed on a Zeiss 880 confocal system using a Plan-Neofluor 40x/1.3 NA (oil) objective. Excitation/emission wavelengths were 488 nm/505–530 nm (GFP), and 543 nm/560–615 nm (tdTomato). Images were analyzed and processed using a combination of the Zen software (Black edition), Adobe Photoshop, and ImageJ (http://imagej.nih.gov/ij/). Spindle angles were measured with the angle tool in ImageJ. Angle measurements were transformed as described in Figure 4.1D and analyzed using JMP Pro Version 13.2.

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CHAPTER 5

THE MITOTIC SPINDLE MEDIATES NUCLEAR MIGRATION THROUGH AN EXTREMELY NARROW INFECTION STRUCTURE OF THE RICE BLAST FUNGUS *MAGNAPORTHE ORYZAE*[1]

[1] Pfeifer, M. A., & Khang, C. H. (2021). The mitotic spindle mediates nuclear migration through an extremely narrow infection structure of the rice blast fungus *Magnaporthe oryzae*. Submitted to *mBIO*.

Abstract

The blast fungus, *Magnaporthe oryzae*, causes severe destruction to rice and other crops worldwide. As the fungus infects rice, it develops unique cellular structures, such as an appressorium and a narrow penetration peg, to permit successful invasion of host rice cells. Fundamental knowledge about these cellular structures and how organelles, such as the nucleus, are positioned within them is still emerging. Previous studies show that a single nucleus becomes highly stretched during movement through the narrow penetration peg in an extreme nuclear migration event. Yet the mechanism permitting this nuclear migration event remains elusive. Here, we investigate the role of the mitotic spindle in mediating nuclear migration through the penetration peg. We find that disruption of spindle function during nuclear migration through the penetration peg prevents development of invasive hyphae and virulence on rice. Furthermore, regulated expression of conserved kinesin motor proteins, MoKin5 and MoKin14, is essential to form and maintain the spindle, as well as, properly nucleate the primary hypha. Overexpression of MoKin5 leads to formation of aberrant microtubule protrusions, which contributes to formation of nuclear fragments within the appressorium and primary hypha. Conversely, overexpression of MoKin14 causes the spindle to collapse leading to the formation of monopolar spindles. These results establish a mechanistic model towards understanding the intricate subcellular dynamics of extreme nuclear migration through the penetration peg, a critical step in the development of rice blast disease.

Introduction

Nuclear migration and proper nuclear positioning are fundamental eukaryotic processes. Disruption of nuclear migration, which can lead to improper nuclear positioning, is linked to developmental defects in lower eukaryotes and disease states in humans and higher eukaryotic organisms (Bone and Starr, 2016). Seminal studies of nuclear migration and positioning in fungi revealed that cellular components, such as microtubules (MTs) and motor proteins (i.e., kinesins and dynein) are required for successful nuclear migration (Eshel et al., 1993; Plamann et al., 1994; Xiang et al., 1994). Studies of nuclear migration in various organisms underscore that mechanisms of nuclear migration can be complex, involving the eloquent coordination of cytoskeletons and various motor proteins within the context of the cell cycle. Mechanisms of nuclear positioning vary in fungi (Gladfelter and Berman, 2009). For example, in mature hyphae of the ascomycete *Neurospora crassa*, cytoplasmic bulk flow passively moves nuclei forward (Ramos-García et al., 2009). Other fungi, like the basidiomycete *Ustilago maydis*, utilize a mitotic nuclear migration event to deliver a newly-divided nucleus to the bud (Straube et al., 2005). In nuclear migration events that occur during mitosis, the spindle is a key player. Spindles are elaborate cellular machines that ensure genetic information is equally divided between mother and daughter cells. Spindles are comprised of MTs, spindle pole bodies (SPBs), and condensed chromosomes called chromatids, along with motor and other MT-associated proteins.

One powerful framework used to explain the intricacy of spindle formation, as well as elongation and maintenance of the spindle throughout mitosis is the force-balance model (Blackwell et al., 2017). The force-balance model establishes that spindles are formed and maintained by motor proteins exerting antagonizing forces upon SPBs. In many fungi and other eukaryotes, these motor proteins are members of the kinesin-5 and kinesin-14 superfamilies. Canonical functions of kinesin-5 and kinesin-14

motor proteins are defined. Kinesin-5 motor proteins walk towards the growing plus-ends of MTs and exert an outward force on SPBs (Waitzman and Rice, 2014). Kinesin-14 motor proteins walk towards the minus-ends of MTs and exert an inward force on SPBs (She and Yang, 2017). However, not all eukaryotes rely on kinesin-5 and kinesin-14 motor proteins to form and maintain a spindle. For example, kinesin-5 is dispensable in the human pathogenic fungus, *Candida albicans* (Chua et al., 2007; Shoukat et al., 2019). Within *Drosophila* embryos, dynein provides the antagonistic inward force instead of kinesin-14 (Sharp et al., 2000). While the mitotic roles of kinesin-5 and kinesin-14 are defined during development of a number of model organisms, much less is known about the functions of these proteins in spindle formation and function in diverse biological contexts. For instance, what are the roles of these motor proteins in forming a spindle within eukaryotic pathogens as pathogens infect hosts?

The blast fungus, *Magnaporthe oryzae* (anamorph *Pyricularia oryzae),* is a plant pathogen capable of grievous damage to cereal crops worldwide, including rice, wheat and finger millet (Islam et al., 2019; Skamnioti and Gurr, 2009; Takan et al., 2004; Tembo et al., 2020). The *M. oryzae* and rice pathosystem serves as a valuable model towards understanding the nuclear migration dynamics of a pathogen during host infection. *Magnaporthe oryzae* is mononuclear, i.e., each cell contains a single nucleus Rice blast infection is initiated when conidia of *M. oryzae* attach to rice leaves. Conidia germinate and develop appressoria. Appressoria are highly-melanized infection structures. Within appressoria, huge amounts of turgor pressure accumulate, and cytoskeletons, such as F-actins and septins, rearrange at the appressorial pore to give rise to the first fungal structure to enter the rice cell, the penetration peg (Dagdas et al., 2012; Howard et al., 1991). The penetration peg is narrow at ~0.7 μm and can reach a maximum length of 3.3 μm (Howard and Valent, 1996). The primary hypha, the first fungal hypha located within the first-invaded rice cell, must be sufficiently developed for

nuclear migration through the penetration peg to occur. For instance, when the average tip diameter of the primary hypha is 3.1 μm only a single nucleus is observed within the appressorium. However, once the average diameter of the primary hypha tip increases to 5.6 μm, a nucleus within the appressorium and a nucleus within the primary hypha is observed (Shipman et al., 2017). As the fungus continues to grow inside the first-invaded rice cell, bulbous invasive hyphae (IH) develop. Once the first-invaded rice cell is completely colonized by the fungus, the fungus seeks pit fields, housing plasmodesmata, to continue proliferating within rice cells (Kankanala et al., 2007). At plasmodesmata in the first-invaded rice cell, the fungus develops another narrow structure called the IH peg (Sakulkoo et al., 2018). The IH peg serves as a conduit to connect IH within the first-invaded rice cell to IH growing within adjacent rice cells. Eventually disease lesions appear on the surface of rice leaves as the fungus spreads throughout the plant.

The nuclear migration dynamics of *M. oryzae* are best characterized during vegetative hyphal growth and during the early events of rice infection (Pfeifer and Khang, 2018). During early rice infection, a single nucleus, referred to here as the mother nucleus, is located within the appressorium (Jenkinson et al., 2017; Shipman et al., 2017). The newly formed migrating nucleus, here called the daughter nucleus, endures an extreme nuclear migration event, while the mother nucleus remains within the appressorium. During this extreme nuclear migration event, the mother nucleus with a diameter of ~ 2 μm begins to divide within the appressorium. Subsequently, the daughter nucleus becomes highly stretched as it transits the constricted penetration peg (Howard and Valent, 1996; Jenkinson et al., 2017). The daughter nucleus then quickly moves to the apical region of the primary hypha located inside the first-invaded rice cell (Jenkinson et al., 2017; Shipman et al., 2017). Typically, this process lasts ~5 minutes,

with the daughter nucleus traveling over 20 μm from the appressorium through the penetration peg into the primary hypha (Jenkinson et al., 2017).

Although the general behavior of the mother and daughter nucleus are characterized during this extreme nuclear migration event, the cytoskeletons involved in this process are unknown. Based on studies of subsequent *M. oryzae* infection stages, it is likely the spindle is involved. During development of bulbous IH inside the first-invaded rice cell, the spindle nucleates newly-formed IH during mitotic nuclear migration (Jones et al., 2016; Pfeifer et al., 2019). The spindle also delivers a newly-formed nucleus to IH growing in adjacent rice cells through the narrow IH peg. During movement through the IH peg, the spindle can adopt a striking geometry to facilitate movement of the nucleus (Pfeifer et al., 2019). Intriguingly, the migrating nucleus with a diameter of ~2 μm becomes highly elongated as it moves through the IH peg with a diameter of ~0.5. This pattern is akin to the nuclear morphology of the migrating daughter nucleus during movement through the penetration peg at earlier stages of rice infection (Jenkinson et al., 2017).

Despite evidence that the spindle is involved in nuclear migration at other *M. oryzae* infection stages, there is no direct evidence that the spindle mediates extreme nuclear migration through the penetration peg during initial rice cell colonization. Moreover, kinesin-5 and kinesin-14 motor proteins are yet to be discovered within *M. oryzae*. The goal of this study was twofold. First, we determined that the spindle is involved in nuclear migration through the penetration peg using confocal live-cell imaging of this cellular phenomenon. Second, we identified kinesin-5 and kinesin-14 motor proteins in *M. oryzae*. Identification of kinesin-5 and kinesin-14 in *M. oryzae* allowed us to develop an approach to genetically perturb spindle function specifically during extreme nuclear migration through the penetration peg. Our live-cell imaging

observations coupled with experiments genetically perturbing spindle function demonstrate that the spindle mediates nuclear migration through the penetration peg.

Results

Dynamics of the spindle and the nucleus during extreme nuclear migration.

We determined that the spindle formed and elongated during nuclear migration through the penetration peg using confocal live-cell imaging of a fluorescent *M. oryzae* strain (Fig.5.1A). In this strain, microtubules (MTs) were labeled with β-tubulin-GFP (pseudo colored cyan throughout figures), and the nucleus was labeled with histone H1-tdTomato (RFP). We inoculated the fungal strain onto susceptible rice sheaths and observed nuclear migration through the penetration peg (Fig.5.1B). Prior to nuclear migration through the penetration peg, the spindle bisected the mother nucleus within the appressorium (Fig.5.1B, 00:00). The spindle and the mother nucleus rotated to become aligned to the axis of the appressorial pore and penetration peg (Fig.5.1B, comparing 00:00 to 03:52). During migration through the penetration peg, the daughter nucleus stretched and separated (Fig.5.1B, 06:24; *See Fig.5.3A*). The mother nucleus remained within the appressorium. The daughter nucleus was delivered to the apical region of the primary hypha as the spindle elongated (Fig.5.1B, comparing 06:24 to 10:12). During this nuclear migration event, the daughter nucleus traveled a total of 22 μm from the site where the spindle first bisected the mother nucleus in the appressorium to the primary hypha. Consequently, nuclei migrating through the penetration peg undergo a longer nuclear migration compared to nuclei migrating in other IH cells. For instance, during nuclear migration in leading IH in wild-type, the maximum spindle length is typically less than 14 μm (*See Fig.5.10E*). From these data, we concluded the spindle is involved in extreme nuclear migration through the penetration peg in wild-type.

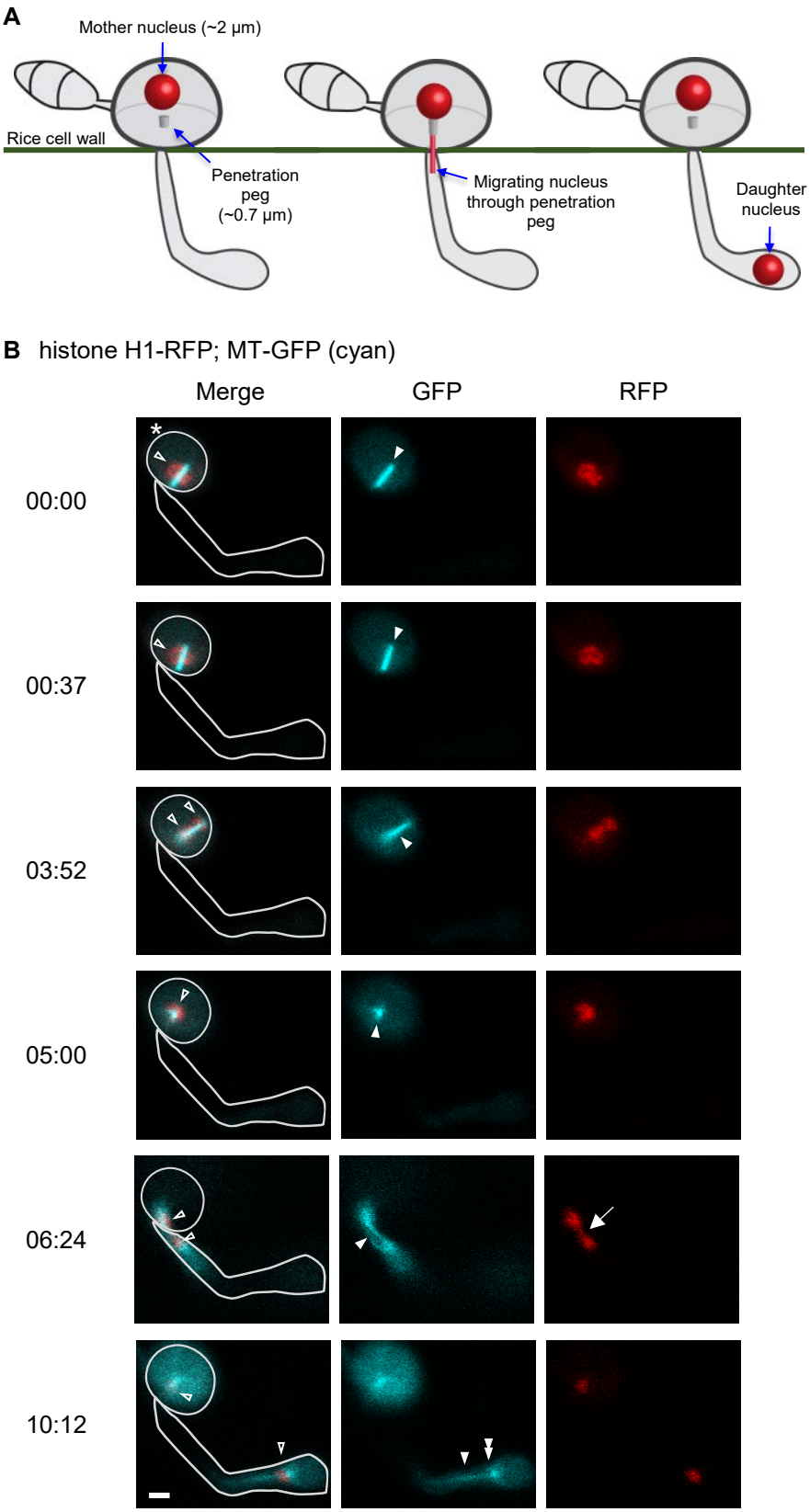

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Fig.5.1. (continued) The spindle mediates nuclear migration through the penetration peg. (A) Schematic representation of extreme nuclear migration in *M. oryzae* during movement through the penetration peg. The nucleus in the appressorium is referred to, here, as the mother nucleus (left). The appressorium forms on the surface of the rice leaf. The migrating nucleus, called the daughter nucleus, becomes elongated as it moves through the penetration peg (middle). Note that the penetration peg spans the rice cell wall, and does not protrude into the appressorium as shown in this depiction. The daughter nucleus is then positioned at the apical region of the primary hypha (right). The primary hypha forms inside the first-invaded rice epidermal cell. (B) Extreme nuclear migration through the penetration peg in *M. oryzae* strain CKF3578. The nucleus is shown in red (histone H1-RFP), and the spindle is shown in cyan (MT-GFP). Times is in minutes: seconds. An overlay to outline the appressorium and the primary hypha is provided in the merged channel. The GFP and RFP channel micrographs are purposively left without an overlay to more clearly display annotations. (00:00) The spindle (filled arrowhead) bisects the mother nucleus (open arrowhead) within the appressorium (asterisk). (00:37) The spindle rotates to become aligned for movement through the penetration peg. (03:52) Condensed chromosomes (chromatids) move towards the polar edges of the spindle (open arrowheads) while the spindle continues to become aligned for movement through the penetration peg. (05:00) the spindle (filled arrowhead) and mother nucleus (open arrowhead) are positioned for movement through the penetration peg. Due to the three-dimensional nature of the appressorium, the mother nucleus and spindle appear to be co-localized at this point. (06:24) The mother nucleus remains within the appressorium (top open arrowhead), while the daughter nucleus begins to separate from the mother nucleus (bottom open arrowhead) in the penetration peg, and is stretched within the penetration peg (arrow). Here the length of dividing nuclei from the top of the mother nucleus to the bottom of the daughter nucleus is approximately 5 μm. Penetration pegs are known to range in length up to 3.3 μm (Howard and Valent, 1996). The spindle (filled arrowhead) propels the daughter nucleus forward. (10:12) The mother nucleus is located within the appressorium (top open arrowhead), the daughter nucleus is delivered to the apical region of the primary hypha (bottom open arrowhead) by the spindle (filled arrowhead). The daughter bound spindle pole body is evident (double filled arrow). Micrographs are single informative focal planes. Scale bar is 2 μm.

Identification of MoKin5-RFP as a marker for spindle pole bodies (SPBs) during mitosis. Since kinesin-5 plays an important role in formation and maintenance of spindles in other fungi, we identified the kinesin-5 homolog, *MoKin5*, in *M. oryzae*. *MoKin5* (MGG_01175) was identified based on protein sequence homology to previously characterized kinesin-5 proteins in *Aspergillus nidulans, Schizosaccharomyces pombe,* and *Saccharomyces cerevisiae* (Fig.5.S1). MoKin5 shared 62% global similarity to BimC (AN3363), which is kinesin-5 in *A. nidulans*. Importantly, MoKin5 contained a predicted kinesin motor domain near its N-terminus. The location of this kinesin motor domain is characteristic of kinesin-5 motor proteins, and indicate that MoKin5 likely walks towards the plus-ends of MTs (Waitzman and Rice, 2014). We cloned MoKin5 to produce a *MoKin5*-tdTomato (RFP) construct driven by the native *MoKin5* gene promoter. We generated *M. oryzae* fluorescent strains to determine the subcellular localization of MoKin5-RFP in wild-type during interphase and mitosis.

The subcellular localization of MoKin5-RFP was first determined relative to MT-GFP during interphase in IH using live-cell confocal imaging. During interphase, MTs were arranged in a cage-like manner around a mass of MoKin5-RFP fluorescence (Fig.5.2A). We hypothesized that the mass of MoKin5-RFP fluorescence represented the nucleus. We confirmed that MoKin5-RFP localized within the nucleus during interphase in an additional *M. oryzae* strain. This strain expressed MoKin5-RFP and histone H1-GFP to label the nucleus. During interphase, MoKin5-RFP and histone H1-GFP co-localized (Fig.5.2B). We concluded that when MoKin5-RFP is expressed from its native promoter it accumulates within the nucleus during interphase.

During mitosis, the localization of MoKin5-RFP changed in wild-type strains. MoKin5-RFP accumulated at the ends of the spindle (MT-GFP) (Fig.5.2A). We observed dividing nuclei (histone H1-GFP) and found MoKin5-RFP accumulated at the polar ends

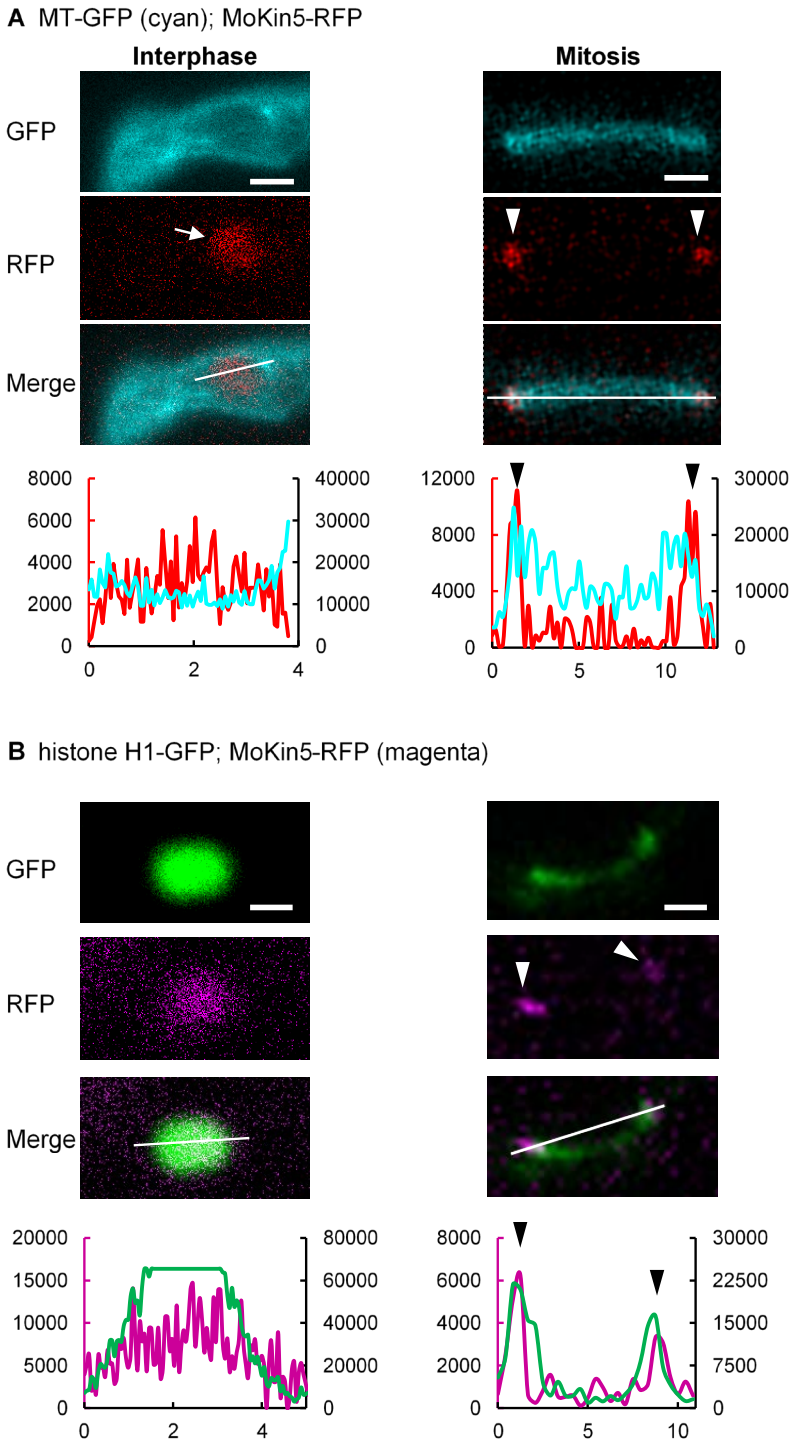

Fig.5.2. The localization patterns of MoKin5-RFP within invasive hyphae during interphase and mitosis in wild-type. All micrographs are single informative focal planes. Scale bars are 2 µm. Corresponding linescans quantify the fluorescence intensity in micrograph above. RFP intensity is displayed on primary vertical axis, GFP intensity is displayed on secondary vertical axis. Distance in µm is shown on horizontal axis.

Fig. 5.2. (continued) (A) The subcellular localization patterns of MoKin5-RFP relative to MT-GFP (cyan) in *M. oryzae* strain CKF4168. In interphase, MoKin5-RFP accumulates in the nucleus (left panel, arrow). During mitosis, MoKin5-RFP accumulates at the ends of the spindle (right panel, arrowheads). (B) The subcellular localization patterns of MoKin5-RFP relative to histone H1-GFP in *M. oryzae* strain CKF4208. In interphase, MoKin5-RFP co-localizes with histone H1-GFP (left panel). During mitosis, MoKin5-RFP accumulates at the ends of a dividing nucleus (right panel, arrowheads).

of the mitotic nucleus (Fig.5.2B). These data suggested MoKin5-RFP localized at the spindle pole bodies (SPBs) during mitosis (Fig.5.2). We corroborated this finding by comparing the subcellular localization of MoKin5-RFP to a known component of SPBs, γ-tubulin. We identified *M. oryzae γ-tubulin* (MGG_00961) based on protein homology to *A. nidulans γ-tubulin* (AN0676, MipA; Fig.5.S2). We generated a reporter strain expressing γ-tubulin-RFP and MT-GFP. Comparing the localization of MoKin5-RFP to the localization of γ-tubulin-RFP relative to the spindle during appressorium development revealed identical subcellular localization patterns at the ends of the spindle (Fig.5.S2). Taken together, these data showed that MoKin5-RFP accumulated at the SPBs during mitosis. MoKin5-RFP was subsequently utilized as a reporter for the SPBs during mitosis.

The dynamics of spindle pole bodies (SPBs) relative to the nuclei and spindle during extreme nuclear migration. We investigated the arrangement of the mother and daughter nuclei in relation to the SPBs during extreme nuclear migration through the penetration peg using live-cell confocal microscopy of a fluorescent fungal strain infecting rice. In this *M. oryzae* strain, the nucleus was labeled with histone H1-GFP and SPBs were labeled with MoKin5-RFP. We observed that the mother nucleus remained within the appressorium, while the migrating daughter nucleus was delivered to the apical region of the primary hypha (Fig.5.3A). The daughter nucleus became highly elongated as it migrated through the penetration peg (Fig.5.3A, 00:00, Lower Focal Plane). MoKin5-RFP, marking the SPB

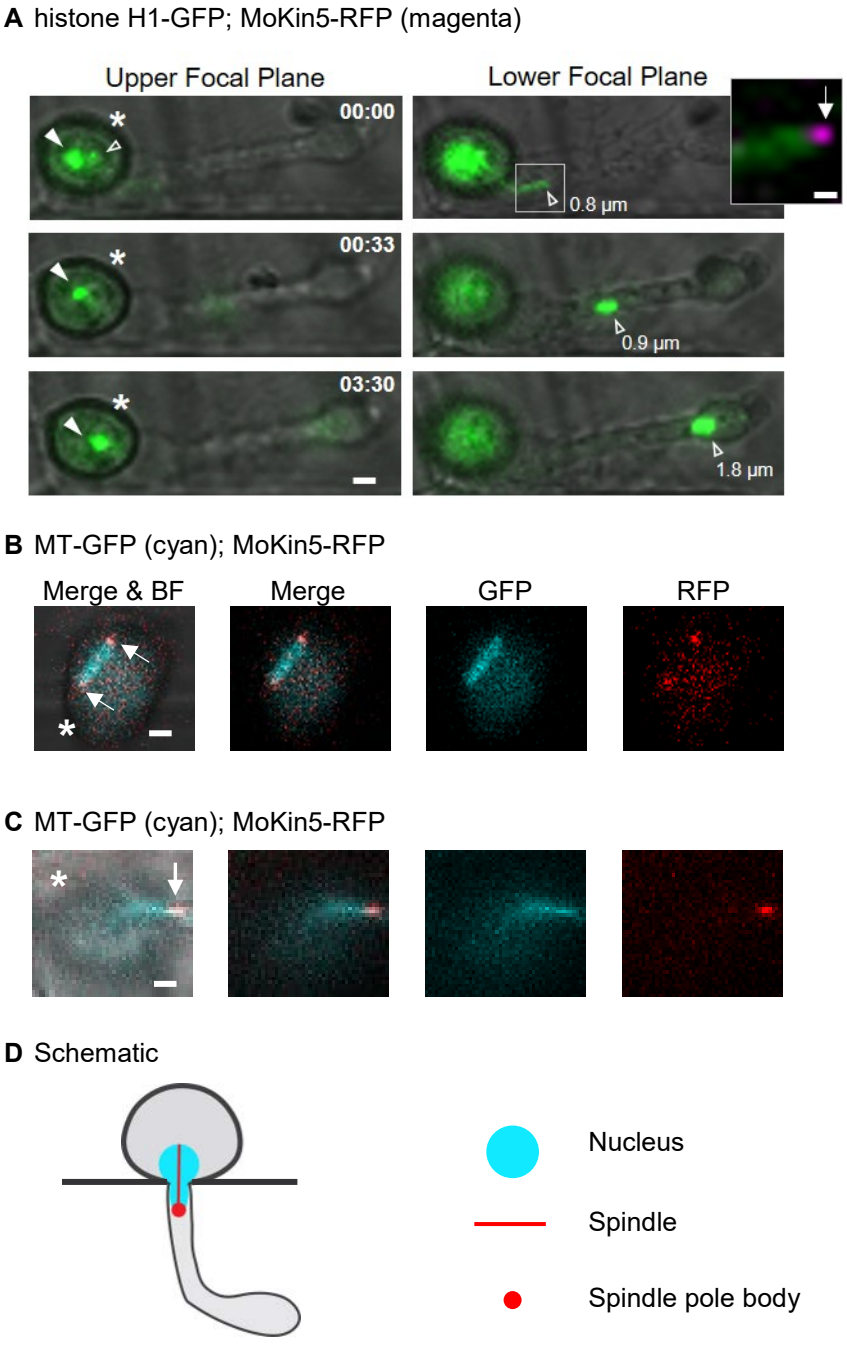

Fig.5.3. Nuclear, spindle, and spindle pole body dynamics during nuclear migration through the penetration peg in wild-type. All micrographs are single informative focal planes. All scale bars are 2 µm, except for the inset in panel 3A (far right; inset scale bar is 0.5 µm). Asterisks indicate the appressorium. Time is in minutes: seconds.

Fig.5.3. (continued) (A) The dynamics of the mother and daughter nucleus (histone H1-GFP) during extreme nuclear migration through the penetration peg in *M. oryzae* strain CKF4208. Two informative focal planes are shown. Micrographs show GFP and brightfield channels. Upper focal plane panels (left) show the localization of the mother nucleus (filled arrowhead) within the appressorium. The mother nucleus remains within the appressorium throughout the event. (00:00) The migrating daughter nucleus is localized within the penetration peg and is approximately 9.7 µm in length from top to bottom (open arrowhead). The lower focal planes show the dynamics of the daughter nucleus (open arrowhead). As the daughter nucleus moves towards the apical region of the primary hypha, it expands in the y-dimension diameter. The inset (far right) corresponds to the white box in the lower focal plane image. The inset is a merged micrograph showing both the GFP and RFP channels. MoKin5-RFP (magenta) localizes at the tip of the dividing nucleus (arrow) as it migrates through the penetration peg. (B) MoKin5-RFP marks the spindle pole bodies (arrows) at the ends of the spindle (MT-GFP (cyan)) within the appressorium of *M. oryzae* strain CKF4168. (C) The daughter bound spindle pole body (marked by MoKin5-RFP; arrow) localizes at the tip of the spindle (MT-GFP) during movement through the penetration peg in *M. oryzae* strain CKF4168. (D) Schematic representation of the position of the nucleus, spindle, and daughter bound spindle pole body during movement through the penetration peg in wild-type.

bound to the migrating daughter nucleus, was localized at the apical tip of the elongated daughter nucleus during movement through the penetration peg (Fig.5.3A, 00:00, Lower Focal Plane, Inset). The SPB bound to the mother nucleus was not detectable in our microscopy, possibly due to relatively strong autofluorescence in the melanized appressorium. The y-dimension diameter of the daughter nucleus expanded throughout the nuclear migration event. The diameter of the apical tip of the daughter nucleus was ~0.8 µm immediately following movement through the penetration peg (Fig.5.3A,00:00, Lower Focal Plane) but increased to ~1.8 µm as it neared the apical region of the primary hypha (Fig.5.3A; 03:30, Lower Focal Plane). We also followed the dynamics of the SPBs in relation to the spindle during extreme nuclear migration through the penetration peg in an additional fluorescent *M. oryzae* strain. The spindle (MT-GFP) and SPBs (MoKin5-RFP) first formed within the appressorium (Fig.5.3B). As expected, the daughter bound SPB preceded the spindle during movement through the penetration peg (Fig.5.3C). These data established the typical wild-type dynamics of the nucleus,

spindle, and SPBs during extreme nuclear migration through the penetration peg (Fig.5.3D; *See Fig.5.11*).

Development of an inducible promoter system to perturb spindle function during nuclear migration through the penetration peg. Our observations in wild-type pointed to the importance of the spindle in mediating extreme nuclear migration through the penetration peg. We hypothesized that genetically perturbing spindle function would impair nuclear migration at this infection stage. Yet we lacked an inducible promoter system to test this hypothesis. Testing our hypothesis would require an inducible promoter system that met two requirements. First, the promoter system needed to allow the fungus to develop so that it could successfully penetrate into the rice cell. This is because the primary hypha must be sufficiently developed to trigger mitosis within the appressorium (Shipman et al., 2017). Second, the promoter system needed to induce a drastic effect on gene expression upon fungal penetration into rice so that phenotypes were noticeable. To meet these requirements, we exploited the effector biology of *M. oryzae*. Effectors are small proteins secreted by pathogens to modulate their hosts during infection (Giraldo and Valent, 2013). We developed an inducible promoter system using the promoter of the *M. oryzae* effector gene, *Bas4*. Bas4 is an apoplastic effector whose promoter activity is highly induced upon initial penetration into plant tissue (Khang et al., 2010; Mosquera et al., 2009). We reasoned that we could generate an inducible overexpression construct by expressing a target gene with the *Bas4* promoter (*p*). The first inducible overexpression construct we generated contained the *Bas4p* fused to the *MoKin5* coding sequence and accompanying terminator region (*Bas4p-MoKin5*; Fig.5.4A).

We conducted RT-qPCRs to determine the expression of *MoKin5* relative to *actin* in two fungal cell types: vegetative mycelia and within IH growing inside the first-invaded rice cell. In wild-type, the *MoKin5* average expression level relative to *actin* was 1 in both

mycelia (± 0.4 margin of error) and IH (± 0.1 margin of error) (Fig.5.4B). In the fungal strain carrying the *Bas4p-MoKin5* construct, the *MoKin5* average expression relative to *actin* was 2.3 (± 0.6 margin of error) in mycelia. In the rice sheath samples infected by the same fungal strain, the relative expression of *MoKin5* to *actin* was 24.3 (± 2.7 margin of error). The relatively high expression of *MoKin5* within IH validated the use of the *Bas4* promoter to induce overexpression of a gene during early rice infection. *Magnaporthe oryzae* strains carrying a *Bas4p-MoKin5* construct were therefore referred to as MoKin5 overexpression (OE) strains.

MoKin5 OE causes defects in nuclear morphology and positioning. The development of the *Bas4p* inducible overexpression system allowed us to test our hypothesis that genetically perturbing spindle function would impair nuclear migration through the penetration peg. We reasoned that one consequence of impaired spindle function would be disruption in nuclear positioning within the appressorium and primary hypha relative to wild-type. We conducted live-cell confocal microscopy of fluorescent fungal strains expressing histone H1-RFP to label nuclei infecting a susceptible rice cultivar at two timepoints, ~28 hpi (early) and ~48 hpi (late). At the early timepoint, a majority of infection sites displayed a single nucleus within both the appressorium and a single nucleus within the primary hypha in wild-type (Fig.5.5A; Fig.5.S3). Nuclear positioning within the MoKin5 OE strain was highly disrupted. In the MoKin5 OE strain, only 2% (n=2) of infection sites displayed a single nucleus within the appressorium and a single nucleus within the primary hypha at the early time point (Fig.5.5A). In the MoKin5 OE strain, 25% (n=31) of infection sites displayed an anucleate appressorium with a single enlarged nucleus within the primary hypha at the early timepoint

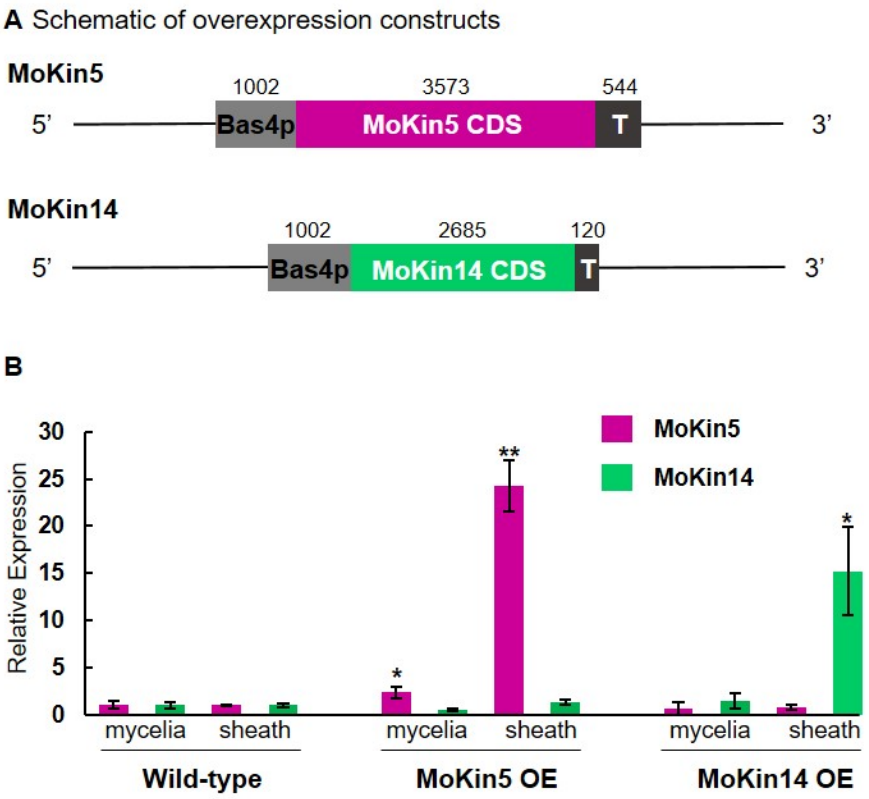

Fig.5.4. Average relative expression levels of *MoKin5* and *MoKin14* driven by the *Bas4* promoter (p). (A) Schematics of *MoKin5* and *MoKin14* overexpression (OE) constructs. Lengths are in base pairs. CDS is coding sequence and T is terminator region. (B) Relative expression of *MoKin5* (magenta) and *MoKin14* (green) in wild-type (CKF3578), MoKin5 OE (CKF4108), and MoKin14 OE (CKF4106) mycelia and YT16-infected rice sheaths. Data from two separate RT-qPCR experiments are shown. Samples were normalized relative to *actin* in each strain. Mycelia were harvested after 5 days growth in complete medium. Infected YT16 rice sheaths were harvested at 30-31 hours post inoculation. Significance was determined using a Student's t-test assuming unequal variance. P-value of *MoKin5* in MoKin5 OE mycelia is 0.04. P-value of *MoKin5* in MoKin5 OE sheaths is 0.003. P-value of *MoKin14* in MoKin14 OE sheath is 0.03. Error bars are 95% confidence intervals.

(Fig.5.5A, Fig.5.S3A). This phenotype was especially striking because all the infection sites scored contained intact appressoria. That is, any infection site that showed a collapsed appressorium in the bright-field channel was excluded from analysis. Additional defects in nuclear morphology and positioning were observed in the MoKin5 OE strains at the early and late timepoints (Fig.5.S3). Prominent defects included nuclear fragments within the appressorium (Fig.5.6B), nuclear fragments within the appressorium and primary hypha (Fig.5.6C), nuclear fragments exclusively within the primary hypha (Fig.5.7), and a single enlarged nucleus that appeared to be stuck within the penetration peg (Fig.5.S3C). We concluded that MoKin5 OE caused failure in extreme nuclear migration through the penetration peg.

MoKin5 OE causes defects in fungal development and virulence on rice. Considering the dramatic defects in nuclear morphology and positioning in the MoKin5 OE strain, we determined the effect of MoKin5 OE on IH development and blast lesion development on whole rice plants. At the late timepoint (~48 hpi), MoKin5 OE strains typically failed to develop beyond the primary hyphal stage of development (Fig.5.S4). We conducted whole-plant spray inoculations to determine if MoKin5 OE strains retained virulence of rice. In whole-plant spray inoculations, the mean percentage of diseased tissue area was 68% (±13% margin of error) in wild-type. The mean percentage of diseased tissue was 0% in the MoKin5 OE strain (representative infected leaves in Fig.5.5B). We concluded that MoKin5 OE strains failed to develop beyond the primary hyphal stage within the first-invaded rice cell, which caused a drastic reduction in virulence on rice.

MoKin5 OE leads to the formation of MT protrusions and nuclear fragments. The severe developmental defects caused by MoKin5 OE warranted a mechanistic explanation. We investigated the effect of MoKin5 OE upon the spindle during nuclear migration through the penetration peg using live-cell confocal imaging of

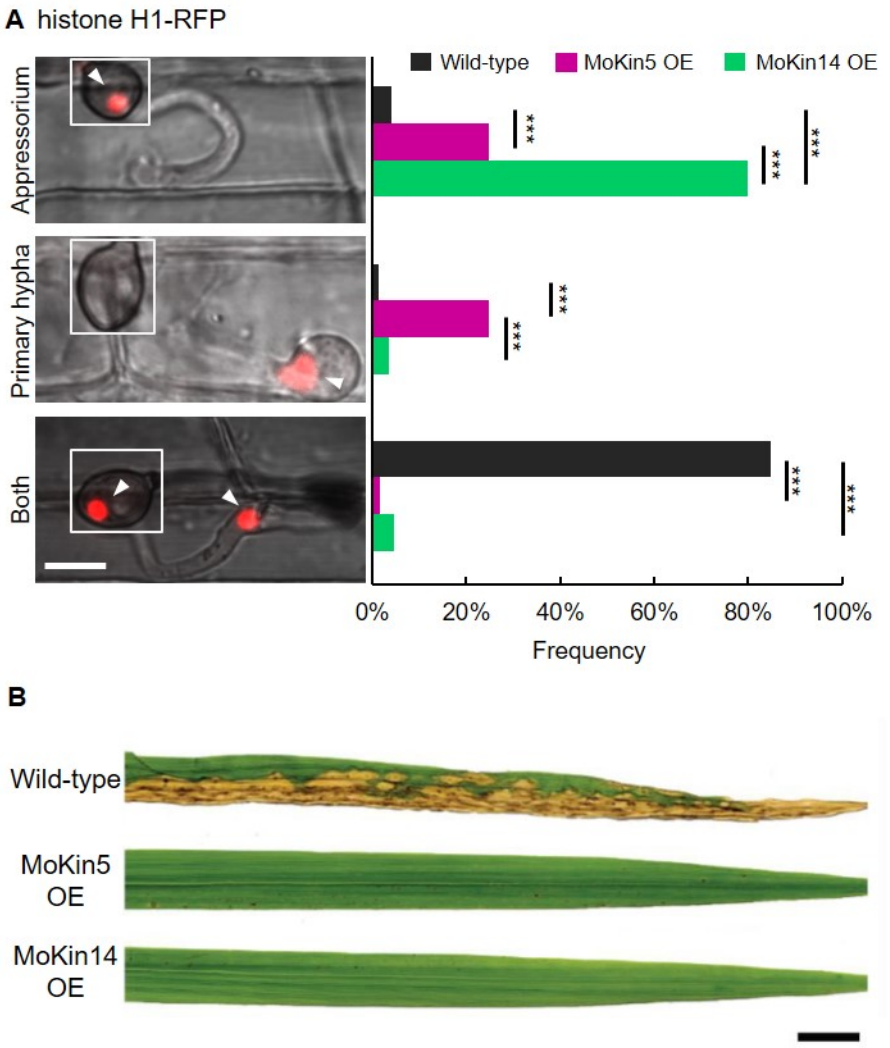

Fig.5.5. MoKin5 OE and MoKin14 OE cause defects in nuclear positioning and morphology, and decreases in virulence on rice. (A) Frequency of most commonly observed nuclear positioning and morphology phenotypes at ~28 hours post inoculation in wild-type (CKF3578 and CKF3971, n=153), MoKin5 OE (CKF4108, n=125), and MoKin14 OE (CKF4106 and CKF4093, n=85) strains. Example micrographs on the left are single focal planes. Only histone-H1 and brightfield channels are shown in example micrographs. The inset shows a single focal plane depicting the position of the mother nucleus within the appressorium. Scale bar is 5 μm. Top panel shows a single nucleus within the appressorium (arrowhead). The middle panel shows a single nucleus within the primary hypha (arrowhead). The bottom panel shows a single nucleus within both the appressorium (arrowhead) and a single nucleus within the primary hypha (arrowhead). Statistical significance of nuclear phenotype frequency was determined using two-tailed Fisher's exact tests. *** represent p-values less than 0.0001. Non-significant p-values are 0.22 for MoKin5 OE relative to MoKin14 OE in the wild-type category and 0.35 for wild-type relative to MoKin14 OE in the primary hypha category. The frequency of all observed nuclear phenotypes at the early timepoint is available in Fig.5.S3A. (B) Images of representative infected leaves from whole-plant spray inoculations. Wild-type is CKF3578, MoKin5 OE is CKF4108, and MoKin14 OE is CKF4106. Scale bar is 1 cm.

a MoKin5 OE strain infecting rice sheaths. We initiated our investigation by examining the dynamics of the spindle (MT-GFP) relative to the nucleus (histone H1-RFP) in the appressoria of a MoKin5 OE strain at the start of mitosis. In these MoKin5 OE infection sites, a bar of MT-GFP extended in an abnormal and persistent manner beyond the circumference of the nucleus, as recognized by histone H1-RFP fluorescence, in appressoria and primary hyphae (Fig.5.6A). These persistent MT-GFP structures, which we refer to as MT protrusions, were not observed in wild-type. We quantified the frequency of single, double, and triple+ MT protrusions relative to the nucleus within appressoria (Fig.5.6A, n=22). We found that 45% (n=10) of infection sites contained a single MT protrusion (Fig.5.6A, top panel), 36% (n=8) of infection sites contained double MT protrusions (Fig.5.6A, middle panel), and 18% (n=4) of infection sites contained three or more MT protrusions (Fig.5.6A, bottom panel). We concluded that MoKin5 OE did impair spindle function by preventing formation of a typical spindle within the appressorium.

Additional MT protrusion and nuclear positioning phenotypes were observed in the MoKin5 OE strain within appressoria and primary hyphae. In appressoria, small nuclear fragments were distributed along the MT protrusions (Fig.5.6B-C). We followed the relative position of the spindle/MT protrusions, and the nucleus/nuclear fragments during extreme nuclear migration through the penetration peg in the MoKin5 OE strain. In the MoKin5 OE strain, the MT protrusion preceded the nuclear fragment (Fig.5.6C-6D). This arrangement was in stark contrast to the arrangement of the spindle and nucleus in wild-type (Fig.5.3C-3D). Nuclear fragments tended to occur more frequently within the primary hyphae of the MoKin5 OE strain. At the early timepoint, only 7% (n=9) of MoKin5 OE infection sites showed nuclear fragments in the appressorium, whereas 12% (n=15) of MoKin5 OE infection sites showed nuclear fragments in the primary hypha exclusively (Group 2 vs Group 4 in Fig.5.S3A). We conducted time-lapse confocal

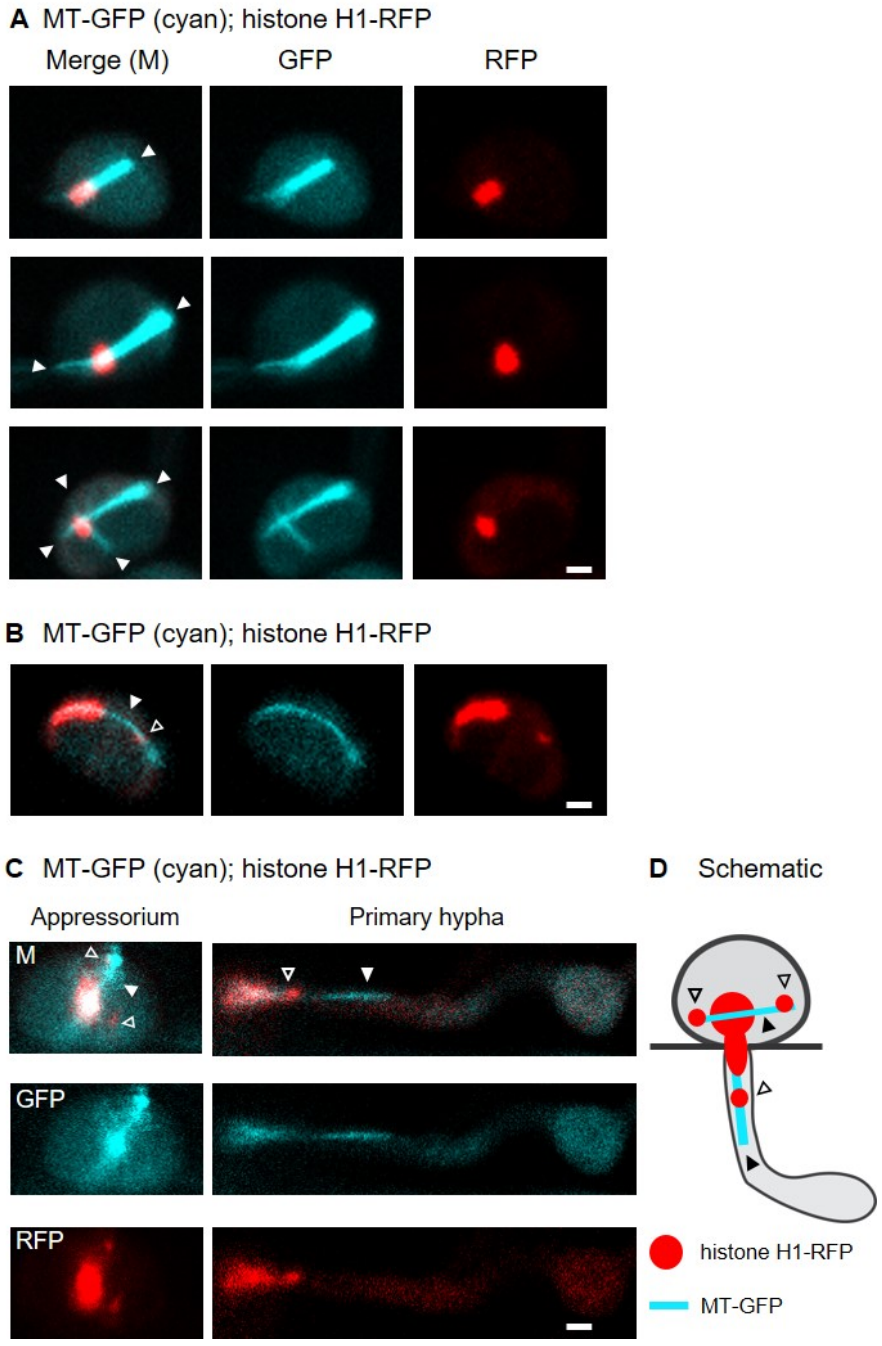

Fig.5.6. Formation of microtubule (MT) protrusions and nuclear fragments within the MoKin5 OE strain CKF4108. All micrographs are informative single focal planes, except Fig.5.6C (appressorium, left panels are maximum intensity projections). All scale bars are 2 µm. (A) Single (top), double (middle), and triple + (bottom) MT protrusions (filled arrowhead) within appressoria. (B) A nuclear fragment (open arrowhead) on a MT protrusion (filled arrowhead) within an appressorium.

Fig.5.6. (continued) (C) The arrangement of the nucleus, nuclear fragments, and MT protrusions during extreme nuclear migration through the penetration peg. Within the appressorium (left panels) two nuclear fragments (open arrowheads) form along MT protrusions. A single MT protrusion is in focus (filled arrowhead), while another MT protrusion is out of focus (not marked). The micrograph on the right shows a lower single informative focal plane. Here, an MT protrusion (filled arrowhead) leads the nuclear fragment (open arrowhead) just emerging from the penetration peg. (D) A two-dimensional schematic representation of the dynamics presented in Fig.5.6C.

microscopy to further investigate the nature of the MT protrusions and nuclear fragments within the primary hyphae. Within primary hyphae, nuclear fragments separated and merged over time along the MT protrusion (Fig.5.7). From these data, we concluded that MoKin5 OE caused formation of MT protrusions. These MT protrusions contributed to the formation of nuclear fragments beginning within the appressorium, and that the nuclear fragments merged together to form a single enlarged nucleus within the primary hypha.

MoKin5 OE causes defects in spindle polarity. We pursued a mechanistic understanding of how the MT protrusions observed in the MoKin5 OE strain contributed to formation of nuclear fragments within the appressorium. We conducted confocal microscopy of appressoria in an *M. oryzae* strain co-expressing three constructs: *MoKin5*-RFP driven from its native promoter, *Bas4p-MoKin5*, and *β-tubulin*-GFP to label the spindle. During mitosis in the appressoria of the MoKin5 OE strain, MoKin5-RFP localized along the spindle (Fig 8A; Fig S5). The MoKin5-RFP localization pattern in the MoKin5 OE strain differed dramatically from wild-type during mitosis (Fig.5.2; Fig.5.3B). In wild-type, MoKin5-RFP accumulated only at the SPBs. We concluded that during mitosis MoKin5 OE caused MoKin5-RFP to inappropriately localize along MTs within the spindle.

We continued to investigate the nature of the spindle and MoKin5-RFP in the MoKin5 OE strain by conducting time-lapse confocal microscopy of mitotic appressoria

MT-GFP (cyan); histone H1-RFP

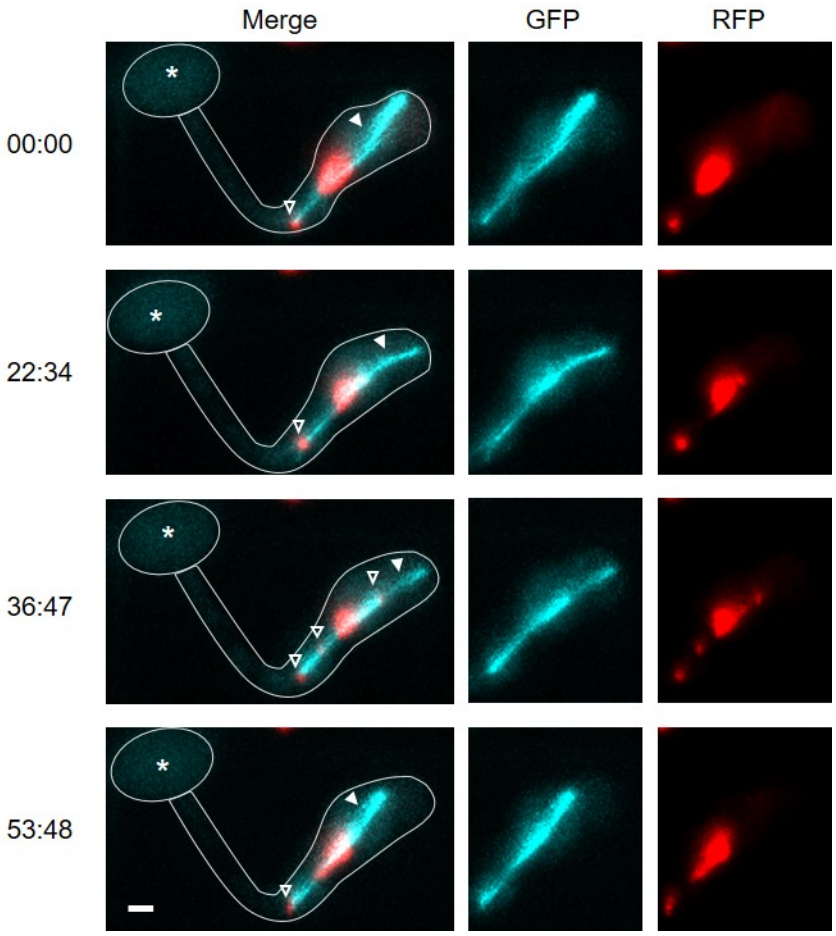

Fig.5.7. The behavior of small nuclear fragments (histone H1-RFP, open arrowheads) along the spindle and MT protrusion (MT-EGFP (cyan); filled arrowhead) in a primary hypha of the MoKin5 OE strain CKF4108. Each micrograph is a maximum intensity projection of informative single focal planes. The scale bar is 2 μm. Asterisks indicate an anucleate appressorium. An overlay outlining the appressorium and primary hypha is present in the merged channel micrographs. Time is in minutes: seconds.

(Fig.5.8C). In these appressoria we made several important observations. First, we observed that the MT-GFP and MoKin5-RFP signal displayed a relatively bright focus at one end of the spindle (Fig.5.8, filled arrowheads in the merge channel). Second, the spindle elongated from only a single end, which we call the growing plus-end (Fig.5.8C, Fig.5.8E, plus symbol). This spindle elongation followed the curvature of the appressorium. Third, at the very early stage of spindle elongation, MoKin5-RFP showed a brief accumulation at the growing plus-end (Fig.5.8C, 00:00, arrow; Fig.5.8D, arrow). Finally, we observed that the MoKin5 OE spindle continued to elongate and rotate within the appressorium for at least 32 minutes (Fig.5.8C). These data suggested that the MoKin5 OE spindle displays aberrant polarity likely due to a combination of excessive outward forces acting on the spindle and excessive polymerization of MTs within the spindle. Recently, monomeric human kinesin-5 was found to act as a promoter of MT polymerization at the plus-ends of MTs (Chen et al., 2019).

MoKin14 OE causes defects in fungal development and virulence on rice. Due to the prominent defects in nuclear migration caused by MoKin5 OE, we investigated the effect of MoKin14 OE on extreme nuclear migration through the penetration peg. We identified a kinesin-14 motor protein in *M. oryzae* (*MoKin14*, MGG_05350) through protein homology to other known kinesin-14 proteins (Fig.5.S6). MoKin14 shared 55.9% global similarity to KlpA (AN6340), which is kinesin-14 in *A. nidulans.* MoKin14 also contained a predicted kinesin motor domain at the C-terminus. This C-terminal kinesin motor domain is a characteristic of the kinesin-14 superfamily, and indicates that MoKin14 likely walks towards the minus-end of MTs (She and Yang, 2017). Like the MoKin5 OE strain, we generated several *M. oryzae* strains of MoKin14 OE (*MoKin14* under control of the *Bas4* promoter; *Bas4*p-*MoKin14*) for subsequent analysis of nuclear positioning, IH development, and virulence on rice.

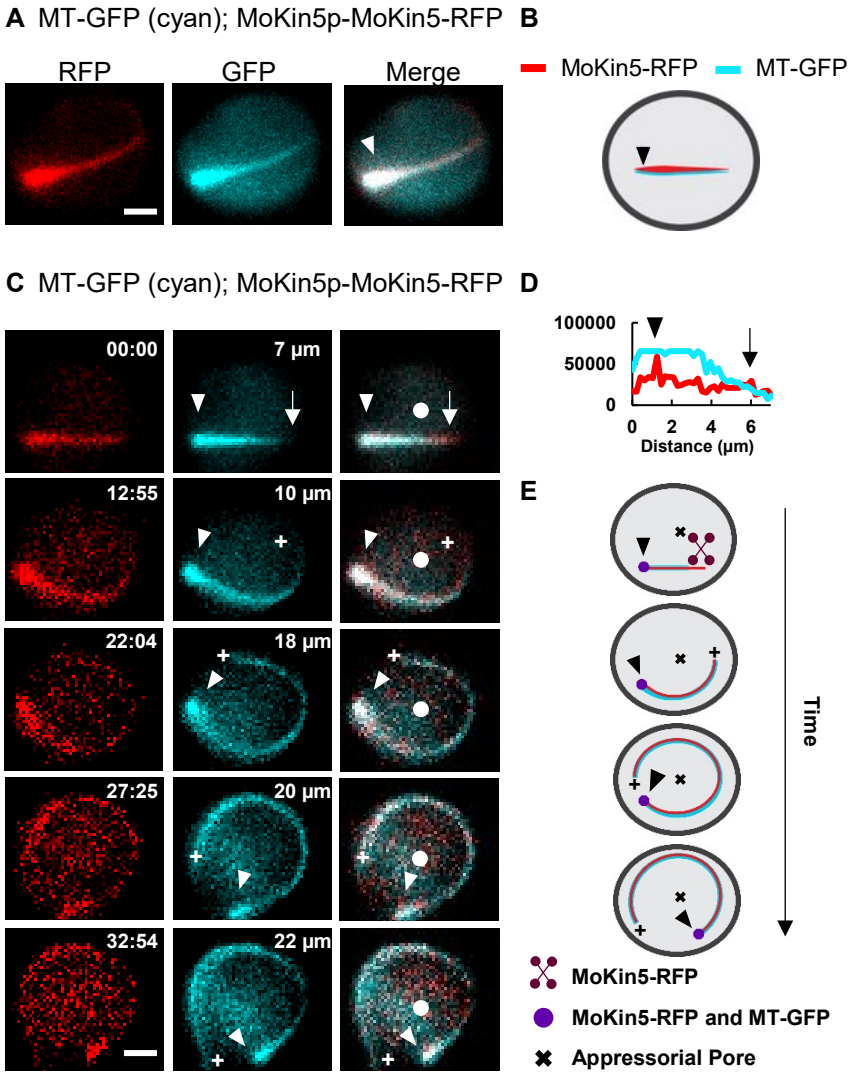

Fig.5.8. The localization of MoKin5-RFP relative to MT-GFP (cyan) in spindles located within the appressoria of MoKin5 OE strain CKF4203. Maximum intensity projections of informative single focal planes are shown. Scale bars are 2 µm. (A) A bright focus (filled arrowhead) of MT-GFP and MoKin5-RFP is found at one end of the MoKin5 OE spindle. (B) A schematic representation of the patterns shown in Fig.5.8A. (C) Time-lapse micrograph series showing spindle elongation in the MoKin5 OE strain (top-down view). Time is in minutes: seconds. (00:00) The bright focus of MT-GFP (cyan) and MoKin5-RFP (filled arrowhead) is opposite a small and transient accumulation of MoKin5-RFP at the other end of the spindle (arrow). The initial length of the MoKin5 OE spindle is 7 µm. Over time, the spindle grows from a single end (plus symbol). The MoKin5 OE spindle elongates in a manner that follows the curvature of the appressorium, and the spindle rotates (compare 12:55 to 32:54). The approximate location of the appressorial pore, which is the location of the penetration peg, is shown (filled white circle).

Fig.5.8. (continued) (D) Linescan quantifying fluorescence intensity of MT-GFP and MoKin5-RFP corresponding to timepoint 00:00 in Fig.5.8C. MT-GFP and MoKin5-RFP signal shows an accumulation at one end of the spindle (black arrowhead). MoKin5-RFP shows a smaller accumulation at the opposite end of the spindle (black arrow). (E) Schematic representation of the MoKin5 OE spindle elongating within the appressorium based on data presented in Fig.5.8C (top-down view). The focus of MoKin5-RFP and MT-GFP at the end of the spindle is shown by a purple circle, and corresponds to the white arrowheads in Fig.5.8C. MoKin5-RFP shows a brief accumulation at the opposite end of the spindle at the start of spindle elongation (top image). The spindle grows from the end marked by the plus symbol. The approximate location of the appressorial pore is shown with a black "X."

Our first step in the analysis of the MoKin14 OE strains was to confirm that expressing *MoKin14* from the *Bas4* promoter increased the relative expression of *MoKin14* within IH (Fig.5.4A). We conducted RT-qPCRs. In both mycelia and IH of wild-type, the average expression of *MoKin14* relative to *actin* was 1 (± 0.3 in margin of error in mycelia and ± 0.2 margin of error in IH). In the *M. oryzae* strain carrying the *Bas4-MoKin14* construct, the average expression of *MoKin14* relative to *actin* was 1.5 (± 0.6 margin of error) in mycelia and 15.2 (± 4.7 margin of error) in IH (Fig.5.4B). Because we validated that a strain carrying the *Bas4p-MoKin14* construct did, indeed, cause an overexpression of MoKin14 in the early stages of rice infection, we then determined the effect of MoKin14 overexpression (OE) on nuclear positioning in appressoria and primary hyphae. At the early timepoint (~28 hpi), 80% (n=68) of the MoKin14 OE sites displayed a single nucleus (histone H1-RFP) within the appressorium, which differed from both the wild-type and MoKin5 OE phenotypes (Fig.5.5A; Fig.5.S3). The MoKin14 OE strains also showed a drastic arrest in IH development at ~48 hpi. At this timepoint, MoKin14 OE strains were typically arrested at the primary hyphal stage of development (Fig.5.S4). We conducted whole-plant spray inoculations and found that the MoKin14 OE strain did not display virulence on rice. The mean percentage of diseased tissue area was 0% in the MoKin14 OE strain compared to 68% (±13% margin of error) in wild-type (representative leaves in Fig.5.5B). From these results, we concluded that MoKin14

OE caused a failure in extreme nuclear migration through the penetration peg. The failure in extreme nuclear migration led to defects in IH development, which prevented virulence on rice.

MoKin14 OE causes formation of monopolar spindles. MoKin14 OE caused failure in extreme nuclear migration through the penetration peg, and resulted in a nuclear positioning phenotype that was unique relative to wild-type and MoKin5 OE strains. This observation suggested that MoKin14 OE induced a distinct effect upon the spindle. We hypothesized that if MoKin14 generated an inward force on SPBs within *M. oryzae,* overexpressing MoKin14 with the *Bas4* promoter would cause formation of monopolar spindles within the appressorium. We conducted live-cell confocal microscopy of *M. oryzae* strains expressing MT-GFP and histone H1-RFP to determine the dynamics of the spindle in relation to the nucleus within the appressorium. Within appressoria, the spindle phenotype of the MoKin14 OE strain differed from both wild-type and the MoKin5 OE strain (Fig.5.9). In wild-type, a spindle bisected the nucleus (Fig.5.9A, top panel). We also detected the asynchronous movement of chromatids towards the ends of the spindle in wild-type (Fig.5.9A, bottom panel). In contrast, MoKin14 OE resulted in a single focus of MT-GFP overlapping with the nucleus within the appressorium (Fig.5.9B, top panel). MTs emanated from this single focus and, at times, the mother nucleus appeared to be arrested in mitosis. For example, within the MoKin14 OE strain, a “butterfly” shaped nucleus was observed within the appressorium, in which chromatids appear to be arrested in the process of dividing (Fig.5.9B, bottom panel). Consistent with our previous observations, the MoKin5 OE spindle did not form a typical spindle, but instead appeared as a half spindle relative to the nucleus within the appressorium (Fig.5.9C). We concluded that MoKin14 OE caused formation of monopolar spindles within the appressorium.

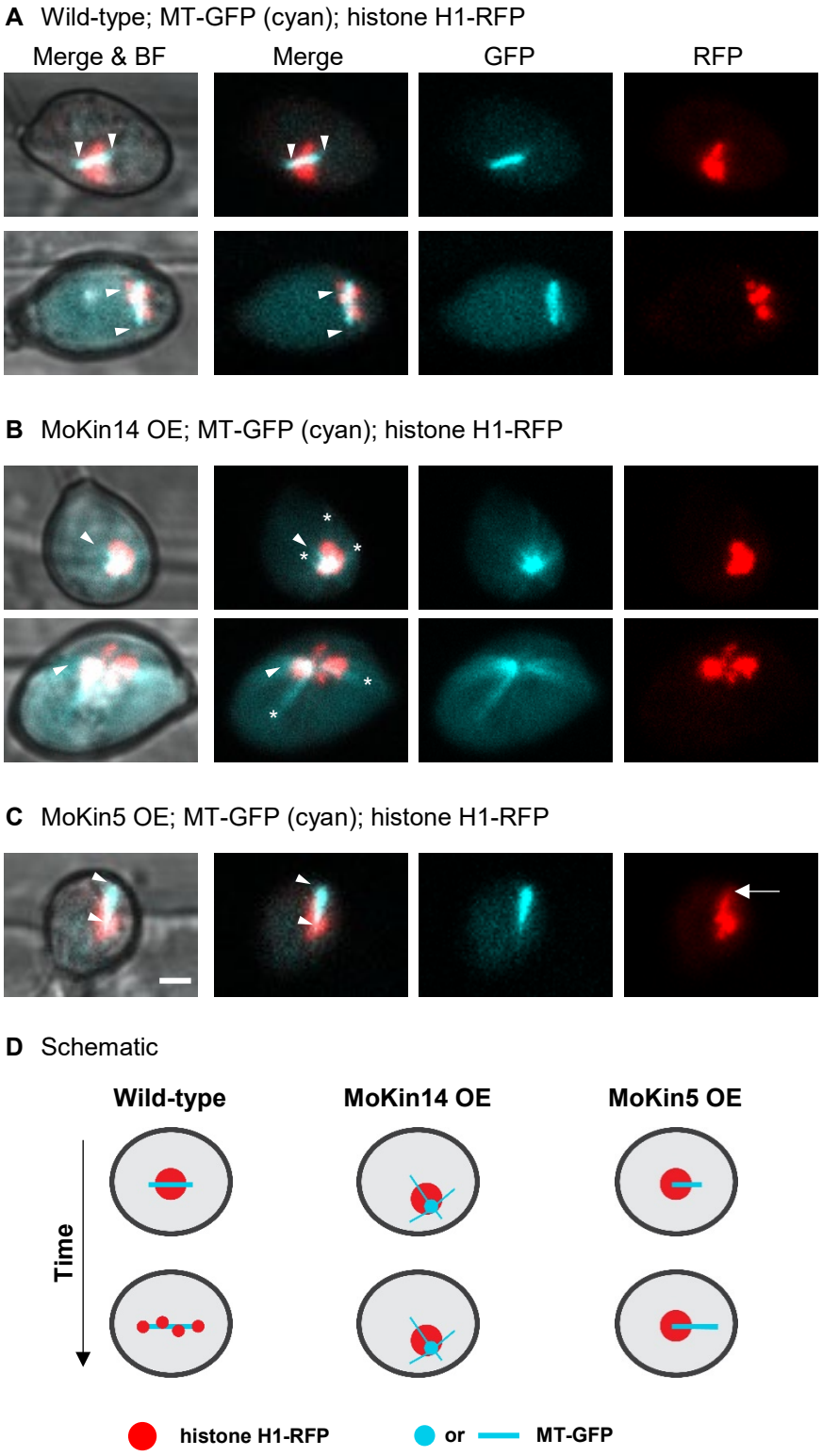

Fig.5.9. The arrangement of the spindle (MT-GFP (cyan)) relative to the mother nucleus (histone H1-RFP) within appressoria of wild-type, MoKin14 OE, and MoKin5 OE strains. All micrographs are single informative focal planes. Scale bar is 2 μm.

Fig.5.9. (continued) (A) In wild-type, CKF3578, the spindle bisects the nucleus within the appressorium. (Top panel) Filled arrowheads indicate the ends of the spindle. (Bottom panel) Chromatids move asynchronously towards the ends of the spindle. (B) In the MoKin14 OE strain, CKF4106, a monopolar spindle forms within the appressorium. In both panels a relatively bright focus of MT-GFP likely represent unseparated spindle pole bodies (filled arrowhead). MTs emanate from this bright MT-GFP focus (asterisks indicate prominent MTs emanating from the unseparated SPBs). (Bottom panel) The nucleus adopts a butterfly shape, suggesting a mitotic arrest. (C) In the MoKin5 OE strain, CKF4108, a typical bipolar does not form. The spindle does not span the entire diameter of the nucleus (spindle ends marked by filled arrowheads), and the nuclear fragmentation process appears to be beginning at one end of the nucleus where the spindle is located (arrow). (D) A schematic representation of the spindle and nuclear dynamics within the appressorium corresponding to data presented in Figs.5.9A-C. When MoKin14 is overexpressed, the monopolar spindle persists over time.

Monopolar spindles can form in two conditions. The first condition is when duplicated SPBs fail to initially separate at mitotic onset. The second condition in when duplicated SPBs fail to maintain their placement at opposite ends of the spindle throughout mitosis. In order to determine the effect of MoKin14 OE on SPBs directly, we analyzed an additional MoKin14 OE strain. This strain contained three constructs: *β-tubulin*-GFP to label the spindle; *Bas4p-MoKin14*; and *MoKin5*-RFP driven off the native *MoKin5* promoter. This particular strain was unique compared to other MoKin14 OE strains because it developed IH within the first-invaded rice cell. In both wild-type and this MoKin14 OE strain, MoKin5-RFP accumulated at the SPBs during mitosis (Fig.5.10A, 10C, arrowheads). We followed the dynamics of MT-GFP and MoKin5-RFP, over time within IH of the MoKin14 OE strain, and observed a captivating pattern. The spindle experienced cycles of elongation and contraction relative to the wild-type (Fig.5.10A-10D). These spindle collapse events tended to occur more frequently when the spindle was less than ~5 μm (Fig.5.10E). Yet the SPBs rapidly separated at spindle lengths exceeding ~5 μm (Fig.5.10E). We concluded that MoKin14 OE induces monopolar spindle formation due to excessive inward forces acting upon duplicated SPBs, primarily in early mitosis when the spindle is at a shorter length. The excessive

inward force generated by MoKin14 OE prevented formation and maintenance of a typical bipolar spindle.

Discussion

In this study, we demonstrated that the spindle was involved in nuclear migration through the penetration peg by genetically perturbing spindle function using an inducible overexpression promoter. We characterized the effects of kinesin-5 and kinesin-14 overexpression upon nuclear positioning, fungal development, and spindle function. Our results shed light on mechanisms permitting successful nuclear migration through the penetration peg, and the roles of kinesin-5 and kinesin-14 in the rice blast fungus, *M. oryzae.* In the following section, we discuss the mechanisms that permit nuclear migration through the penetration peg.

Mechanisms permitting nuclear migration through the penetration peg. Our results revealed that nuclear migration through the penetration peg is initiated at the onset of mitosis within the appressorium. We observed chromatids moving towards the polar ends of the spindle in an asynchronous manner, consistent with previous studies (Row et al., 1985; Shah et al., 2019; Yadav et al., 2019). In our study, the spindle rotated to become aligned for movement through the appressorial pore and penetration peg. We did not observe astral MTs emanating from the spindle within the appressorium or penetration peg, although we cannot rule out that astral MTs

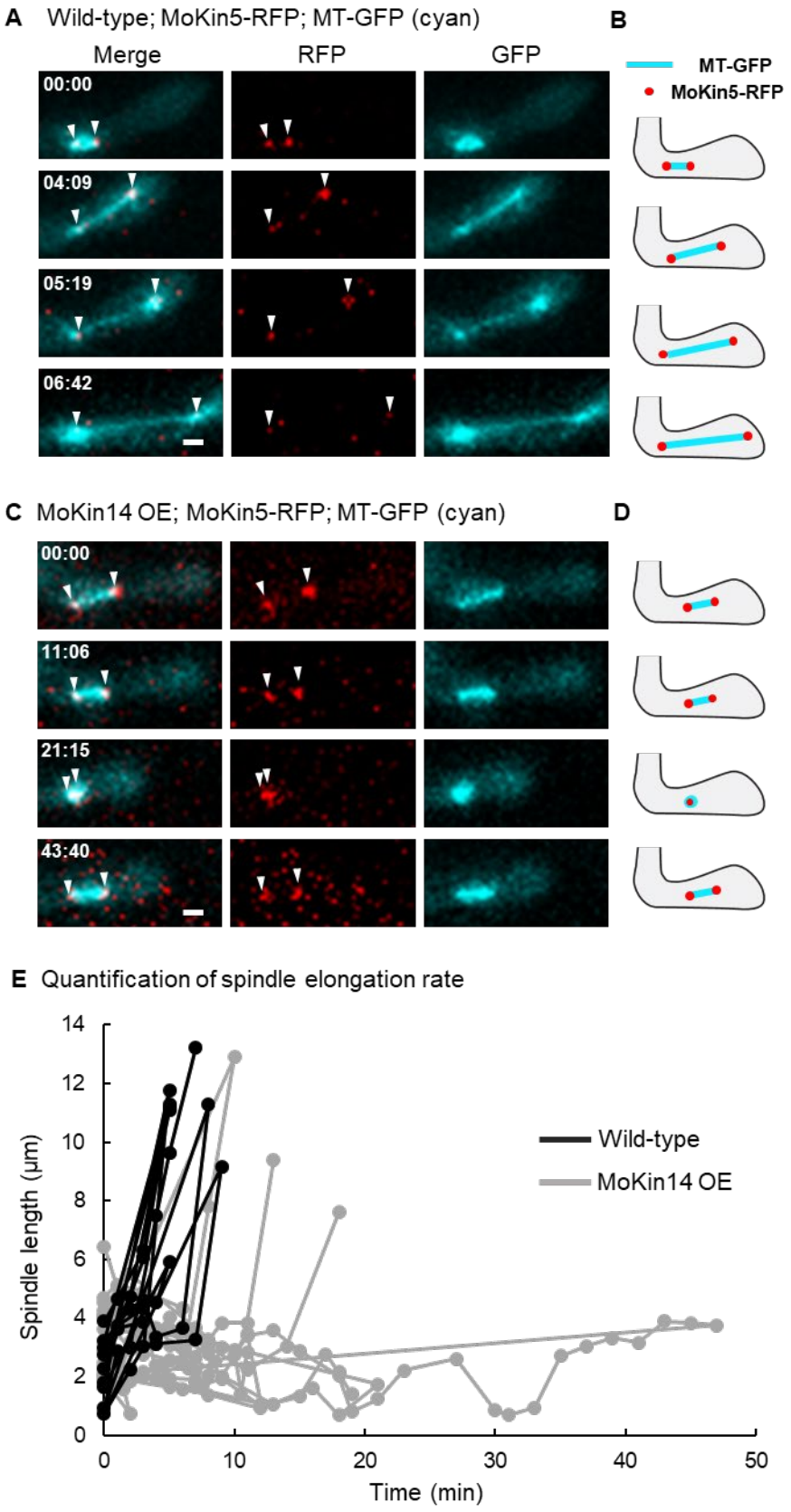

Fig.5.10. Legend continues on following page.

Fig.5.10. (continued) MoKin14 OE causes the spindle (MT-GFP (cyan)) to collapse and form monopolar spindles. MoKin5-RFP accumulates at the spindle pole bodies (arrowheads). All micrographs are maximum intensity projections of informative single focal planes. Scale bars are 2 μm. Time is in minutes: seconds. (A) Representative time-lapse of spindle and spindle pole body dynamics in a leading invasive hypha of wild-type *M. oryzae* strain, CKF4168. (B) Schematic representation of the spindle and spindle pole bodies dynamics as shown in Fig.5.10A. (C) Time-lapse of spindle and spindle pole body dynamics in a leading invasive hypha of the MoKin14 OE strain, CKF4182. The spindle experiences several rounds of spindle collapse due to excessive MoKin14. (D) Schematic representation of the spindle and spindle pole bodies dynamics as shown in Fig.5.10C. (E) Quantification of spindle length over time in invasive hyphae of the wild-type strain (CKF4168, n = 9) and the MoKin14 OE strain (CKF4182, n=24). Spindle length was determined by measuring the distance between MoKin5-tdTomato foci at the SPBs. Total time is calculated from the time the first image was acquired. The cell cycle was not synchronized; thus, the spindle is not at the same spindle length at the 00:00 timepoint for each time-lapse series.

were present but not detectable. We found live-cell imaging within the appressorium to present unique challenges in terms of visualizing fluorescently-tagged proteins that are clearly visible in other cell types of *M. oryzae.* The challenge in visualizing these fusion proteins is likely due to the highly-melanized nature of the appressorium (Howard and Ferrari, 1989). Nonetheless, our data clearly demonstrated that during nuclear migration through the penetration peg, the SPB bound to the migrating daughter nucleus precedes the nucleus and the spindle through the penetration peg. The dynamics of the nucleus, spindle, and SPBs are summarized in Fig.5.11. In migrating myoblasts from mice, the positioning of centrosomes, a type of microtubule organizing centers like SPBs, is critical for effective nuclear movement (Chang et al., 2015). Interestingly, one consequence of MoKin5 OE was disruption to the arrangement of the DNA (nuclear fragments) in relation to the spindle, likely altering the position of the daughter bound SPB. The time required for the spindle to navigate towards the penetration peg was drastically increased in the MoKin5 OE strain relative to wild-type.

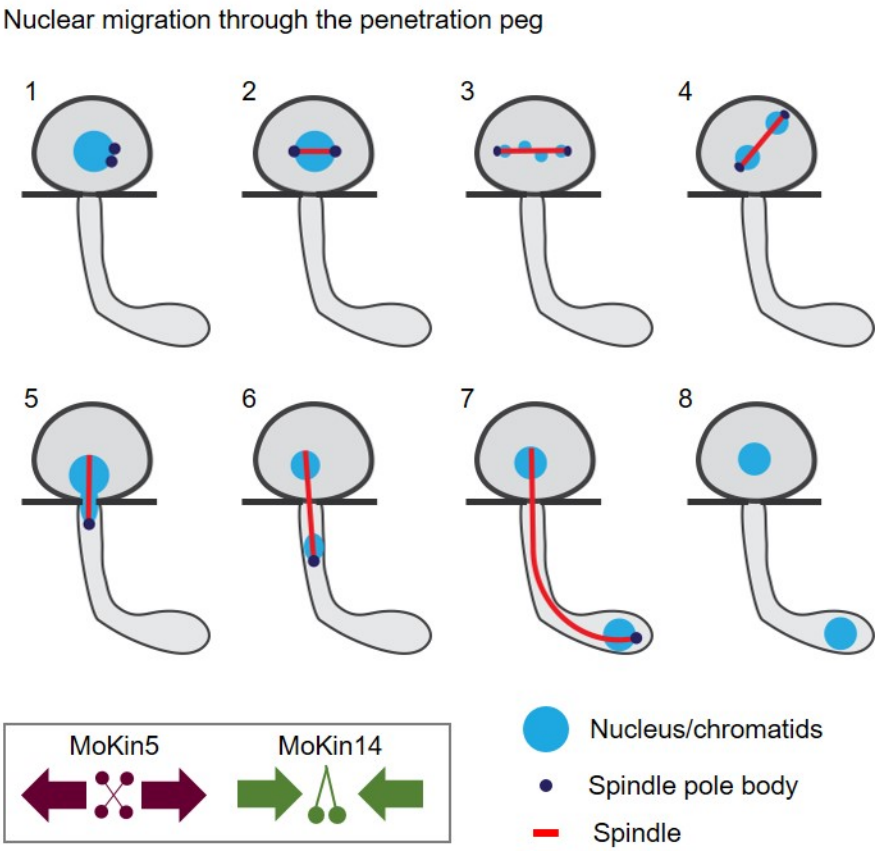

Fig.5.11. Proposed model of nuclear migration through the penetration peg. The early phases of mitosis occur within the appressorium (1-3). (1) In prophase, duplicated spindle pole bodies begin separation. (2) A bipolar spindle bisects the mother nucleus located in the appressorium in prometaphase. (3) Chromatids move asynchronously towards the spindle pole bodies in metaphase/anaphase A. (4) The spindle and divided chromatids located at the spindle pole bodies rotate to become aligned to the axis of the penetration peg in anaphase A/B. (5) The daughter nucleus begins transiting the penetration peg with the daughter bound spindle pole body leading in anaphase B. The daughter nucleus becomes highly elongated during this event. (6-7) The spindle continues to elongate, propelling the daughter nucleus towards the apical region of the primary hypha in anaphase B. (8) The daughter nucleus is positioned at the tip of the primary hypha and the spindle has collapsed, indicating exit from mitosis. The inset shows MoKin5 generating an outward force on the spindle. MoKin5 also acts as a promoter of MT nucleation. MoKin14 generates an inward force on the spindle primarily during early mitosis.

From these data, we propose that the daughter bound SPB plays an important role in guiding the spindle to the appressorial pore for subsequent movement through the penetration peg. The daughter bound SPB may display an enrichment of polarity determinants that help guide the spindle to the penetration peg. Similarly, the daughter bound SPB could be enriched in motor proteins, such as dynein or MoKin5, that may generate forces needed to propel the nucleus through the penetration peg. In yeast, dynein is asymmetrically distributed to one SPB, and this asymmetry is required for dynein-dependent spindle positioning at the bud neck (Grava et al., 2006). In *M. oryzae*, MoTea1 is associated with the septin and F-actin ring present near the appressorial pore where the penetration peg emerges (Dagdas et al., 2012). The spindle may be connecting to other cytoskeletons present at the appressorial pore via a Tea1-like mechanism as occurs in *S. pombe* (Mata and Nurse, 1997). In *Ustilago maydis*, nuclear division defects were found in Tea1 knockout mutants in the yeast-like cells (Woraratanadharm et al., 2018). More research is needed to elucidate the mechanisms that guide the daughter bound SPB efficiently to the appressorial pore in *M oryzae*.

The stretched daughter nucleus observed during movement through the penetration peg in this and a previous study is highly intriguing (Jenkinson et al., 2017). We interpret this nuclear morphology to represent the movement of individual chromatids or clusters of chromatids through the narrow penetration peg. Recent studies show that heterochromatin levels influence nuclear migration through constricted spaces (Gerlitz, 2020). One advantage of undergoing a mitotic nuclear migration through the penetration peg could be that DNA is already highly compacted into chromatids. This would allow efficient and protected movement of the nucleus through a constricted space. In *M. oryzae*, the daughter nucleus expanded in diameter immediately following movement through the penetration peg. This suggests that regions of heterochromatin within the migrating daughter nucleus relax following transit through the penetration peg.

In the future, experiments altering DNA condensation within the migrating daughter nucleus may offer insight into the role DNA condensation plays in extreme nuclear migration events in *M. oryzae*.

In migratory cancer and immune cells, nuclei moving through constricted 3D spaces during interphase rely upon DNA and nuclear envelope repair mechanisms for survival (Denais et al., 2016; Raab et al., 2016). In *M. oryzae*, which uses an intermediate form of mitosis, the outer nuclear envelope and core nucleoporins remain intact during appressorium development (Pfeifer and Khang, 2021; Saunders et al., 2010b), yet the behavior of the inner nuclear membrane remains undetermined. It could be that the inner nuclear membrane remains intact during nuclear migration through the penetration peg as a means to protect the nucleus. Moreover, our results suggest that nuclear migration through the penetration peg occurs during the later stages of mitosis. In other eukaryotes, ESCRT (endosomal sorting complexes required for transport) machinery is known to remodel the nuclear envelope at the later stages of mitosis (Gu et al., 2017; Vietri et al., 2015). Snf7, a component of the ESCRT-III complex, was implicated in *M. oryzae* pathogenicity on rice (Cheng et al., 2018). Yet the localization of Snf7 during nuclear migration is not yet characterized. Undergoing mitosis during extreme nuclear migration through the penetration peg may allow transient nuclear envelope ruptures to be rapidly repaired by the ESCRT machinery already mobilized for mitotic function in *M. oryzae*.

Although nuclear migration through the penetration and IH peg share similar features, some evidence suggests nuclear migration through the penetration peg may be a mechanistically distinct event during *M. oryzae* development. In both nuclear migration through the penetration and IH peg, the migrating nucleus tends to cover a longer distance than during nuclear migration within bulbous IH (Jenkinson et al., 2017; Jones et al., 2016; Pfeifer et al., 2019; Shipman et al., 2017). In both events the migrating

nucleus becomes highly-elongated as it transits the narrow peg. However, nuclear migration through the penetration peg may be unique based on a knockout study of a gene involved in nuclear positioning in *M. oryzae*. MoAnd1, a homolog of Num1 in *Saccharomyces cerevisiae* and ApsA in *A. nidulans*, likely plays a role in anchoring cytoplasmic dynein at the cell cortex permitting proper spindle positioning (Jeon et al., 2014). The MoAnd1 knockout mutant displayed nuclear positioning defects in vegetative hyphae, conidia, and appressoria. Notably, the MoAnd1 knockout showed reduced disease lesion formation in spray inoculations, whereas disease lesion development was comparable to wild-type in wound inoculations (Jeon et al., 2014). Wound inoculations involve physically damaging the cuticle of the rice leaf, which allows *M. oryzae* to enter rice cells without forming an appressorium. Since wound inoculations circumvent nuclear migration through the penetration peg, it is possible that nuclear migration through the penetration peg is more susceptible to defects in spindle function compared to nuclear migration in bulbous IH and through IH pegs. Recently, cooperation between microtubules, actin, and septins was discovered during appressorium morphogenesis (Dulal et al., 2021). It could be that a similar degree of cooperation between these cytoskeletons is required for successful nuclear migration through the penetration peg. More research is needed to conclusively determine if nuclear migration through the penetration peg is a distinct cellular phenomenon.

The roles of kinesin-5 and kinesin-14 in *M. oryzae.* While we provide *in vivo* evidence of MoKin5 and MoKin14 function within the spindle in *M. oryzae* during extreme nuclear migration through the penetration peg, we lack *in vitro* data to make definitive claims of the directionality of these motor proteins along MTs. In the future, *in vitro* experiments coupled with knockout experiments of MoKin5 and MoKin14 will fully elucidate whether the force-balance model of bipolar spindle formation applies to *M. oryzae*. We hypothesize that MoKin5 is an essential gene because in many organisms it

is required for viability (Enos and Morris, 1990; Hagan and Yanagida, 1990; Heck et al., 1993; Hoyt et al., 1992; Roof et al., 1992). Follow-up experiments will likely require forming double MoKin5/MoKin14 knockouts in the MoKin14 knockout background. Nonetheless, our results do provide information about the function of kinesin-5 and kinesin-14 in *M. oryzae* spindle formation and function during nuclear migration through the penetration peg. We begin this section of our discussion examining the likely roles of MoKin5 in *M. oryzae*.

Kinesin-5 in M. oryzae. Overexpression of MoKin5 by the *Bas4* promoter appears to promote excessive MT polymerization and excessive outward force generation, which leads to the formation of nuclear fragments. Within the appressoria of the MoKin5 OE strain, the length of the spindle continually increased in length over time. This finding is consistent with other studies of kinesin-5 overexpression. For example, overexpression of Cin8, one of two kinesin-5 motor proteins present in *S. cerevisiae*, resulted in extended spindles (Saunders et al., 1997) as did kinesin-5 overexpression in the spindles of *Drosophila* embryos (Brust-Mascher et al., 2009). In mice, kinesin-5 overexpression caused the formation of multipolar and monopolar spindles, and kinesin-5 overexpression was associated with polyploidy (Castillo et al., 2007). In our study, MoKin5 OE caused formation of an anucleate appressorium with a single enlarged nucleus within the primary hypha. We believe it is likely this single enlarged nucleus represents a polyploid state. In mice, it is proposed that kinesin-5 overexpression prevented attachments of the chromatids to the spindle due to the generation of excessive outward forces (Castillo et al., 2007). In our study, the nucleus and nuclear fragments appeared to be attached to the spindle, evident in the movement of nuclear fragments along the MT protrusions. We, therefore, favor a different mechanistic model to explain how aberrant nuclear phenotypes, and possible polyploidy, arises in the MoKin5 OE strain.

We favor a model that in the MoKin5 OE strains SPBs fail to separate. This failure in SPB separation coupled with excessive MT polymerization and excessive outward force causes the dramatic spindle and nuclear phenotypes observed in the MoKin5 OE strains. Key data from the early stages of mitosis in the appressoria support this model. We observed that a typical spindle fails to form within the MoKin5 OE strain. This was evident when a bar of MT-GFP signal spanned only approximately half the mother nucleus, and in the formation of single, double, and three or more MT protrusions. Moreover, we observed that the MoKin5 OE spindle elongates from a single plus end. If the MoKin5 OE spindle were a bipolar spindle, with each SPB maintained at the opposite end of the spindle, we would anticipate nearly equal growth from both ends of the spindle. An additional prediction is that if the MoKin5 OE spindle was a true bipolar spindle, the chromatids would move towards both poles of the spindle. This is not the pattern we found. In the MoKin5 OE strain, nuclear fragments formed along the MT protrusions, and the fragments only moved towards the growing plus-end of the spindle. We speculate that the excessive polymerization of MTs in the MoKin5 OE strain causes kinetochores to become precociously attached to MTs within the spindle. The combination of excessive MT polymerization and outward force generation causes formation of nuclear fragments. Over time, the disrupted polarity of the spindle and excessive MoKin5 causes the entire nucleus and nuclear fragments to migrate to the primary hypha.

In sum, we conclude that MoKin5 in *M. oryzae* likely generates an outward pushing force acting within the spindle. We also provide data that excessive MoKin5 causes consistent polymerization of MTs within the spindle. MoKin5 OE induced distinct defects in nuclear morphology and positioning, and in spindle function compared to MoKin14 OE. We discuss the likely role of MoKin14 in the following section.

Kinesin-14 in M. oryzae. Our study revealed that when MoKin14 is overexpressed, spindles fail to form and maintain bipolarity throughout the early stages of mitosis. In *Aspergillus nidulans*, kinesin-14 overexpression prevents nuclear division and causes formation of monopolar spindles, consistent with our results (O'Connell et al., 1993). Overexpression of kinesin-14 proteins in *S. pombe* causes formation of monopolar spindles (Pidoux et al., 1996; Yukawa et al., 2018), and overexpression of kinesin-14 in *S. cerevisiae* leads to shorter spindles (Saunders et al., 1997). Given that MoKin14 OE resulted in similar spindle phenotypes, it is likely that MoKin14 generates an inward force that acts upon duplicated SPBs in early mitosis in the appressorium and in IH. However, the later stages of mitosis were relatively unaffected by MoKin14 OE. Our preliminary analysis of MoKin14 knockout mutants support the conclusion that MoKin14 plays a role in early mitosis, but not late mitosis (unpublished). Together these data suggest that other motor proteins, such as dynein, may be generating the antagonizing force needed to maintain the spindle in the later stages of mitosis in *M. oryzae*. There is some evidence that supports this idea. While the function of dynein in *M. oryzae* is not yet determined, knocking out MoAnd1, a cortical anchor protein for dynein, impairs nuclear positioning in diverse cell types (Jeon et al., 2014). Conducting a functional study of dynein is an important future direction towards illuminating further details of nuclear migration within *M. oryzae.*

Conclusion

The major contribution of this study is the direct evidence that the mitotic spindle mediates nuclear migration through the penetration peg in the blast fungus during colonization of the host rice cell. This knowledge is important because this is a critical step in the successful colonization of the fungus within rice tissue. Previously, the dynamics of the spindle were reported in *M. oryzae* during vegetative growth, appressorium development, IH growth, and during cell-to-cell movement through the IH

peg (Czymmek et al., 2005; Pfeifer et al., 2019; Pfeifer and Khang, 2021; Row et al., 1985; Saunders et al., 2010a; Shah et al., 2019; Yadav et al., 2019). From these studies, we can see that delivery of a single daughter nucleus into incipient cells involves the spindle and likely occurs during the later stages of mitosis. While this finding may appear intuitive, not all fungi sync nuclear migration to nuclear division (Gladfelter and Berman, 2009). Defining the contribution of the spindle during nuclear migration through the penetration peg provides fundamental knowledge about the biology of the rice blast fungus and establishes new avenues for research. Presently, anti-rice blast efforts focus on mobilizing resistance genes into commercial rice cultivars, identifying chemical inhibitors to thwart *M. oryzae* development, and exploiting mechanisms of RNA interference through the application of artificial siRNAs or through *in vivo* host-induced gene silencing (e.g., Guo et al., 2019; He et al., 2020; Sharma et al., 2012; Sugahara et al., 2019). Our study shows that overexpressing two kinesin motor proteins using an effector gene promoter prevents development of blast disease lesions on rice. There is possibility of translating this general overexpression approach towards development of novel anti-fungal strategies to combat blast disease.

Materials and Methods

Fungal and rice strains. Transgenic *M. oryzae* strains were generated by transforming wild-type O-137 (CKF558) using *Agrobacterium*-mediated transformation (Khang et al., 2005). Fungal transformants were selected on media containing either: 200 µg/mL Hygromycin (Hyg, Hyg^R), 800 µg/mL G418 Sulfate (G418, $NTPII^R$), 400 µg/mL Nourseothricin (NTC, $Nat1^R$), and 200 µM of cefotaxime (bactericide for *Agrobacterium*). Transformants were purified by single spore isolation and two to twelve independent transformants were analyzed per gene. A summary of the fungal strains, primers, constructs, and unique PCR fragments used in this study are provided in Supplemental Tables 5.1-5.4. Fungal strains were stored at -20 °C and propagated on either oatmeal agar or V8 juice agar, using standard techniques, at 24 °C with

continuous light. Rice (*Oryza sativa*) cultivar YT16 was grown in a Conviron PGC20 growth chamber with daytime temperature of 28°C and nighttime temperature of 24 °C under long day conditions (14 hours/day, 10 hours/night).

RNA isolation and gene expression analysis. The expression of *MoKin5* and *MoKin14* relative to *actin* was determined in mycelia and infected YT16 rice sheaths in two independent reverse transcription quantitative (RT-q) PCRs of wild-type (CKF3578), MoKin5 OE (CKF4108), and MoKin14 OE (CKF4106) strains. Fungal mycelia were grown in 1% sucrose complete media at 25°C for five days in a dark environment, snap frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Twenty infected rice sheaths for each biological replicate (n=60 sheaths per fungal strain) were hand-trimmed and snap frozen in liquid nitrogen at 30-31 hours post inoculation. We confirmed that each fungal strain had penetrated into the rice tissue by conducting confocal microscopy two hours prior to harvesting the sheath samples (data not shown). For mycelia and infected sheath samples, total RNAs were extracted using the Trizol method combined with the RNA Clean and Concentrator -5 kit (Zymo), according to manufacturer's instructions. Genomic DNA was removed using Turbo™ DNase (Ambion) using manufacturer's instructions. Complementary DNA (cDNA) was synthesized following manufacturer's instructions using the ImProm II Reverse Transcriptase system (Promega) from 500 ng of total RNAs for mycelial samples and 650 ng of total RNAs for sheath samples. Applied Biosystems SYBR Green qPCR 2X Master Mix (Thermo Fisher) was used to perform the RT-qPCRs with a CFX96 Touch Real-Time PCR Detection System (BioRad). Reactions contained 7 µL Applied Biosystems SYBR Green qPCR Master Mix, 1.5 µL each of the forward and reverse primer (3.3 nM concentration), 1.5 µl cDNA, and 2.5 µL distilled water, for a final volume of 14 µL. Standard thermocycling conditions for primers ≥ 60°C per the Applied Biosystems SYBR Green qPCR Master Mix manufacturer's instructions were used. Thermocycler conditions were: 2 minutes at 50°C, 2 minutes at 95°C, and 40 cycles of 15 seconds at

95°C and 1 minute at 60°C. Relative expression levels of *MoKin5* and *MoKin14* were calculated using the *M. oryzae actin* gene (MGG_03982) as reference (Che Omar et al., 2016). The $2^{-\Delta\Delta Ct}$ was used to calculate relative expression levels (Livak and Schmittgen, 2001). Average threshold cycle (Ct) values from three technical replicates were normalized to *actin* for each strain (ΔCt**)**. This value was subtracted from the calculated mean ΔCt value of the wild-type (CKF3578) in the respective mycelia or sheath condition, yielding the ΔΔCt value. These values were transformed using the equation $2^{-\Delta\Delta Ct}$. Mean $2^{-\Delta\Delta Ct}$ values, along with 95% confidence intervals, were calculated for each strain from three biological replicates.

Rice sheath inoculations. Susceptible rice cultivar YT16 was inoculated with fungal spores as described previously (Jones and Khang, 2018). Leaf sheaths 3-8 cm in length from 2 to 3-week old plants were inoculated with either 3-4 X 10^4 spores per mL for ~48 hour post inoculation (hpi) observation, or 7-10 X10^4 spores per mL for ~28 hpi observation. All spore inoculum was filtered using Miracloth. Inoculated sheaths were prepared for microscopy by hand trimming with razor blades.

Appressorium development assay. Spores were harvested and diluted to a final concentration of 2-4X10^4 spores per mL. Spores were inoculated onto a hydrophobic coverslip and incubated for 3-4 hours at room temperature prior to microscopy.

Whole-plant spray inoculations. Spores were collected from 7 to 10-day old V8 juice agar plates and diluted to a final concentration of 1X10^5 spores per mL in 0.2% gelatin. 17-day old YT16 rice plants were sprayed with 5 mL of spores. Sprayed rice plants were placed in clear plastic bags overnight at room temperature. The next day sprayed plants were removed from the plastic bags and placed in a Conviron PGC20 growth chamber with daytime temperature of 28°C and nighttime temperature of 24 °C under long day conditions. Infected leaves were harvested 7 days after inoculation. Infected leaves were harvested and collected on notecards that were

scanned with an Epson Perfection 4870 Photo Scanner to generate digital images for analysis of lesion development. Lesion development was analyzed using ImageJ to determine the percentage of diseased tissue area. Briefly, scanned leaf images were color adjusted to find the total area of the leaf and then again adjusted to find the total area of the diseased tissue. The resulting ratio was converted to a percentage for each biological replicate, and mean values along with margins of error were calculated for each fungal strain. Figures of infected rice leaves were compiled with Adobe Photoshop and Adobe Illustrator.

Confocal microscopy and analysis. Live-cell confocal microscopy of developing appressoria and infected rice sheaths was conducted using a Zeiss 880 confocal system equipped with a Plan-Neofluor 40×/1.3 NA (oil) objective. Excitation/emission wavelengths were 488 nm/505–530 nm (GFP), and 543 nm/560–615 nm (RFP). Analyses of resulting micrographs were done using combinations of the Zen software (Black and Blue editions). Figures were compiled using Zen software (Black and Blue editions), Adobe Illustrator, and Microsoft PowerPoint.

Quantification of nuclear phenotypes. Informative micrographs collected from wild-type, MoKin5 overexpression (OE), and MoKin14 OE strains at approximately 28 hours post inoculation and 48 hours post inoculation were analyzed. Only infection sites with an intact appressorium and developed primary hypha were considered for quantification. Observed patterns of nuclear positioning within the appressorium and primary hypha were quantified. Phenotype frequency was compiled and graphed using a combination of Microsoft Excel and Adobe Illustrator.

Quantification of rate of mitosis. Rate of mitosis in wild-type and MoKin14 OE strains was determined using the time the first micrograph in a time-lapse series was acquired as the 00:00 timepoint. The spindle length was calculated by selecting a single informative focal plane, and measuring the length of the spindle from SPB to SPB

(marked by MoKin5-RFP) using the line tool in Zen Black. In monopolar spindles, the length of spindle was measured using the MT-GFP fluorescence signal. The resulting time and spindle length intervals were analyzed and plotted in Microsoft Excel.

Quantification of spindle length in the MoKin5 OE strain. The length of the spindles observed in strain CKF4203 was determined using the Closed Bezier tool in Zen software (Black edition). The length of the spindle was measured from the minus-end to the plus-end.

Sequence information. Gene identification numbers, except for *actin* and *Bas4*, were determined using *Aspergillus nidulans* or *Schizosaccharomyces pombe* protein sequences as query sequences in NCBI BlastP searches of the non-redundant protein sequence database using the *Magnaporthe oryzae 70-15* reference genome. Protein sequences were obtained from FungiDB. Gene identification numbers for *M. oryzae* were identified and gene sequence information along with 2 Kb upstream and downstream was downloaded from FungiDB and analyzed using Geneious Prime 2019.2.3. Reciprocal NCBI BlastP searches using the protein sequences from *M. oryzae* to either *A. nidulans* or *S. pombe* were conducted as a quality control step. A list of resulting gene identification numbers is available in Supplemental Table 5.2. Sequence information is available from FungiDB.

Statistical analysis and reproducibility. Significance of gene expression levels was determined using a Student's two-tailed test assuming unequal variance in Microsoft Excel. Significance of nuclear positioning phenotypes at the early timepoint was determined using Fisher's exact test (McDonald, 2009) in in GraphPad QuickCalcs (accessed March 19, 2021). Confocal micrographs are representative of at least three biological replicates. Representative examples of each strain are presented throughout the figures.

Data availability. Data supporting the conclusions of this study are available upon reasonable request from the corresponding author. Key constructs generated in this study will be made available from Addgene. Mutant fungal strains are available from corresponding author with appropriate permits.

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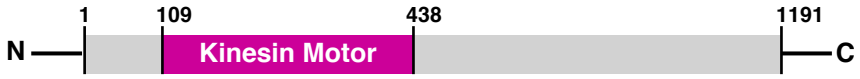
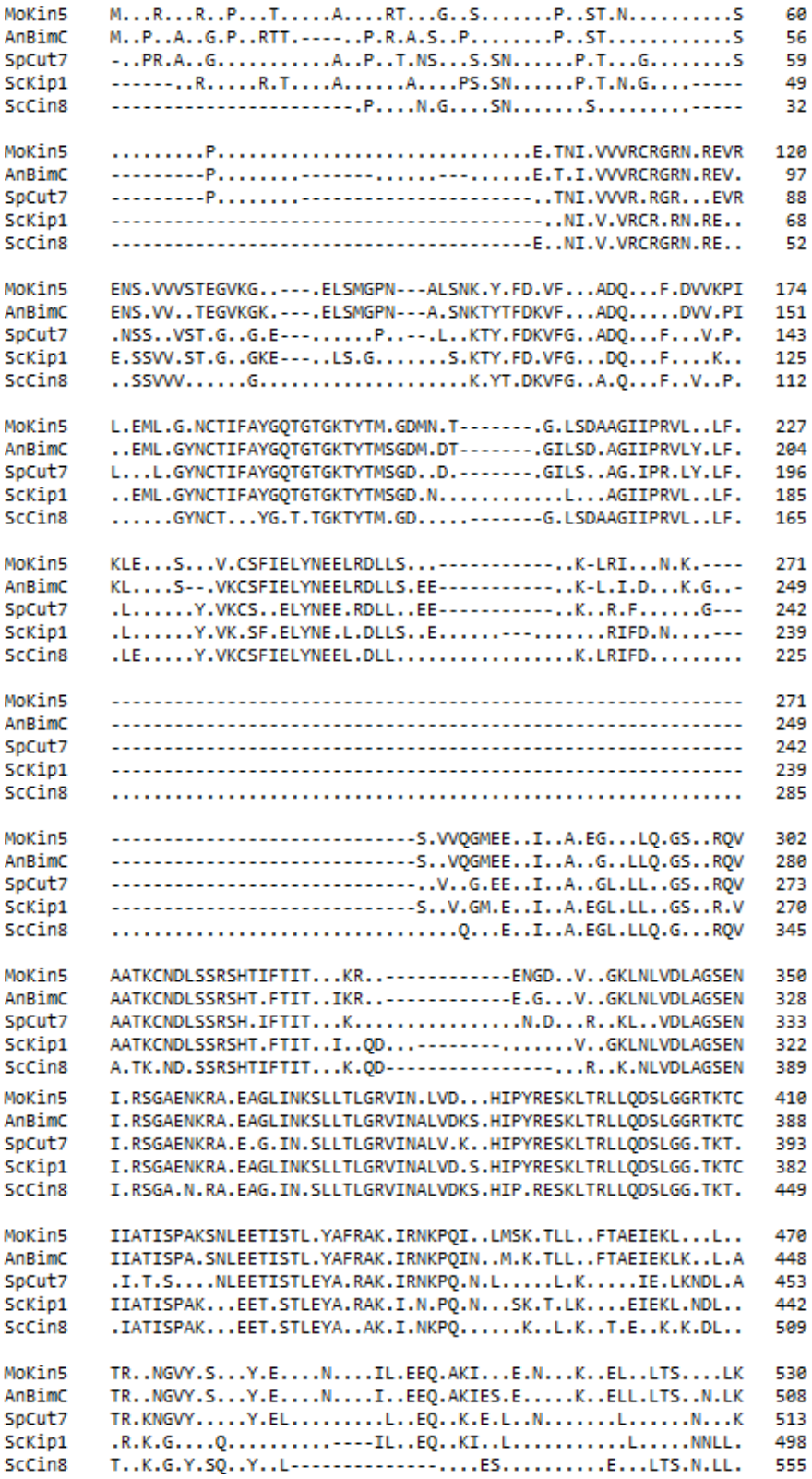
A Schematic structure of MoKin5 protein (MGG_01175)



B Sequence alignment of MoKin5 and AnBimC (AN3363)

```
MoKin5    M.........LP...T..........R................P..ST............S   60
AnBimC    M.........LP...T..---------R................P..ST............S   56

MoKin5    .........P.E..S..A.......KRKERD....E....E.T.I.VVVRCRGRN.REV.  120
AnBimC    ---------P.E..S..A-------KRKERD---.E....E.T.I.VVVRCRGRN.REV.   97

MoKin5    ENS.VV..TEGVKG..VELSMGPNA.SNK.Y.FD.VFS.AADQ.....DVV.PI..EML.  180
AnBimC    ENS.VV..TEGVKG..VELSMGPNA.SNK.Y.FD.VFS.AADQ.....DVV.PI..EML.  157

MoKin5    G.NCTIFAYGQTGTGKTYTM.GDM..T.G.LSD.AGIIPRVL..LF.KL...ES...V.C  240
AnBimC    G.NCTIFAYGQTGTGKTYTM.GDM..T.G.LSD.AGIIPRVL..LF.KL...ES--.V.C  215

MoKin5    SFIELYNEELRDLLSA....KL.IY....KK---ST.VQGMEE..I..A..GIK.LQ.GS  297
AnBimC    SFIELYNEELRDLLSA....KL.IY....KK...ST.VQGMEE..I..A..GIK.LQ.GS  275

MoKin5    ..RQVAATKCNDLSSRSHT.FTITV..KR..E.G..YV..GKLNLVDLAGSENI.RSGAE  357
AnBimC    ..RQVAATKCNDLSSRSHT.FTITV..KR..E.G..YV..GKLNLVDLAGSENI.RSGAE  335

MoKin5    NKRA.EAGLINKSLLTLGRVIN.LVD...HIPYRESKLTRLLQDSLGGRTKTCIIATISP  417
AnBimC    NKRA.EAGLINKSLLTLGRVIN.LVD...HIPYRESKLTRLLQDSLGGRTKTCIIATISP  395

MoKin5    A.SNLEETISTLDYAFRAK.IRNKPQI...M.K.TLLREFTAEIEKL..EL..TR.RNGV  477
AnBimC    A.SNLEETISTLDYAFRAK.IRNKPQI...M.K.TLLREFTAEIEKL..EL..TR.RNGV  455

MoKin5    Y.S.E.YEE....NESRRI..EEQ.AKI...E..LR.KVQEL..LTS.F..LKK....T.  537
AnBimC    Y.S.E.YEE....NESRRI..EEQ.AKI...E..LR.KVQEL..LTS.F..LKK....T.  515

MoKin5    ..L..T...L.QT..VL..TR..L..E..LR.AH.ETE..L...G..LI.TLG.TV....  597
AnBimC    ..L..T...L.QT..VL..TR..L..E..LR.AH.ETE..L...G..LI.TLG.TV....  575

MoKin5    .L..K..RK..L...N...W..S...V.DVT.....R...FQ..........S.....F.  657
AnBimC    .L..K..RK..L...N...W..S...V.DVT.....R...FQ..........S.....F.  635

MoKin5    ..E......T...L.........................M..VLEEIK..R...K..VGE.  717
AnBimC    ..E......T...L.........................M..VLEEIK..R...K..VGE.  695

MoKin5    L.....AA.RI...V..E....H.QLHTSF..LGKD.KSIFE....HL..Q..E..RLR.  777
AnBimC    L.....AA.RI...V..E....H.QLHTSF..LGKD.KSIFE....HL..Q..E..RLR.  755

MoKin5    .L.......I......SA.......EE...A..ER..L..QI..L.......Q..RL..K  837
AnBimC    .L.......I......SA.......EE...A..ER..L..QI..L.......Q..RL..K  815

MoKin5    ..........S....E...TQ.......WV.K......D.N.S.....TK...DW.A...  897
AnBimC    ..........S....E...TQ.......WV.K......D.N.S.....TK...DW.A...  875

MoKin5    ....I...T.SVH.ETVR.V..Q..D...QM..LD.FV..ARSQN......H.A......  957
AnBimC    ....I...T.SVH.ETVR.V..Q..D...QM..LD.FV..ARSQN......H.A......  935

MoKin5    ..V..SY..I.........R......E.............PL...V..PL..L..S....  1017
AnBimC    ..V..SY..I.........R......E.............PL...V..PL..L..S....  995

MoKin5    .L.EY..TG.TP.K..Y.Y...LP.T..HE.L...L.-------------...L..PS.S-  1064
AnBimC    .L.EY..TG.TP.K..Y.Y...LP.T..HE.L...L.................L..PS.S.  1055

MoKin5    --.P....V..D.E..................GLREV..NV...................  1122
AnBimC    ...P....V..D.E..................GLREV..NV...................  1115

MoKin5    P....D............-S.R..S.......K..........A....GRENVPP.G....  1181
AnBimC    P....D............S.R..S.......K..........A....GRENVPP.G--..  1173

MoKin5    .GRR...R..-                                                  1191
AnBimC    .GRR...R..P                                                  1184
```

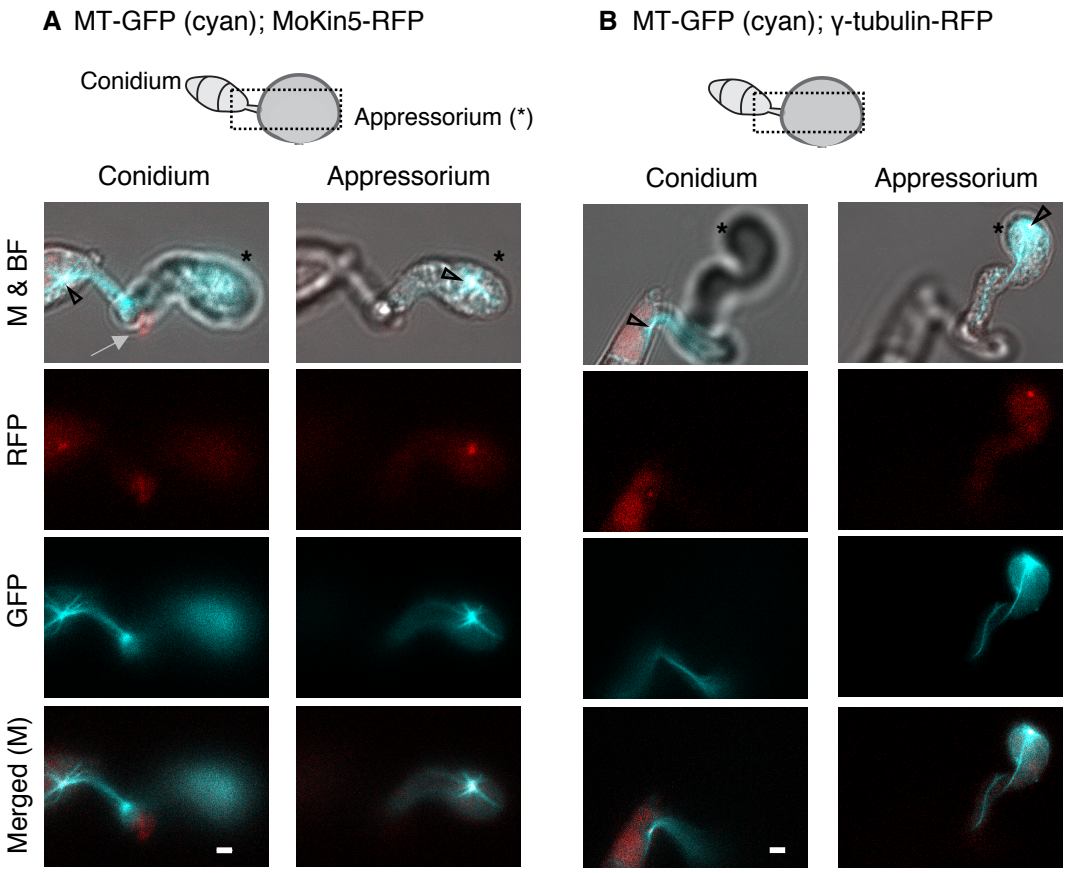
C Sequence alignment of kinesin-5 proteins



C Sequence alignment of kinesin-5 proteins, continued

```
MoKin5    KE.E....QL..T...L..T...L..TR..L..E..LRKAH.ETE.KL...G..LI..LG   590
AnBimC    K........L..TN..L..T...LQ.TR..L..E..LR.AHEETE..L..VG..LI..LG   568
SpCut7    KE.E....QL......LE..KS...K....L..E...RK..E..E.K...V...L.Q...   573
ScKip1    .EKEK.....Q..N.......S..QK.....-----L....E..............Q...   553
ScCin8    K.K.K.....Q..N...E..K.-------------------------------------   577

MoKin5    ETV.H...L..K..R...LQS.N...W......V.DVTE....R...FQ...E....S.S   650
AnBimC    .TVE...SLQ.KLDR...L...N...W...S..V.DVT.....RV..FQ..H...L...S   628
SpCut7    E..E...SL..KLDR.E.....N.N.....................D........L....   633
ScKip1    .........Q..L.....LQ...NN...K.SEV...VTE.....V.....KH...L.S..   613
ScCin8    -T..H......K....E...S..NN...K..EV..........R..D...K.E.......   636

MoKin5    ..M.......L..L.....LL..N..--......K..........Q....VLEEIK..R.   708
AnBimC    .K......T......R..S.L.E...--SLD.A.........S......NVLEEIKD.R.   686
SpCut7    ..M...L.T..N.L.........E.FQ--SLD.A.....................E.KD...   691
ScKip1    ......L....N.L.R..S...E.FQ..S.....K.......S..Q...N....IK....   673
ScCin8    .K....L...LN.......L...N.Q.-----------------.........VL.......   681

MoKin5    .IK..VGESL..I..A...I...V.SE.......LH.S...LGK..KSIFEDL..HL..Q   768
AnBimC    ..KS.VGE.LN..S.A...I...VI.E...L.S.LH.S..NLGK.LKSIFE....HL..Q   746
SpCut7    S.......SL..IS.......N...SE...L......S...L...L.S.......H...Q   751
ScKip1    SI.S....S.N.IS.......N.........S......K......K....DL.......   733
ScCin8    ............I........N.VI..............KN....L..I.E.........   741

MoKin5    ..E..RLR..L.......I......SA.......EE...A..ER..L.A.I....N....   828
AnBimC    KNE..RLR..L.......I.....ASA.L...I.EE...AE.ER..L...IK..V.E..Q   806
SpCut7    K......R..............N.A...L...I...K.K.E...QDL.A.I.K.V...LQ   811
ScKip1    ......L.....................LN..ID..K.K..K..QD.........NE...   793
ScCin8    KN----------------....N.....LN...D......EK.........KK..N..L.   785

MoKin5    .Q..RL.DK...L...L....K..E....Q........V.K......D.N.S.E...TK.   888
AnBimC    .Q..RL..K..............LEQA..Q......E.V.K....A.DVNASK..I.TK.   866
SpCut7    EQN..L..K...L.S.L.D.......AN....N.S.E..R.....A..V.A.KE.I....   871
ScKip1    ..NK...D....L........K...Q.N....N......R...........S........   853
ScCin8    E..K..........S...D....L..........S.....----------------------   825

MoKin5    K.DW.A.......I.....SV..E..R.V..QK.D...QM..LDS.V..ARSQ..L....   948
AnBimC    .NDW.A.D.....I..A..SV..E..R.V..Q..DM..QM..LD..V..ARSQ.......   926
SpCut7    .N.....DS....I.....S...........QK...NL....LD..............E   931
ScKip1    K.....C.........A...V.............D..L......S......SQ.....NE   913
ScCin8    -----.CDS...................V......MN.........V........L..N-   879

MoKin5    H.A..........NSY..I....K....R.....DE...E.......L.PL...V..PLS  1008
AnBimC    H.A.L.........SY..I.....N...R....Q.E....H.T.E.S..PL.NDV..PL.   986
SpCut7    ....L.D..E..........D.IK...T...K..D.VL.......N....L.......L.   991
ScKip1    .N..L....E......N.I.......I....K.QDEVL.EH.....SLK.LG.D.....S   973
ScCin8    -N....D..E...N..N...D.IKN.IT........V.....T.EN..K..GN..------   933

MoKin5    .L..SI.DT.L.EY..TG.TP.K.......ELP.T..........------------..  1056
AnBimC    .L..S....SL.EY..TG.TP.KR.......LP.TE.........................  1046
SpCut7    .LE..I.DTSL.....TG.TP.KR------ELP.T.-------------------------  1021
ScKip1    -.E.........E.......P..............E-------------------------  1008
ScCin8    ------------------------------------------------------------   933

MoKin5    ........---..........D....T...E......S.....LREVN.N.....S.....  1113
AnBimC    ...................ND..DE......T..N.......LREV..N....P......  1106
SpCut7    -------------------..T.D.....ETT..NL.S.K...RE.....N.........  1062
ScKip1    -------------------N...D.T....T...NLS................P....P.  1049
ScCin8    -------------------N.T.DE............S.K..LR..N.N.N...S...P.   974

MoKin5    .........P....D...S.M.....-S.R..S.......K....G.....A....GREN  1172
AnBimC    .K......SP..S.D.A.......G..S.R..S......TK...K......A....GREN  1166
SpCut7    ........S...SS..A.S.M..-------------------------------------  1122
ScKip1    .K...........S..........G..............T....KG..............  1109
ScCin8    ..............D...S.......-------------------------------------  1034

MoKin5    VPP.G.....GRR...R..-                                          1191
AnBimC    VPP.G--...GRR...R..P                                          1184
SpCut7    --------------------                                          1085
ScKip1    ..------------------                                          1111
ScCin8    --------------------                                          1000
```

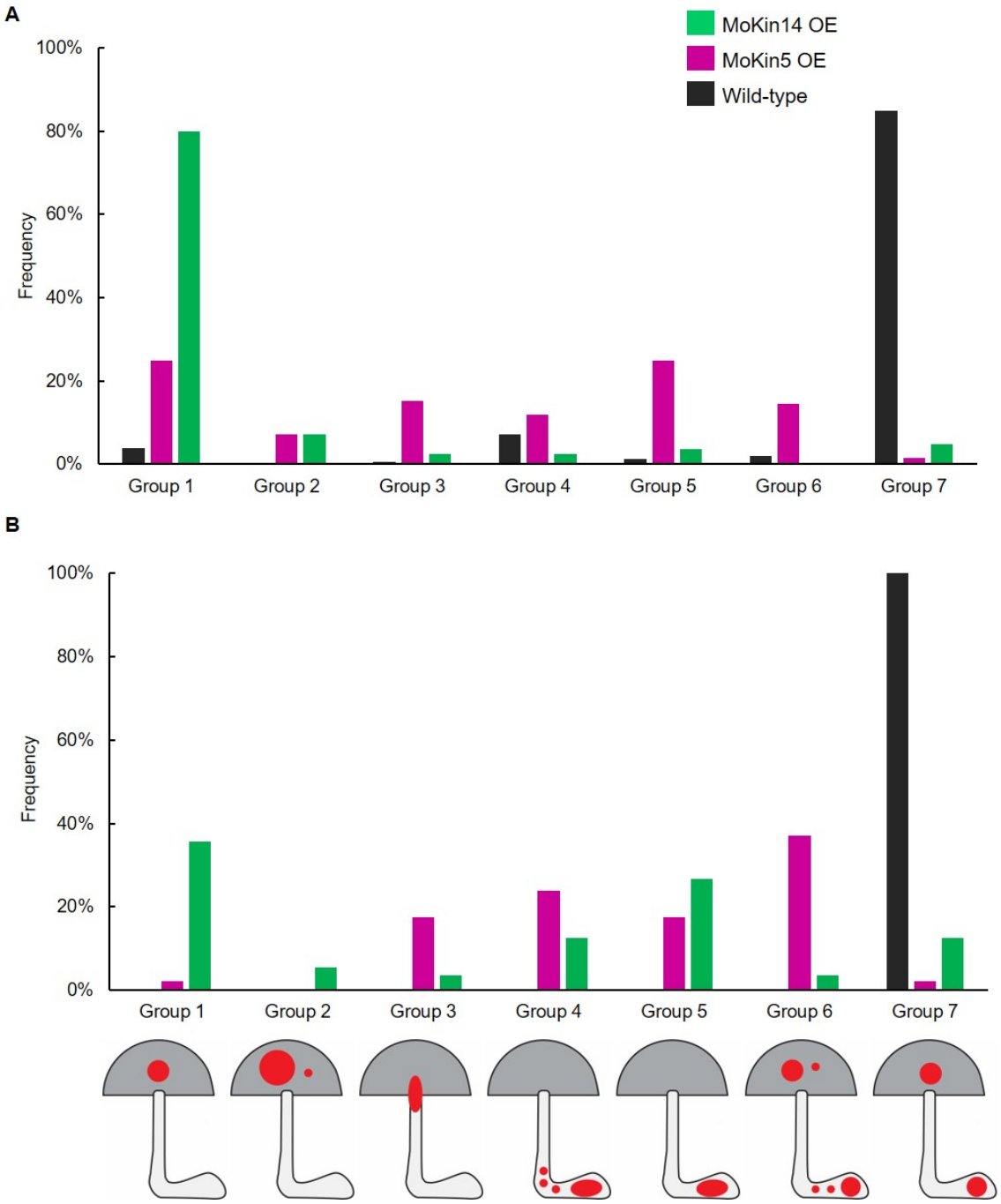
Fig.5.S1. MoKin5 is a conserved kinesin-5. (A) Schematic of MoKin5 protein structure. A PFAM kinesin motor domain (PF00225) is predicted at positions 109 to 438. (B) Protein sequence alignment of MoKin5 and kinesin-5 in *Aspergillus nidulans* (AnBimC; AN3363). Dashes represent indels, and dots represent mismatched amino acids. Magenta box corresponds to predicted kinesin motor domain in Fig. 5.S1A. (C) Protein sequence alignment of MoKin5 to select kinesin-5 proteins in other fungi, including *A. nidulans* (AnBimC), *Schizosaccharomyces pombe* (SpCut7), and *Saccharomyces cerevisiae* (ScKip1, ScCin8). Other kinesin homologs besides MoKin5 and MoKin14 likely exist in *M. oryzae* based on BlastP searches of the non-redundant protein database, however, these proteins are not yet characterized.


A MT-GFP (cyan); MoKin5-RFP
Conidium
Appressorium (*)
Conidium
Appressorium
M & BF
RFP
GFP
Merged (M)
B MT-GFP (cyan); γ-tubulin-RFP
Conidium
Appressorium

C Sequence alignment of Mo-γ-tubulin (Mogamma; MGG_00961) to An-γ-tubulin (Angamma; AN0676)

```
Mogamma   M..MPREIITIQAGQCGN..GSQFWQQLC.EHGI.QDGNLE.FATEGGDRKDVF.YQSDD   60
Angamma   -..MPREIITIQAGQCGN..GSQFWQQLC.EHGI.QDGNLE.FATEGGDRKDVF.YQSDD   59

Mogamma   TRYIPRAIL.DLEPRV.NGIQ.GPYKNIYNPENF..G..G.GA.NNWG.GY..GE.V.E.  120
Angamma   TRYIPRAIL.DLEPRV.NGIQ.GPYKNIYNPENF..G..G.GA.NNWG.GY..GE.V.E.  119

Mogamma   ...MIDREADGSDSLEGFM.LHSIAGGTGSGLGSFLLER.NDRFPKK.IQTYSVFPDTQ.  180
Angamma   ...MIDREADGSDSLEGFM.LHSIAGGTGSGLGSFLLER.NDRFPKK.IQTYSVFPDTQ-  178

Mogamma   A.DVVV.PYNS.LAMRRLTQNADSVVVLDN.ALS.I.ADRLHVQEPSFQQTN.LVSTVMS  240
Angamma   A.DVVV.PYNS.LAMRRLTQNADSVVVLDN.ALS.I.ADRLHVQEPSFQQTN.LVSTVMS  238

Mogamma   ASTTTLRYPGYMHNDLV.I.ASLIPTP..HFL.TSYTPFTGD...QAKTVRKTTVLDVMR  300
Angamma   ASTTTLRYPGYMHNDLV.I.ASLIPTP..HFL.TSYTPFTGD...QAKTVRKTTVLDVMR  298

Mogamma   RLLQPKNRMVS..P.K.SCYISILN.IQGE.DPTDVHKSLLRIRERRLA.FIPWGPASIQ  360
Angamma   RLLQPKNRMVS..P.K.SCYISILN.IQGE.DPTDVHKSLLRIRERRLA.FIPWGPASIQ  358

Mogamma   VALTK.SPYI...HRVSGLMLANHTS.ATLFKRIV.QYD..RKRNAF.E.YKK..PF...  420
Angamma   VALTK.SPYI...HRVSGLMLANHTS.ATLFKRIV.QYD..RKRNAF.E.YKK..PF...  418

Mogamma   LDEFDEAR.VV.DL..EYEAAE..NYL.PDAG......ETDRRMG                465
Angamma   LDEFDEAR.VV.DL..EYEAAE..NYL.PDAG.....--------                456
```

Fig.5.S2. Relative localization of MoKin5-RFP and Mo-γ-tubulin-RFP in spindles (MT-GFP) spanning the germtube of developing appressoria (asterisks). Micrographs are single informative focal planes. Scale bars are 2 μm. (A) Localization of MoKin5-RFP (black arrowheads) relative to MT-GFP in a developing appressorium of *M. oryzae* strain CKF4168. Gray arrow shows red autofluorescence in micrograph. (B) Localization of Mo-γ-tubulin-RFP (black arrowheads) relative to MT-GFP in a developing appressorium of *M. oryzae* strain CKF4117. CKF4117 displayed severe developmental defects and was not used for subsequent analysis. (C) Protein sequence alignment of Mo-γ-tubulin (Mogamma; MGG_00961) to γ-tubulin in *Aspergillus nidulans* (Angamma; MipA; AN0676). Dashes represent indels, and dots represent mismatched amino acids.


A
MoKin14 OE
MoKin5 OE
Wild-type
100%
80%
60%
40%
20%
0%
Frequency
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
B
100%
80%
60%
40%
20%
0%
Frequency
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7

C histone H1-RFP

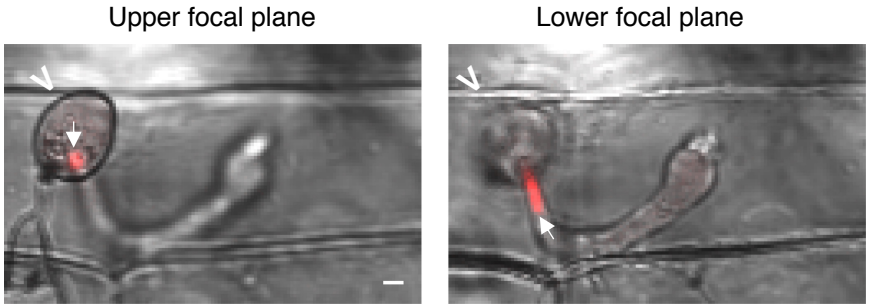
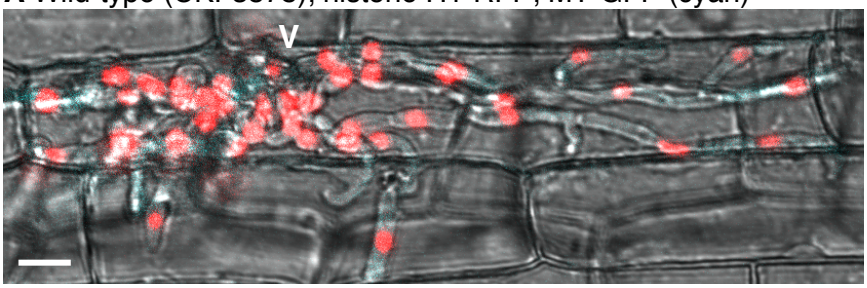
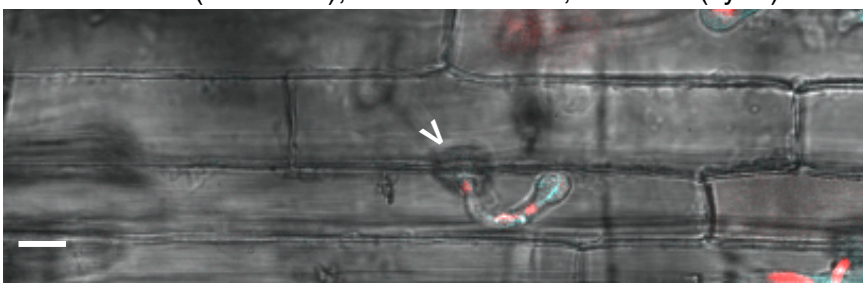


Fig.5.S3. Summary of observed nuclear positioning and morphology phenotypes. Only infection sites with intact appressoria were considered for analysis. For each strain, only micrographs with histone H1-RFP and brightfield channels were scored. (A) Frequency of all nuclear positioning and morphology phenotypes at ~28 hours post inoculation in wild-type (CKF3578, CKF3971, n=153), MoKin5 OE (CKF4108, n=125), and MoKin14 OE (CKF4106; CKF4093, n=85) strains. Schematic representations of nuclear positioning and morphology for each group are found at the bottom of Fig. 5.S3B. (B) Frequency of all nuclear positioning and morphology phenotypes at ~48 hours post inoculation in wild-type (CKF3971, n=26), MoKin5 OE (CKF4108, n=46), and MoKin14 OE (CKF4093, n=56) strains. Schematic representations summarize key nuclear positioning and morphologies for each group. Group 1, Group 5, and Group 7 example micrographs can be found in Fig. 5.5A. A Group 2 example micrograph is found in Fig. 5.6B. A Group 3 example micrograph is found in Fig. 5.S3C. A Group 4 example micrograph is found in Fig. 5.7. A Group 6 example micrograph is found in Fig. 5.6C. (C) An example micrograph of a Group 3 nuclear phenotype. Single focal planes are shown. A nucleus (arrow) appears to be stuck in the penetration peg. The appressorium is indicated by an arrowhead. Scale bar is 2 μm.

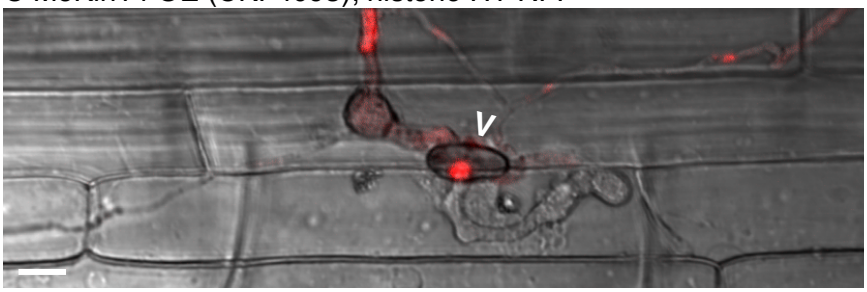
A Wild-type (CKF3578); histone H1-RFP; MT-GFP (cyan)



B MoKin5 OE (CKF4108); histone H1-RFP; MT-GFP (cyan)



C MoKin14 OE (CKF4093); histone H1-RFP



D MoKin14 OE (CKF4106); histone H1-RFP; MT-EGFP (cyan)

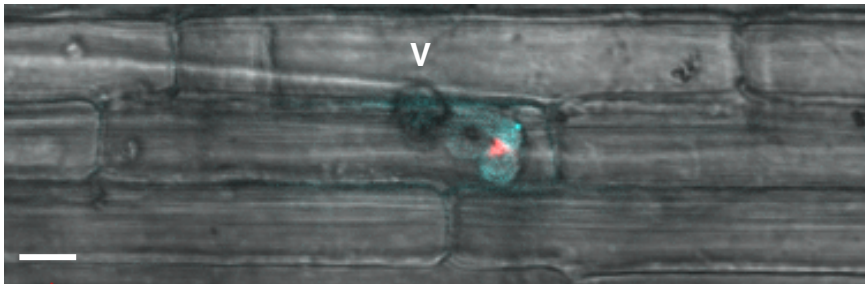


Fig.5.S4. Representative examples of wild-type, MoKin5, and two independent MoKin14 OE strains in infected rice sheaths at ~48 hours post inoculation. Micrographs are single focal planes. Scale bars are 10 μm. Arrowheads point to appressoria.

MoKin5 OE. MoKin5-RFP; MT-GFP (cyan)

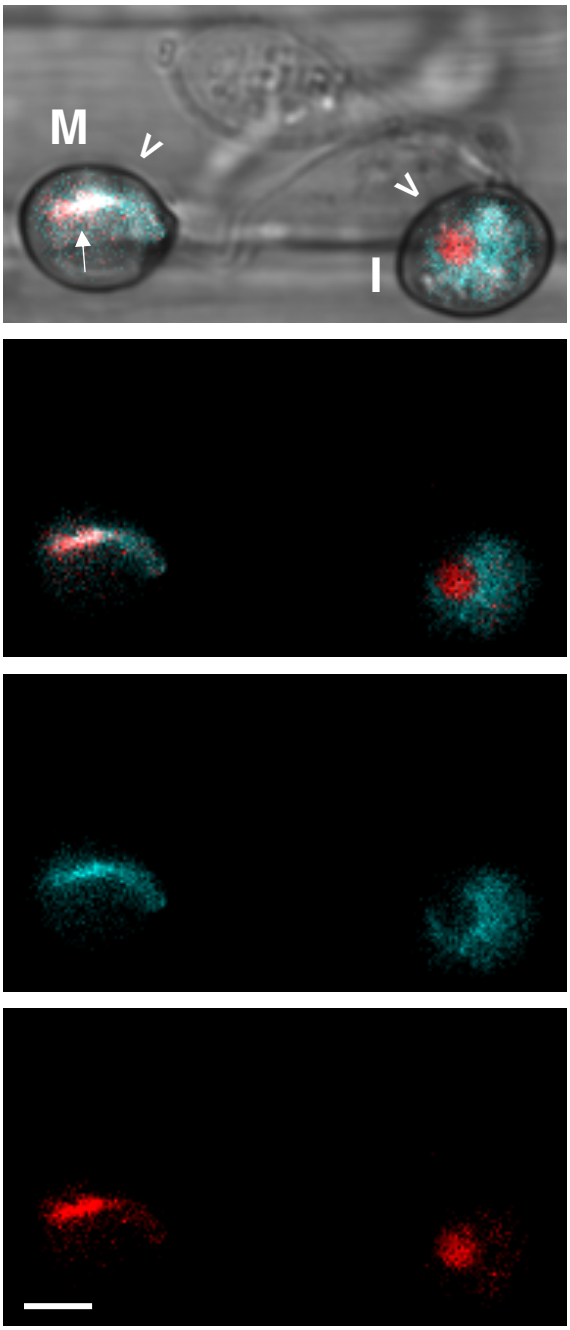


Fig.5.S5. Representative example of localization of MoKin5-RFP and MT-GFP in a MoKin5 OE strain CKF4203. Arrowheads point to appressoria. The appressorium designated with “M” is in mitosis, the spindle is evident (arrow) and MoKin5-RFP fails to localize at the ends of the spindle, as occurs in wild-type. The appressorium designated with “I” is in interphase, and MoKin5-RFP localizes within the nucleus, as occurs in wild-type. Micrograph is a single informative focal plane. Scale bar is 5 μm.

A Schematic structure of MoKin14 protein (MGG_05350)

B Sequence alignment of MoKin14 and AnKlpA (AN6340)

```
MoKin14   MKSSCTGGSGTGRCSNGAEPIPSFPFTRLFPHLHHPSGFTVLHYVSSVTTTPSPIASGAL   60
AnKlpA    ------------------------------------------------------------

MoKin14   LLASNFALQRELIPRFRSLSMDGS.EN...R..G.....RI..L.....S..NAR.....  120
AnKlpA    ------------------------.EN...R..G.----RI..L.....S..NAR..----   29

MoKin14   LP..G........K....PQ............R..............A.R.V.....A..  180
AnKlpA    LP..G----....K....PQ-----------R..............A.R.V.....A..   77

MoKin14   .AN.............TR.....S...G....S..GR..T...S....P.G...PRPAT.  240
AnKlpA    .AN......------..TR.....S...G....S..GR..T-..S....P.G...PRPAT.  131

MoKin14   ................G-WD.DER.Q..ES......S.......E........E....R.  299
AnKlpA    ................G.WD.DER.Q..ES......S.......E........E....R.  191

MoKin14   ..LE........Q...L...L.....---.....L....R.H....D.L....R.E.E..  356
AnKlpA    ..LE........Q...L...L.............L....R.H....D.L....R.E.E..  251

MoKin14   ...........E.QK...A.......E..E..R...R.L.....................  416
AnKlpA    .----------.E.QK...A.......E..E..R...R.L...----------....---  288

MoKin14   .RE............Q.....L.....E....R.....L...LDRE..............  476
AnKlpA    .RE............Q.....L.....E....R.....L...LDRE..............  348

MoKin14   ......LE..I..L...I.FLES....Q.............A......AK.KL..EET.R  536
AnKlpA    ......LE..I..L...I.FLES....Q.............A......AK.KL..EET.R  408

MoKin14   R.L.N..QELKGNIRV.CRVRP.L..-.....A....PD-...S..I....-EE.S..GT  593
AnKlpA    R.L.N..QELKGNIRV.CRVRP.L........A....PD....S..I.....EE.S..GT  468

MoKin14   VTR....F.FD.VF.P..QN..VF.EISQLVQSALDG.NVCIFCYGQTGSGKT.TMSS.D  653
AnKlpA    VTR....F.FD.VF.P..QN..VF.EISQLVQSALDG.NVCIFCYGQTGSGKT.TMSS.D  528

MoKin14   GMIPRA...IYET...L..K.W.YTMEG.FVEVYNE.L.DLL--A.E....KL.I.HD..  711
AnKlpA    GMIPRA...IYET...L..K.W.YTMEG.FVEVYNE.L.DLL..A.E....KL.I.HD..  588

MoKin14   R..TT.....TV.L..PE.VE..L..A..NRSVAATKANERSSRSHS.F.LKL.G.N..T  771
AnKlpA    R..TT.....TV.L..PE.VE..L..A..NRSVAATKANERSSRSHS.F.LKL.G.N..T  648

MoKin14   GER.EGTLNLVDLAGSERL.HS.A.GDR..ETQ.IN.SLSCLGDVI.ALG.........H  831
AnKlpA    GER.EGTLNLVDLAGSERL.HS.A.GDR..ETQ.IN.SLSCLGDVI.ALG.---.....H  705

MoKin14   .PYRNSKLT.LLQFSLGGNSKTLMFVMVSPL.AHL.ET.TSL.FATKVHNTHIGTAK...  891
AnKlpA    .PYRNSKLT.LLQFSLGGNSKTLMFVMVSPL.AHL.ET.TSL.FATKVHNTHIGTAK...  765

MoKin14   ....-                                                        895
AnKlpA    ....V                                                         770
```

C Sequence alignment of kinesin-14 proteins

```
MoKin14   MKSSCTGGSGTGRCSNGAEP........L..HL...S.......................   60
AnKlpA    ------------------------------------------------------------
SpKlp2    --------------------M..E..K.L..HL...S-----------------------   17
ScKar3    ------------------------------------------------------------
SpPkl1    --------------------M..E..K.......--------------------------   14

MoKin14   ...S.....RE....F.S............R..GL.P..RI..LN....S..NA.....M  120
AnKlpA    ------------------------....QSR..G.----RI..L.E.N.S..NA..R---   29
SpKlp2    ---S.....RE...EF.S..P...I..N.S....L.P--R....NEVNQ...-A.....M  71
ScKar3    ------------------------------------------------------------
SpPkl1    --............E.....P...I..NQ..............N.V.QS..N...R...    72

MoKin14   LP.AG........K..SLPQ...S........RA.E.......A.....R.V.AS..A..  180
AnKlpA    LP..G----.IA.K....PQ..R.--------R...........PS..RSV.....A..   77
SpKlp2    LP...------....K..SL....R.--------..A.........A.P...RSV.AS....R  120
ScKar3    --------------.ESLP.....--------------....Q....PS......A.....R  34
SpPkl1    ...A......IA...ES......S----------...E....Q.............S..A..  124

MoKin14   .AN...S.........TR.....S...G....S..GR...........P.G...P.PAT.  240
AnKlpA    RAN....S.------..TRS.S..S...G...SS..GR...-S....R.P.G..LP.PAT.  131
SpKlp2    .A.A..SS...--..D.RS.S..S..S.....S.N...L.......R..E.AY....A.V  178
ScKar3    R.....................S..S....S.S..R.L......T.............V   94
SpPkl1    ...A..SS.......D..S..............SNG.....S...T...E.AYL......  184

MoKin14   ..SY....E.D...Q.G-WD.DERL.K..S...E.........SE....E.E.....KRA  299
AnKlpA    ....QE..........G.WD.DER...L.S.........IS....E...L.....V...R.  191
SpKlp2    ..S..........Y.........E.L....S...E..E..S..L.E....E..K.V..K..  238
ScKar3    ...Y.E........Q..L......L..L.....ET.E.I.........L..EK.......  154
SpPkl1    ....Q...E.D..Y...L....E...K......ET.E..S..LS.......E.......A  244

MoKin14   NALENEL.........L...LE.-----.-----.HK..L....RE.....DNL...LR.  351
AnKlpA    ..LE.......E.N..LK..L..------.K...A...D.L....R.....ID.L....R.  246
SpKlp2    ..LE..L.S.K.EN......LEE............H..EL.............L......  298
ScKar3    N...NEL.S.KEE....K....E.....LK...AS.K.EL.....E....I..L......  214
SpPkl1    NA....L.....E..............L.....S..D..............N....LR.  304

MoKin14   E.E.A...........E..KE..A.......E..E......R.L.E..............  411
AnKlpA    E.ES..----------.E..K.L.A......SEL.E.....ER.L..E-------------  284
SpKlp2    EL.SA...------...KIKEL........S.LQEE......K..E.-------------  339
ScKar3    ..E....S-------...KI.E...KI....S.LQE...D.E.K...E-------------  255
SpPkl1    .L.....S-------..EKI.....KI.......Q.E..D.......E.------------  346

MoKin14   .....D.E.E.........E.....LL..K..EN.....Q.E.-L...L.RE..LKE...  470
AnKlpA    -......E...L..K........Q..L.....E.....E...S-L..EL.RE.K...NLR  342
SpKlp2    ----...........K........Q........EN.S...QIE.-L..EL..E...KENL.  394
ScKar3    --------.E..............EL......E..S....IE..L..EL....KL....R  307
SpPkl1    -----D.....L.......E....E....K........E.I.S-L..EL.........LR  400

MoKin14   .A..........LE..I..L...I.FLES.N..Q.....E.E.....A....DE.K.KLI  530
AnKlpA    ..LD........LE.TI.AL...I.FLES...EQ.E....L.....DA..E....KEKL.  402
SpKlp2    ..LD.....V..LE.T..AL...I..LE........KI.ELE.....A..E.D...EKLI  454
ScKar3    NA.......V...E.....L....A.....N.E..EKI.ELE....D.E....E..E.LI  367
SpPkl1    N.L................L....A..............EL.......E........KL.  460

MoKin14   .EET.RR.L.N..QELKGNIRV.CRVRP.L..---.....A..AFPD-...S.Q-I....  585
AnKlpA    .EETLRRKLHN..QELKGNIRVFCRVRP.L.N.--....AAQ...PD....S..-I.I.G  459
SpKlp2    .EETLRRKLHN.IQELKGNIRVFCRVRP.L..------...AQ.AFPD........-I.I..  508
ScKar3    .EET.RR.LHN..QEL.GNIRV.CR.RP.L.N.--E............D..S..-.....  424
SpPkl1    .EE..RRKLHN.IQELKGNIRVFCRVRP.L.....E...A....FPD.D....Q.....G  520
```

C Sequence alignment of kinesin-14 proteins, continued

```
MoKin14   -EE.S..GTVTR....F.FDRVFAP...N..VF.EISQLVQSALDG.NVCIF.YGQTGSG  644
AnKlpA    PEE.SS.GTVTR....FSFD.VF.P...N.DVF.EISQLVQSALDGYNVCIF.YGQTGSG  519
SpKlp2    ....SSL.........F.FDRVF.PE..N.DVF.E.SQL.QSA.DGYNVCIFAYGQTGSG  568
ScKar3    ..........-T....EF.FD..F.....N.DVF.E..QLVQS.LDGYNVCIFAYGQTGSG  479
SpPkl1    P...SSLG.......EFSFDRVFAPE..N..VF.EISQL.QSA.DGYNV.IFAYGQTGSG  580

MoKin14   KT.TMSSP-DGMIP.....IY.TI..LK.K.W.Y.MEG.F.E.YNE...DLL--A.E...  701
AnKlpA    KT.TMSS.-DGMIP.....IY.T.TSL.EKGW.Y.MEG.F.E.YNE...DLL..A.EL.K  578
SpKlp2    KT.TMSS.-.GMIP.....IYN..TSLKE.GW.Y.MEGQF.EIYNE.I.DLL....E..K  627
ScKar3    KT.TM..P.DG.IP.....I.N.I..LK.KGW.Y.....F.EIYNE.I.DLL........  539
SpPkl1    KT.TMSS.-DGMI......I.N....L.EKGW.Y...GQF.EIYNE.I.DLL..A..L..  639

MoKin14   -----.KL.I.HDE.R..TT..N...V.L..PE.VE..L..A..NRSVAATKANERSSRS  756
AnKlpA    -----KKLEIRHD..R..TTIT..T.V.L.SPEMVE..LK.A..NRSVAATKANERSSRS  633
SpKlp2    ..---KKLEI.HD.K..RTTITN.T...L..PE.V...L..A.KNRSVAAT.ANE.SSRS  684
ScKar3    ......K.EIRHD.....TTITNVT...L.S.EMVE..LK.A.K.RS.A.T..NE.SSRS  599
SpPkl1    -----.K..I.HDEK..RTT..NV..........V...L..A..NR..AATKANERSSRS  694

MoKin14   HSVFML.L.G.NS.TGERCEGTLNLVDLAGSERL.HSQA.GDR.RETQ.INKSLSCLGDV  816
AnKlpA    HS.F.L.L.G.N..TGER.EGTLNLVDLAGSERLSHS.A.GDRL.ETQ.IN.SLSCLGDV  693
SpKlp2    HSVFML.L.G.NS.TGE.C..TLNL.DLAGSERLS.SQ.VG.RL.ETQ.INKSLSCLGDV  744
ScKar3    HS.F...L.G.N..TG....GTLNLVDLAGSER...SQ.VGDRLRETQ.INKSLSCLGDV  659
SpPkl1    H.VFML...G.NS.T...C.GTLNLVDLAGSERLS.SQAVGDRLRETQ.INKSLSCLGDV  754

MoKin14   I.ALG...--.....H.PYRNSKLT.LLQ.SLGGNSKTLMFV.VSPL..HL.ET..SLR  874
AnKlpA    I.ALGQ-----GK...HIPYRNSKLTYLLQ.SLGGNSKTLMFV.VSPL..HL.ETL.SL.  748
SpKlp2    IHALG.-----GKE...IPYRNSKLT.LLQYSLGGNSKTLMFVN.SPLK.H..ETL.SLR  799
ScKar3    IHALGQ----.....HIP.RNSKLTYLLQYSL.G.SKTLMFVN.SP...H..ETL.SLR  715
SpPkl1    IHALG.......KE..HIPYRNSKLTYLL.YSLG...KTLMFVNVSPLK.....TL.SLR  814

MoKin14   FATKV.NTHIGTAK.......-                                       895
AnKlpA    FATKV.NTHIGTAKK......V                                       770
SpKlp2    FATKVNNT.IGTA.K...----                                       817
ScKar3    FA.KVN.T.....K--------                                       729
SpPkl1    FATKVN.T..G..K....----                                       832
```

Fig.5.S6. MoKin14 is a conserved kinesin-14. (A) Schematic of MoKin14 protein structure. A PFAM kinesin motor domain (PF00225) is predicted at positions 554 to 881. (B) Protein sequence alignment of MoKin14 and kinesin-14 in *Aspergillus nidulans* (AnKlpA; AN6340). Dashes represent indels, and dots represent mismatched amino acids. Green box corresponds to predicted kinesin motor domain in Fig. 5.S6A. (C) Protein sequence alignment of MoKin14 to select kinesin-14 proteins in other fungi including *A. nidulans* (AnKlpA), *Schizosaccharomyces pombe* (SpKlp2, ScPkl1), and *Saccharomyces cerevisiae* (ScKar3). Other kinesin homologs besides MoKin5 and MoKin14 likely exist in *M. oryzae* based on BlastP searches of the non-redundant protein database, however, these proteins are not yet characterized.

Supplemental Table 5.1. Fungal strains used in this study. Most fluorescent protein fusions are C-terminal fusions. Hyg^R cassette confers resistance to Hygromycin. $NTPII^R$ cassette confers resistance to G418 sulfate (G418). $Nat1^R$ cassette confers resistance to nourseothricin (NTC). Promoter is abbreviated as p. Coding sequence abbreviated as CDS, terminator region abbreviated as T.

Name(s)	Purpose	Description	Reference
CKF558 O-137	Recipient strain	Field isolate from rice in China	Orbach et al., 2000
CKF193	Recipient strain	Transformant generated in 0-137 background expressing histone H1 fused to EGFP under control of the constitutive *M. oryzae* ribosomal protein 27 (RP27) promoter into binary vector pBV229 pBV229 Hyg^R	
CKF3545	Recipient strain	Transformant generated in 0-137 background expressing β-tubulin (Bml) at the 5' end of GFP under control of *Neurospora* ccg-1 promoter from pMF309 (Freitag et al., 2004) in binary vector pBHt2 pCK1722 $G418^R$	This study
CKF3578	Fig.5.1 Fig.5.4 Fig.5.5 Fig.5.9 Fig.5.S3 Fig.5.S4	Transformant generated in 0-137 background expressing: (1) NLS fused to tdTomato under control of the constitutive RP27 promoter in binary vector pBGt (pCK1528) (2) histone H1 fused to tdTomato under control of the constitutive RP27 promoter and β-tubulin (Bml) at the 5' end of GFP under control of *Neurospora* ccg-1 promoter from pMF309 in binary vector pBHt2 pCK1528, pCK1728 $G418^R$, Hyg^R	Pfeifer et al., 2019
CKF3971	Fig.5.5 Fig.5.S3	Transformant generated in 0-137 background expressing histone H1 fused to tdTomato under control of the constitutive RP27 promoter into binary vector pCK1806 pCK2001 NTC^R	This study

CKF4093	Fig.5.5 Fig.5.S3 Fig.5.S4	Transformant generated in CKF3971 background containing an overexpression plasmid (pCK2060) made by cloning Bas4 promoter (from pCK1454) to the MoKin14 CDS (MGG_05350) and to the 3' MoKin14 T into binary vector pBV1 pCK2060 NTC^R, Hyg^R	This study
CKF4106	Fig.5.4 Fig.5.5 Fig.5.9 Fig.5.S3 Fig.5.S4	Transformant generated in CKF3578 background containing an overexpression plasmid (pCK2081) made by cloning Bas4 promoter (from pCK1454) to the MoKin14 CDS (MGG_05350) and to the 3' MoKin14 T into binary vector pCK1806 pCK1454, pCK2081 $G418^R$, Hyg^R, NTC^R	This study
CKF4108	Fig.5.4 Fig.5.5 Fig.5.6 Fig.5.7 Fig.5.9 Fig.5.S3 Fig.5.S4	Transformant generated in CKF3578 background containing an overexpression plasmid (pCK2082) made by cloning Bas4 promoter (from pCK1454) to the MoKin5 CDS (MGG_01175) and to the 3' MoKin5 T into binary vector pCK1806 pCK1454, pCK2082 $G418^R$, Hyg^R, NTC^R	This study
CKF4117	Fig.5.S2	Transformant generated in CKF3545 background expressing γ-tubulin fused to tdTomato under control of the native γ-tubulin promoter into binary vector pCK1806 pCK2086 $G418^R$, NTC^R	This study
CKF4168	Fig.5.2 Fig.5.3 Fig.5.10 Fig.5.S2	Transformant generated in CKF3545 background expressing: MoKin5 CDS (MGG_01175) fused to tdTomato under control of the MoKin5 native promoter (pCK2099) into binary vector pCK1806 pCK1722, pCK2099 $G418^R$, NTC^R	This study

CKF4182	Fig.5.10	Transformant generated in CKF4168 background containing an overexpression plasmid (pCK2060) made by cloning Bas4 promoter (from pCK1454) to the MoKin14 CDS (MGG_05350) and to the 3' MoKin14 T into binary vector pBV1 pCK2060 $G418^R$, NTC^R, Hyg^R	This study
CKF4203	Fig.5.8 Fig.5.S5	Transformant generated in CKF3545 background containing an overexpression plasmid (pCK2108) made by cloning Bas4 promoter (from pCK1454) to the MoKin5 CDS (MGG_01175) and to the 3' MoKin5 T into binary vector pBV1 pCK1722, pCK2108 $G418^R$, Hyg^R	This study
CKF4208	Fig.5.2 Fig.5.3	Transformant generated in CKF193 background expressing MoKin5 CDS (MGG_01175) fused to tdTomato under control of the MoKin5 native promoter (pCK2099) into binary vector pCK1806 pCK2099 Hyg^R, NTC^R	This study

Supplemental Table 5.2. Primers used in this study.

Primer Description		Sequence[a] (5' → 3')	Length; Tm[b]
Cloning			
MoKin5 (MGG_01175)			
	F: CKP767M	GA AGA TCT ATG AGC TCC TTG CGG GAG	26 nt; 57°C → 60°C
	R: CKP768M	CCC AAG CTT GTT AGC GTT GAG CCA TGG	27 nt; 54°C → 60°C
	F: CKP803M	AA CAA TTG GGA CAA CTT GTC CTG GCC	26 nt; 57°C → 62°C
	R: CKP804M	AA ACT AGT CCT GAG CCT GGG GCT CTT C	27 nt; 60°C → 63°C
MoKin14 (MGG_05350)			
	F: CKP733M	AAA GGA TCC ATG AAA TCC TCA TGC ACC	27 nt; 50°C → 60°C
	R: CKP734M	GGG TCT AGA GAT CCA GTG CTC ATC AAG	27 nt; 50°C → 60°C
Mo-γ-tubulin (MGG_00961)			
	F: CKP791M	GC TCT AGA CGA AGC CGT GTG TTT CAG TG	28 nt; 57°C → 63°C
	R: CKP792M	AA GGA TCC CCC CAT TCT CCG ATC CGT C	27nt; 58°C → 65°C
Bas4 promoter (MGG_10914)			
	F: CKP110	GAA TTC GGT AGC TTC TAC GGA TGC	24 nt; 53°C → 58°C
	R: CKP234	GGA TCC CAT TGT GAA AAG ATT CGT TGT GG	29 nt; 53°C → 60°C
Primers used for RT-qPCR assays[c]			
Actin (MGG_03982)			
	F: CKP333	CGA CGT CCG AAA GGA TCT GT	20 nt; 57°C
	R: CKP334	TGC ATA CGG TCC GAA AGA CC	20 nt; 57°C
MoKin5			
	F: CKP873M	GTT GAG AAG GAT GGT CGG GTT	21 nt; 60°C
	R: CKP874M	CAG ACT TGG TAG TCG CTT GGA	21 nt; 60°C
MoKin14			
	F: CKP830M	GAA GGA GAC GGT GAC GAG TT	20 nt; 59°C
	R: CKP831M	GCC GTG CCA ATA TGC GTG	18 nt; 60°C
Primers used for sequence confirmation of clones[d]			
pCK2060 pCK2081 pCK2082 pCK2108	CKP335	GAA TTC ATA CAC TTT ATG CAT TCC CCTTGCG	31 nt, 60°C

pCK2099	CKP806M	GGC TTG AGG GAG GTT AAT CC	20 nt, 55°C

[a]Underlined sequences correspond to restriction enzyme sites introduced for cloning: BamHI (GGATCC), EcoRI (GAATTC), XbaI (TCTAGA), HindIII (AAGCTT), BglII (AGATCT), MfeI (CAATTG), SpeI (ACTAGT).

[b]Melting temperature (Tm) changed after first two cycles of PCR. The first Tm is the Tm of the primer sequence without the restriction enzyme site and additional nucleotides for efficient digestion of the PCR product. The second Tm is the Tm of the entire primer. The average Tm of the forward and reverse primer was used.

[c]One to four primer pairs were tested before use in RT-qPCRs for specificity to intended target. Only primers amplifying a single band of expected size from wild-type (CKF558) complementary DNA and no bands from rice (YT16) complementary DNA were used in gene expression analysis.

[d]Listed primers were designed for sequencing of clones. Plasmids not listed here were confirmed with other previously described cloning primers in combination with restriction enzyme digests.

Supplemental Table 5.3. Plasmids used in this study. Promoter is abbreviated as p. Coding sequence abbreviated as CDS, terminator region abbreviated as T. Bold font indicates plasmids listed in Supplemental Table 5.1. PCR products are described in Supplemental Table 5.4.

Plasmid name(s)	Description	Reference	Available from Addgene
pBV1 pBHt2	Hyg^R binary vector for *Agrobacterium*- mediated transformation (ATMT), pBHt2 $Kanamycin^R$, $Hygromycin^R$ pBHt2 (Plasmid #104175; pBV1)	Mullins et al., 2001	https://www.addgene.org/104175/
pBV108 pGKO2	Knockout binary vector for ATMT. Carries positive selection marker (HygR) and negative selection marker, a herpes simplex virus thymidine kinase gene (HSVtk). $Kanamycin^R$, $Hygromycin^R$	Khang et al., 2005	https://www.addgene.org/Seogchan_Kang/
pBV141 pBGt	$NTPII^R$ binary vector for ATMT $Kanamycin^R$, $G418^R$	Kim et al., 2011	
pBV229	For expression of RP27-histone H1-EGFP. Derived from pBV126 (P27-EGFP) and pBV202 (hH1). $Kanamycin^R$, $Hygromycin^R$	Shipman et al., 2017	
pCK1282 pCK1283	For isolation/ligation of Bas4 promoter (Bas4p).1 Kb EcoRI-BamHI fragment generated by PCR of pCK1271 with primers CKP110 & CKP234. $Carbenicillin^R$	This study	
pCK1454	For isolation/ligation of Bas4p. Derived from pCK1349, pCK1282, and pCK1305. $Kanamycin^R$, $G418^R$	This study	

pCK1528	For expression of NTPII^R-RP27-tdTomato-Nuclear Localization Signal (NLS).1 Kb EcoRI-BamHI of pBV126 (NTPII^R):1.4Kb BamH1-BsrGI of pBV359 (RP27 promoter): 10.4Kb BsrG1-HindIII of pBV578 (3XNLS-tdTomato) into EcoR1-HindIII sites of pBV141 (binary vector). Kanamycin^R, G418^R	Pfeifer et al., 2019	Will be made available
pCK1722	For expression of NTPII^R-ccg1-Bml (β-tubulin)-EGFP. Derived from pCK1718 and pCK1305. Kanamycin^R, G418^R	Freitag et al., 2004	Will be made available
pCK1728	For expression of Hyg^R-RP27-histone h1-tdTomato-Ter-ccg1- Bml (β-tubulin)-EGFP. Derived from pCK1287 and pCK1718. Kanamycin^R, Hygromycin^R	Pfeifer et al., 2019	Will be made available
pCK1806	Nat1^R binary vector for ATMT. 0.9Kb Xho1-EcoR1 of pCK796 (TrpC promoter: Nat1^R) into Xho1-EcoRI sites of pBV141 (binary vector). Kanamycin^R, Nourseothricin^R	Original plasmid (pCK796) containing Nat1 construct was a gift from Ane Sesma.	
pCK2001	For expression of Nat1^R-RP27-histone H1-tdTomato. 2.2 Kb EcoRI-BamHI fragment of pBV229 (RP27-histone H1): 1 Kb BamHI-HindIII fragment of pCK1292 (tdTomato) into EcoRI-HindIII sites of pCK1806 (binary vector). Kanamycin^R, Nourseothricin^R	This study	Will be made available

pCK2060	For expression of Hyg^R-Bas4p-MoKin14 CDS & 3'T. 1 Kb EcoRI-BamHI fragment derived from pCK1283 (Bas4 promoter): 2.8 Kb BamHI-XbaI fragment generated by PCR of CKF558 genomic DNA with primers CKP733M & CKP734M into EcoRI-XbaI sites of pBV1 (binary vector). Kanamycin^R, Hygromycin^R	This study	Will be made available
pCK2081	For expression of Nat1^R-Bas4p-MoKin14 CDS & 3'T. 1 Kb EcoRI-BamH1 fragment of pCK1454 (Bas4 promoter): 2.8 Kb BamH1-Xba1 fragment generated by PCR amplification of CKF558 genomic DNA with primers CKP733M & CKP734M (MGG_05350) into EcoRI-XbaI sites of pCK1806 (binary vector). Kanamycin^R, Nourseothricin^R	This study	Will be made available
pCK2082	For expression of Nat1^R-Bas4p-MoKin5 CDS & 3' T. 1 Kb EcoRI-BamH1 fragment of pCK1454 (Bas4 promoter):4.1 Kb BglII-HindIII fragment generated by PCR amplification of CKF558 genomic DNA with primers CKP767M & CKP768M into EcoRI-HindIII sites of pCK1806 (binary vector). Kanamycin^R, Nourseothricin^R	This study	Will be made available
pCK2086	For expression of Nat1^R-Native promoter + γ-tublin CDS-TdTomato. 2.3 Kb XbaI-BamHI fragment generated by PCR of CKF 558 genomic DNA with primers CKP791M & CKP792M: 1.7 kB BamHI-HindIII of pCK1292 (tdTomato) into XbaI-HindIII sites of pCK1806 (binary vector). Kanamycin^R, Nourseothricin^R	This study	

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CHAPTER 6

SPEAKING UP: A MODEL OF SELF-ADVOCACY FOR STEM UNDERGRADUATES WITH ADHD AND/OR SPECIFIC LEARNING DISABILITIES[1]

[1] Mariel A. Pfeifer, Eve Melanie Reiter, McKenna Hendrickson and Julie Dangremond Stanton. 2020. *International Journal of STEM Education* 7, 33. https://doi.org/10.1186/s40594-020-00233-4. Reprinted here with permission of the publisher.

Abstract

Background. Students with disabilities are underrepresented in undergraduate science, technology, engineering, and mathematics (STEM) courses. Students with disabilities who engage in self-advocacy earn higher GPAs and are more likely to graduate from college compared to students with disabilities who do not engage in self-advocacy. We utilized Test's conceptual framework of self-advocacy, which breaks self-advocacy into four components: knowledge of self, knowledge of rights, communication, and leadership to investigate how students with invisible disabilities practice self-advocacy in undergraduate STEM courses. Through a partnership with a disability resource center (DRC), we recruited and interviewed 25 STEM majors who received accommodations for attention-deficit/ hyperactivity disorder (ADHD) and/or a specific learning disorder (SLD). Data were collected using semi-structured interviews and analyzed using content analysis.

Results. We found evidence of all components of Test's conceptual framework of self-advocacy and operationalize each based on our participants' experiences. We identified novel components of self-advocacy for students with ADHD/SLD in undergraduate STEM courses, including knowledge of STEM learning contexts and knowledge of accommodations and the process to obtain them, as well as, a novel self-advocacy behavior, filling gaps. Filling gaps involved participants taking action to mitigate a perceived limitation in either their formal accommodations from the DRC or a perceived limitation in the instructional practices used in a STEM course. We also identified beliefs, such as view of disability and agency, which influenced the self-advocacy of our participants. We incorporated the emergent forms of self-advocacy into Test's conceptual framework to propose a revised model of self-advocacy for students with ADHD/SLD in undergraduate STEM courses.

Conclusions. We developed a revised conceptual model of self-advocacy for students with ADHD/SLD in undergraduate STEM courses. This conceptual model provides a foundation for researchers who wish to study selfadvocacy in undergraduate STEM courses for students with ADHD/SLD in the future. It also offers insights for STEM instructors and service providers about the self-advocacy experiences of students with ADHD/SLD in undergraduate STEM courses. We propose hypotheses for additional study based on our conceptual model of self-advocacy. Implications for research and teaching are discussed.

Introduction

Background. Students with disabilities are underrepresented in science, technology, engineering, and mathematics (STEM) majors and this underrepresentation of individuals with disabilities persists in STEM workforce settings (National Science Foundation, 2019). In college, students with disabilities encounter many challenges influencing their retention in STEM majors (Carabajal, Marshall, & Atchison, 2017; Dunn, Rabren, Taylor, & Dotson, 2012; Hong, 2015). One important challenge students with disabilities in the US face during college is a shift in legislation guiding the accommodation process (Hadley, 2007; Janiga & Costenbader, 2002). In public high schools, accommodations are guaranteed primarily through an educational law called the Individuals with Disabilities Education Act (IDEA) (Smith, 2001). Under IDEA, public schools are responsible for identifying and accommodating students with disabilities. As students with disabilities matriculate into college, IDEA no longer applies. In college, civil rights legislation, specifically the Americans with Disabilities Act (ADA) and its amendments, work in tandem with Section 504 of the Rehabilitation Act of 1973[2] to ensure access to accommodations (Eckes & Ochoa, 2005; Smith, 2001). The ADA calls colleges and universities to provide "reasonable accommodations" that do not "fundamentally alter" the nature of the academic program (Americans with Disabilities Act of 1990). Importantly, in college, students themselves become solely responsible for

[2] Section 504 of the Rehabilitation Act of 1973 applies to students with disabilities in public and private high schools (Taylor, 2005). No existing research examines if there are differences in the self-advocacy experiences of students who attend public or private high school. We hypothesize that self-advocacy is an essential skill for any student, regardless of whether they attend a public or private high school.

seeking and managing their own accommodations (Hadley, 2007; Janiga & Costenbader, 2002). Thus, as students with disabilities begin college, many are learning not only how to navigate life as a college student, but also how to navigate the academic accommodation process on their own for the first time. Successful navigation of the accommodation process in college requires self-advocacy (Hadley, 2007).

Self-advocacy for students with disabilities. Self-advocacy for students with disabilities has been defined and conceptualized in many ways[3] (Gelbar et al., 2019; Test et al., 2005). One well-accepted definition of self-advocacy is the "ability to assertively state wants, needs and rights, determine and pursue needed supports, and conduct your own affairs" (Izzo & Lamb, 2002, p. 6). In studies of students with disabilities, self-advocacy emerged as a critical factor related to the success and retention of students with disabilities in college (e.g., Kinney & Eakman, 2017; Lombardi, Gerdes, & Murray, 2011). While self-advocacy is identified as an important skill that can be taught to students with disabilities, it is less clear how students with disabilities practice self-advocacy in the context of their undergraduate courses (Holzberg, Test, & Rusher, 2019; A. R. Walker & Test, 2011). Few studies describe how students with disabilities practice self-advocacy in their day-to-day lives as college students, and little research exists investigating how students with disabilities practice self-advocacy in undergraduate STEM courses.

[3] Self-advocacy has been conceptualized as an educational goal, a political movement, and as a component of the broader theoretical framework self-determination for students with disabilities (Gelbar et al., 2019; Test, Fowler, Wood, Brewer, & Eddy, 2005; Ward & Meyer, 1999; Wehmeyer, Abery, Mithaug, & Stancliffe, 2003).

A conceptual framework for self-advocacy for individuals with disabilities. Fortunately, a conceptual framework of self-advocacy exists. Test et al. (2005) generated a conceptual framework of self-advocacy through a meta-analysis of 20 research studies of individuals with disabilities, along with input from stakeholders. Stakeholder feedback on working drafts of Test's conceptual framework consisted of responses from seven individuals, including two adults with disabilities known to be active self-advocates, three researchers in the field, and two adult self-advocacy training organizations. In this conceptual framework, self-advocacy contains four components: knowledge of self, knowledge of rights, communication, and leadership (Test et al., 2005). Knowledge of self is awareness of one's own strengths and weaknesses as a student and as an individual with a disability. Knowledge of rights is awareness of relevant federal laws and policies that govern the accommodation process for college students with disabilities. Communication entails behaviors that ensure successful communication about accommodations. An example of successful communication involves engaging in assertive, yet not aggressive, communication with instructors and service providers regarding accommodations and accommodation-related issues. Notably, knowledge of self, knowledge of rights, and communication are considered to be essential for self-advocacy in Test's conceptual framework, whereas leadership is not considered to be essential for self-advocacy. Leadership is broadly defined in Test's conceptual framework and encompasses many subcomponents, ranging from an awareness of individual roles and responsibilities within a group during accommodation meetings to taking political action on behalf of other people with disabilities.

The research that informed development of Test's conceptual framework of self-advocacy involved studies of individuals that ranged greatly in terms of age, disability type, and context (Test et al., 2005). While development of the conceptual framework represented a key step in the delineation of self-advocacy from the broader theoretical

framework of self-determination, few, if any subsequent studies, have empirically tested if and how Test's conceptual framework of self-advocacy applies to subpopulations of individuals with disabilities. For example, the lived experiences of a college student with an apparent disability, such as a visual impairment, can be much different than the lived experiences of a college student with an invisible or hidden disability, such as attention-deficit/hyperactivity disorder (ADHD) (Daly-Cano, Vaccaro, & Newman, 2015; Vaccaro, Kimball, Wells, & Ostiguy, 2015). Given the importance of self-advocacy in the success and retention of students with disabilities in college, we sought to understand how Test's conceptual framework of self-advocacy applied to STEM majors with disabilities.

Self-advocacy in the context of undergraduate STEM. Although self-advocacy is recognized as a critical determinant in academic success for students with disabilities, very few, if any, existing studies examine how students with disabilities engage in self-advocacy within specific academic disciplines (Fleming, Plotner, & Oertle, 2017; Holzberg et al., 2019; Kinney & Eakman, 2017; Lombardi et al., 2011). Within higher education research, studies about students with disabilities are uncommon (Peña, 2014). Similarly, studies about students with disabilities are uncommon within undergraduate STEM education research (e.g. Schreffler, Vasquez Iii, Chini, & James, 2019; Thurston, Shuman, Middendorf, & Johnson, 2017). Students with disabilities encounter unique challenges in undergraduate STEM courses (Moon, Todd, Morton, & Ivey, 2012). For example, the use of ambiguous language in chemistry and other STEM contexts can impede learning for students with certain types of disabilities (Isaacson & Michaels, 2015). Students with disabilities in STEM are less likely than their counterparts in other academic disciplines to use accommodations in their courses (Lee, 2011; Lee, 2014). The reasons fewer students with disabilities in undergraduate STEM courses use accommodations are not well characterized. We hypothesize this phenomenon is related to self-advocacy. We sought to study self-advocacy in the context of undergraduate

STEM courses to define what encompasses self-advocacy for students with disabilities in this academic context. We were particularly interested in how students with two types of invisible disabilities[4], ADHD and specific learning disorders, also called specific learning disabilities (SLD), practiced self-advocacy. We were interested in how students with ADHD/SLD practice self-advocacy because the invisible, or non-apparent, nature of these disabilities requires students to disclose their disability status in order to receive accommodations. For instance, a STEM instructor would not necessarily be able to tell based on a student's outward appearance if they had a disability or if they needed accommodations in a course, whereas a student with an apparent disability may be more readily identified as an individual who may need accommodations in a course. In the following section, we explain the rationale for our decision to aggregate multiple disability types into one study by defining ADHD, SLD, and briefly outline documented similarities and differences between students with ADHD/SLD (Vaccaro et al., 2015).

ADHD and SLD. Two of the most commonly occurring invisible disabilities on college campuses are ADHD and SLD (Raue & Lewis, 2011). Both ADHD and SLD are examples of neurodevelopmental disorders (American Psychology Association, 2013). ADHD is comprised of two major subtypes: predominantly inattentive and predominantly hyperactive/impulsive. The inattentive form of ADHD is characterized by challenges in maintaining focus in day-to-day life and may manifest when individuals with ADHD overlook details, do not listen when spoken to directly, or do not follow through on instructions. Individuals with the inattentive form of ADHD may also experience

[4] We elected to use the term disability throughout our study because this was the term most familiar to our participants. However, other terms such as impairment or learning difference may be the preferred term for some individuals.

challenges in organizing tasks and activities or be easily distracted by outside stimuli and unrelated thoughts. Individuals diagnosed with the hyperactive/impulsive subtype of ADHD can be described as “on the go” (American Psychiatric Association, 2013). They may experience intense feelings of restlessness that can be evident in fidgeting, excessive talking, difficulty in waiting for turns, and interrupting or intruding upon others.

SLD are divided into three major subtypes: impairment in reading (dyslexia), impairment in written expression (dysgraphia), and impairment in mathematics (dyscalculia) (American Psychiatric Association, 2013). SLD can be identified when an individual experiences difficulty in learning and using academic skills, such as reading comprehension, spelling, written expression, number sense, number facts, calculation, and mathematical reasoning. A key determinant of SLD is that the academic skill affected by an SLD is substantially below the expected level given the chronological age of the individual. Typically, SLD are diagnosed at a young age, but an individual may be diagnosed with an SLD later in life when they experience increased academic rigor.

Students with ADHD and students with SLD are often studied together because of their prevalence in college students and because these conditions often co-occur at a rate of 31–45% (DuPaul, Gormley, & Laracy, 2013; Pham & Riviere, 2015; Raue & Lewis, 2011; Wolf, 2001). SLD and ADHD also share cognitive factors such as impaired processing speed and working memory (Budd, Fichten, Jorgensen, Havel, & Flanagan, 2016; Costello & Stone, 2012). Additionally, students with ADHD and students with SLD tend to show similar disparities compared to students without disabilities in terms of motivation, anxiety, information processing, and monitoring understanding (Reaser, Prevatt, Petscher, & Proctor, 2007). Despite commonalities between students with ADHD and students with SLD, researchers have documented very few differences between students with ADHD and students with SLD. For example, one study found that students with only ADHD self-report lower grades and lower course-related self-efficacy

than students with only SLD, but higher confidence to read textbooks compared to students with only SLD (Budd et al., 2016). Although differences between students with ADHD and SLD exist, we found that including both disability types in one study was appropriate considering the purpose of our study was to investigate self-advocacy in students with two common invisible disabilities on college campuses (Vaccaro et al., 2015).

Guiding theoretical framework. We are broadly guided by the social model of disability. The social model of disability distinguishes impairment from disability (Berghs, Atkin, Graham, Hatton, & Thomas, 2016; Haegele & Hodge, 2016). Impairments are biological differences, and disability is the hardship an individual with an impairment experiences due to societal expectations (Berghs et al., 2016; Haegele & Hodge, 2016). For example, blindness resulting from macular degeneration is a form of visual impairment. If we consider an individual with a visual impairment in an elevator without Braille numbers on the call buttons navigating to a specific floor, we would say that the individual has a biological difference, their visual impairment, but they are not disabled because of their impairment. Instead, they are disabled because the elevator was not designed for people with visual impairments. From the social model standpoint, disabilities are addressed through political and social change (Berghs et al., 2016; Haegele & Hodge, 2016). For example, disability could be addressed by adopting policy mandating all call buttons include corresponding Braille numbers. The social model of disability prompts individuals to enact political and social change to address disability, and this notion translates into educational contexts. We find the social model of disability is appropriate for our study because it calls individuals with impairments to take action to improve their own conditions within society. We consider self-advocacy to be the construct that empowers individuals to take these types of actions. For instance, STEM majors with ADHD/SLD can practice self-advocacy to ensure access to academic

accommodations, which may improve their own condition within a microcosm of society, the undergraduate STEM classroom.

The purpose of our study is to characterize the self-advocacy experiences of students with ADHD/SLD in undergraduate students. We utilized Test's conceptual framework of self-advocacy (Test et al., 2005) that outlined four components of self-advocacy (Figure 6.1.) to begin addressing our primary research question. We asked: What components of self-advocacy are evident in students with ADHD/SLD in undergraduate STEM courses?

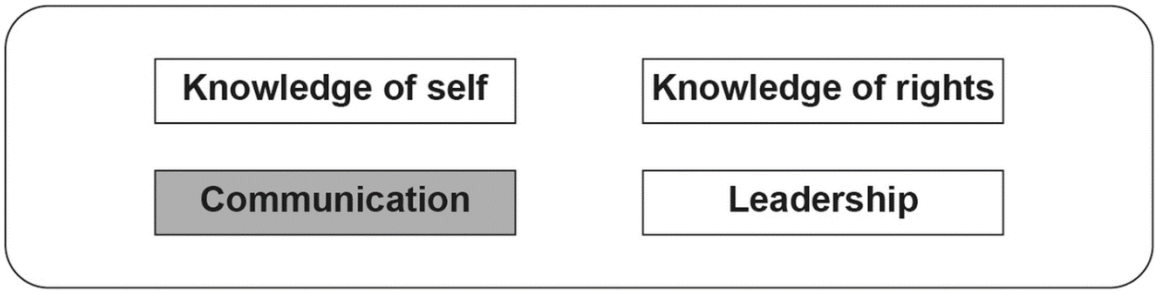

Figure 6.1. Test's conceptual model of self-advocacy (Test et al., 2005). Knowledge of self and knowledge of rights are foundational, communication is described as essential (shaded box), and leadership is seen as non-essential for self-advocacy. We refer to these components of self-advocacy as "Test's components" or "original components" of self-advocacy.

Methods

Context of study. We conducted our study at a large public university with the highest research activity in the southeastern USA. This study was approved for exempt status by the University of Georgia Institutional Review Board (STUDY00004663) and is part of a larger study of students with ADHD and SLD in undergraduate STEM courses. All participants in the study were registered with the campus Disability Resource Center (DRC) and all participants were STEM majors. Most of the participants were actively using academic accommodations at the time of the study. However, one participant had

not used accommodations for several semesters, and one participant had never formally used accommodations in college.

Participant identification and recruitment. We established a partnership with our institution's Disability Resource Center (DRC) to recruit students currently registered to receive academic accommodations. Our partners at the DRC distributed a recruitment email to all students meeting the criteria of the study to ensure confidentiality of registered students. Our recruitment method also preserved student confidentiality because only those students interested in participating in the research study contacted us. Eligibility requirements included that the participant must (1) be currently registered with the DRC and eligible to receive accommodations for either ADHD or SLD as a primary or secondary condition and (2) have completed at least one course which fulfilled the science and quantitative reasoning core curriculum requirement. A round of recruitment emails was sent by our DRC partner to all eligible students in Fall 2018 and again in Spring 2019. Our recruitment email included standard recruitment language and a video with closed-captions to provide multiple means of representation to our potential participants (CAST, 2020). In Spring 2019, we also advertised the study by hanging flyers at the DRC with our contact information. Students interested in participating in the study were invited to contact us directly.

Once initial contact with potential participants was established, we sent a brief screening survey to the student to ensure that each participant had completed at least one undergraduate STEM course at the institution where data collection took place. We then invited participants to schedule an interview at their convenience. Participation was incentivized by providing $20 cash for completion of one interview. We recruited 13 participants in Fall 2018 and 12 participants in Spring 2019. All participants provided written informed consent.

Development of the interview protocol. We conducted semi-structured interviews to characterize the self-advocacy experiences of students with ADHD and/or specific learning disabilities (SLD) in undergraduate STEM courses. Semi-structured interviews utilize a formal interview protocol, but researchers are able to ask follow-up questions as needed to elicit rich detail from participants. Development of our interview protocol was informed by Test's framework of self-advocacy (Test et al., 2005), along with other previous research regarding the experiences of students with learning disabilities and ADHD in college (e.g., Hadley, 2007). An initial interview protocol was piloted with three students with SLD and one student with a traumatic brain injury. Refinements to the wording and order of interview questions were made based on the results of the pilot study and feedback from our DRC partners. The final interview protocol contained two major sections. The first section was designed to characterize the self-advocacy experiences of students with ADHD/SLD in undergraduate STEM courses and the second section was designed to explore the role active learning in undergraduate STEM courses plays on self-advocacy, the results of which will be published separately. Interview questions related to the current study are available in Supplementary File 6.1.

Data collection and survey. One researcher interviewed all 25 participants using the final interview protocol. The average length of an interview was 80 min. At the end of each interview, participants completed a short demographic survey. Demographic information of our participants is summarized in Table 6.1. Each interview was audio-recorded. Immediately following each interview, the interviewer wrote analytic memos regarding overall impressions of self-advocacy for each participant. All interviews were professionally transcribed. Transcripts were checked to ensure fidelity of the data.

Qualitative data analysis. Data were analyzed by a diverse research team, including at least one or more researchers who were a STEM major with ADHD/SLD,

and a researcher who had worked as a DRC coordinator at another institution. We used MaxQDA 2018 (VERBI Software, 2017) for qualitative analysis. We open-coded (sometimes referred to as initial coding) all 25 transcripts (Saldaña, 2015). In our open-coding process, we sought to find nuances in our data and to remain open to emergent ideas related to self-advocacy by reading each transcript. After reading each transcript, members of the research team wrote analytic memos regarding their impressions of the data. Members of the research team met extensively throughout the open-coding process to share thoughts about the data and to discuss ideas and concepts that emerged from the open-coding process. We developed our codebook using a set of five interviews that represented the range of our data. The first four codes of our codebook were a priori (or deductive or theory-driven), originating from Test's conceptual framework of self-advocacy. These a priori codes were knowledge of self, knowledge of rights, leadership, and communication. We identified relevant segments of interviews that represented these a priori codes.

Table 6.1. Summary of participant (n = 25) demographic information.

Participants (n=25)	Number (%)
Gender	
Female	11 (44%)
Male	14 (56%)
Race	
White	23 (92%)
Black or African American	2 (8%)
STEM major	
Life Sciences	13 (52%)
Engineering	7 (28%)
Physical Science	2 (8%)
Mathematics	2 (8%)
Computer Science	1 (4%)
Year in college	
First year	3 (12%)
Second year	3 (12%)
Third year	8 (32%)
Fourth year	4 (16%)

Fifth year	5 (20%)
Sixth year +	2 (8%)
Participant diagnoses	
ADHD	15 (60%)
Specific Learning Disability	5 (20%)
ADHD and Specific Learning Disability	5 (20%)
Age at Official Diagnosis	
College	8 (32%)
Before College	17 (68%)
Type of High School Attended	
Public	14 (56%)
Private	11 (44%)
Other	
Transfer students	6 (24%)
First-generation students	2 (8%)
Pell grant recipients	5 (20%)

We relied on a constant comparative method to develop our emergent or inductive codes (Charmaz, 2006; Fram, 2013). This involved three members of the research team proposing codes to each other after reading the same set of interviews. Initially, our research team generated over 100 possible proposed codes. Given the large number of proposed codes, we met to discuss these proposed codes and to come to an agreement on the codes that aligned with our research questions. We sought to refine our proposed codes by reading additional interviews individually and meeting as a research team to add or remove codes and to redefine existing codes as needed. We used the most current iteration of the codebook to code one interview individually and then meet as a research team to discuss how each individual applied the codes. Through this process, our codebook stabilized. Codes related to this study are provided in Supplemental File 6.2. Using our stabilized codebook, two researchers coded all 25 interviews, meeting after intervals of three to four interviews to discuss coding, and to code to consensus. This involved resolving any coding disagreements by discussing the code and the data until an agreement was reached. Subsequently, a third researcher

coded all 25 interviews to give insights as a person who was a STEM major with ADHD/SLD. One researcher involved in the analysis from the beginning then discussed coding with the third coder. From this iterative process, all first-cycle codes applied were reviewed and approved by at least two members of our research team.

In our analysis, we elected to code to consensus in an effort to attain reliability and validity. In qualitative research, reliability is the dependability of the research, while validity addresses the degree to which the findings are trustworthy and defensible (Golafshani, 2003; Lincoln & Guba, 1985). Coding to consensus is considered one of the most rigorous analytic strategies by many qualitative researchers, especially when analyzing complex phenomenon, such as self-advocacy, or when using intricate codebooks (Curry, Nembhard, & Bradley, 2009; Foster, Urquhart, & Turner, 2008; Morse, 1997; Richards & Hemphill, 2018). Coding to consensus by a diverse research team brings “richness to data interpretation” (Olson, McAllister, Grinnell, Gehrke Walters, & Appunn, 2016, p. 30). When coding to consensus, differences between researchers are acknowledged, discussed, and resolved, thereby accounting for diverse viewpoints in the output of the analytic process. Moreover, studies show that calculated measures of interrater reliability may actually function to reduce reliability and validity of a qualitative study in practice (Eisner, 1991; Sandelowski & Barroso, 2003). In these situations, researchers find themselves making coding decisions in an effort to agree with one another, instead of considering the actuality of the data. In our view, coding to consensus as opposed to calculating a measure of interrater reliability was an appropriate decision given our participants, the construct of self-advocacy, and our study design.

We transitioned to second-cycle coding by conducting axial and pattern coding to identify emergent themes from our analysis. Axial coding involves describing the properties and dimensions of a code and determining how these attributes relate to one

another, while pattern coding organizes similarly coded data into themes (Saldaña, 2015). Second-cycle coding was headed by one researcher with input from two additional members of the research team. If disagreements regarding second-cycle codes emerged, we discussed differences until members of the research team agreed. From this process, we identified emergent themes related to self-advocacy of our participants in the context of undergraduate STEM courses. We incorporated these themes into a model that included the four components of Test's conceptual framework.

Trustworthiness of study. Our study establishes trustworthiness in several ways (Krefting, 1991; Tracy, 2010). We provide transparency in our research by describing our methods in detail. Our study design and interview protocol were reviewed by DRC coordinators and staff with extensive experience working with the target population of our study. We also provide rationalization for aggregating students with ADHD and/or SLD into a single study (Vaccaro et al., 2015). Furthermore, our interview protocol was piloted and refined based on feedback from students with similar disabilities to our participants. We formed a diverse research team to analyze our data by coding to consensus. Our research team included one or more researchers who were STEM majors with ADHD/SLD, and a researcher who had worked as a DRC coordinator at another institution (Vaccaro et al., 2015).

Results

Test's conceptual framework of self-advocacy outlined four components of self-advocacy: knowledge of self, knowledge of rights, communication, and leadership (Test et al., 2005; Figure 6.1). We first asked: *What components of Test's self-advocacy framework are evident in students with ADHD and/or SLD in undergraduate STEM courses?* We found evidence of each component of self-advocacy from Test's framework. Besides these components of the framework, we identified emergent components of self-advocacy based on the experiences of our participants. From our

analysis, we generated a model of self-advocacy for students with ADHD/SLD in the context of undergraduate STEM courses based on the experiences of our participants (Figure 6.2). In the following sections, we describe self-advocacy knowledge, self-advocacy behaviors, and beliefs influencing self-advocacy. We use headers to differentiate components of Test's framework from the emergent components of our analysis. Although the components sometimes overlapped and intersected within the data, we characterize each self-advocacy component separately for clarity.

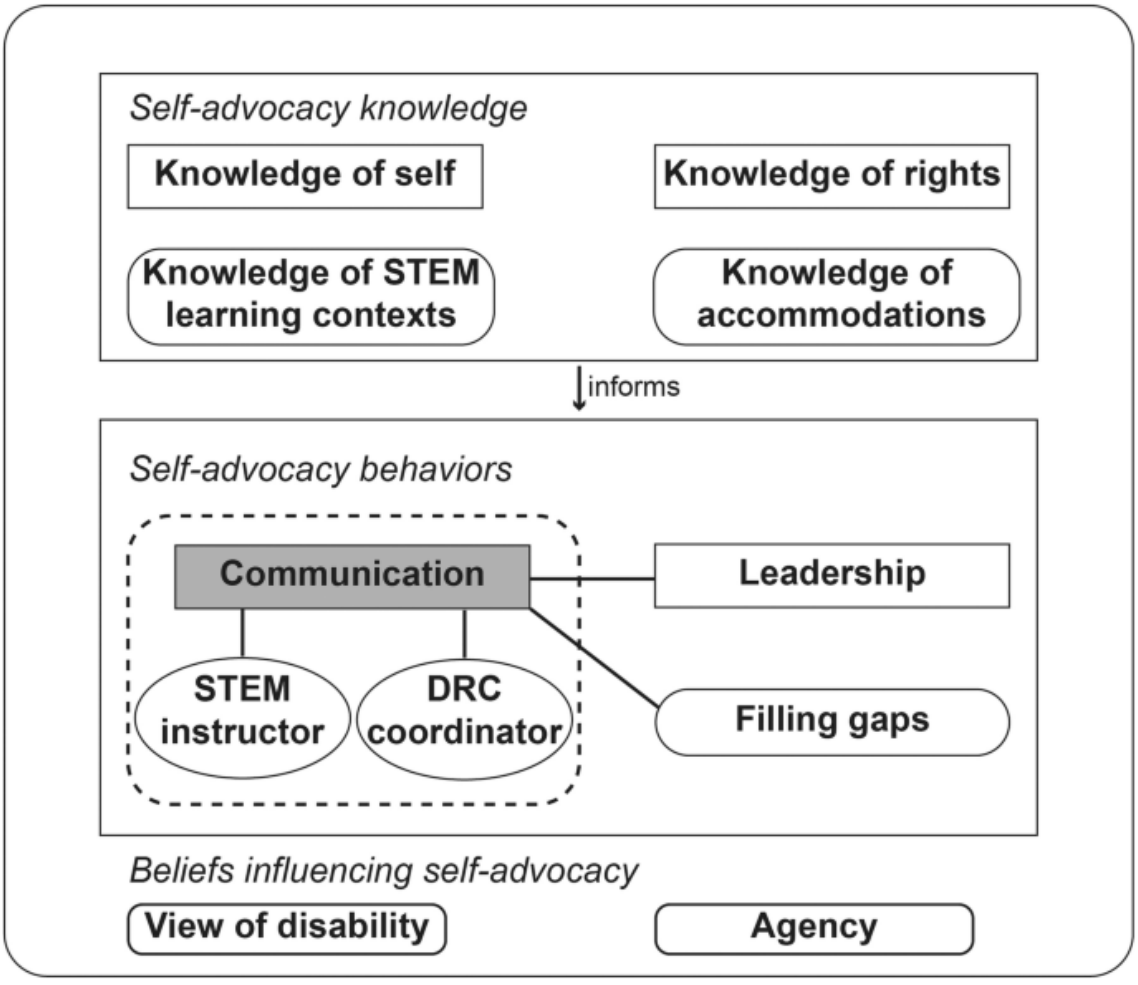

Figure 6.2. Proposed model of self-advocacy for students with ADHD and/or SLD in the context of undergraduate STEM courses, based on our participants' experiences. Square-edged boxes represent components of Test's conceptual framework (Test et al., 2005). Rounded-edged boxes represent emergent themes from our qualitative analysis. Ovals represent individuals our participants interacted with to practice self-advocacy.

Figure 6.2. (continued) The shaded box represents a required component of self-advocacy. The dashed line box surrounding communication represents communication with STEM instructors and DRC coordinators. Lines connecting communication to leadership and filling gaps represent the integral role of communication in self-advocacy, e.g., communication is required for leadership and filling gaps. Components of self-advocacy can overlap due to their intersecting nature.

Participant quote data were lightly edited for clarity. For example, brackets indicate words we edited for readability, and ellipses represent portions of the interview we excluded for conciseness. All names are pseudonyms.

Overview of accommodation process for our participants. One strength of qualitative research is that it allows researchers to develop a detailed understanding of phenomenon situated in a specific context. We provide an overview of the steps involved in the accommodation process for our participants to contextualize their experiences requesting accommodations in their undergraduate STEM courses (Figure 6.3). Participants formally register with the campus DRC by providing documentation of their disability. This documentation is reviewed and once it is approved, participants are officially registered with the DRC. Participants are then invited to make an initial accommodation meeting with their assigned DRC coordinator. In this meeting, the participant and their DRC coordinator agree upon what accommodations the participant will request from their instructors for that semester. The formal accommodation letters are then generated in an online accommodation system and sent to the instructors of each course. The accommodation letters disclose the name of the participant and the type of accommodation(s) the participant requests in a course. No additional information about the participant's disability is disclosed to the instructor in the letters. The instructor acknowledges and approves the participant's accommodations through the online accommodation system. Once approved, the participant can then manage their accommodations through the online accommodation system. Participants are only

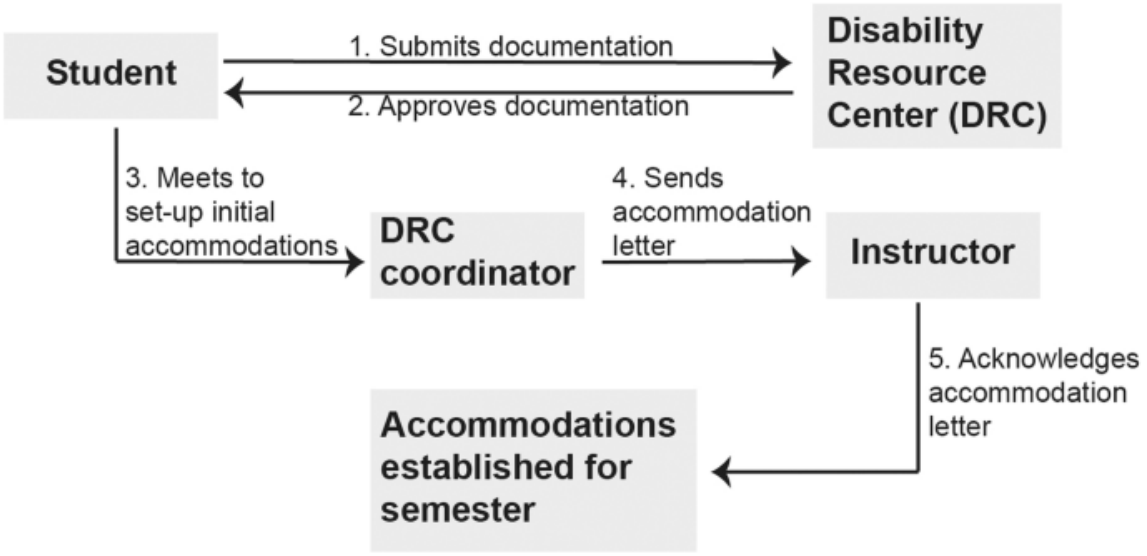

Figure 6.3. Overview of the accommodation process our participants experienced to initially establish and use accommodations in undergraduate STEM courses. (1) Students with qualifying disabilities submit official documentation to the campus Disability Resource Center (DRC). (2) The DRC reviews and approves the documentation, and subsequently (3) the student can schedule an initial accommodation meeting with their assigned DRC coordinator. During this initial meeting, the student and their DRC coordinator agree on the accommodations the student will request in each of their courses, and (4) the DRC sends the instructors of these courses an official accommodation letter through the online accommodation system. (5) The instructor receives and acknowledges the accommodation letter, and the student's accommodations are established for the semester.

required to meet one time with their DRC coordinator throughout their college career because their approved accommodations roll-over each semester. For example, once approved for 1.5× extended-time exams, the participant can select this accommodation for all of their classes in a new semester without meeting each semester with their DRC coordinator. Conversely, any changes to a participant's accommodations require communication with their DRC coordinator.

Self-advocacy knowledge. ***Test's component: Knowledge of self.*** Knowledge of self was defined as the awareness of individual strengths and weaknesses as a learner with a disability (Test et al., 2005). All our participants demonstrated knowledge

of self by describing their strengths and weaknesses while in undergraduate STEM courses. For example, our participants detailed their strengths in math and science. Oakley stated, “I know that a lot of people, [especially] with…dyslexia, they sometimes struggle with math… But I happen to be better at math.” Claudia also identified her strength in math explaining, “I always have been naturally better at math as opposed to English.” One way our participants realized their strengths in math and science was through their previous success in STEM in high school. These realizations served as motivation to pursue a STEM major in college.

While our participants described their strengths as STEM majors, they also outlined their weaknesses. We found the weaknesses described by our participants to be consistent with the functional limitations associated with ADHD/SLD. Participants in our study mentioned issues with focus and attention, processing speed, reading, and organizing thoughts. In this section, we include the reported disability of each participant to inform the association between the knowledge of self and the type(s) of disability(ies) reported by the participant.

Several participants described issues with focus and attention that they experience while in an undergraduate STEM course. Challenges in focus and attention are a characteristic of ADHD (American Psychiatric Association, 2013). Our participants demonstrated knowledge of self when they explained their experiences with focus and attention in the classroom. For example, Isabel shared the challenges she encounters with focus during a lecture.

> When the professor is lecturing, I need for them to repeat what they just said because I may have caught part of it, I may have been distracted and working on a problem and I’m not able to work on a problem and listen to them at the same time.—Isabel, a student with ADHD

Isabel was aware that she may miss portions of a lecture because of challenges in maintaining focus and attention throughout the class period. Another participant, Opal

detailed how her strong desire not to miss any of the lecture affects her as a learner with ADHD.

> I'm trying to hold on to [the instructor's] words, while also holding on to what I'm writing down. It makes me feel like I left something behind. You can feel when you leave for the airport and you feel like you're leaving something behind, you get there and it's like your I.D. That's what it feels like to me…With my notes, I feel like I haven't gathered everything.—Opal, a student with ADHD

Opal described how taking notes during a lecture is stressful because she knows she is missing what the instructor says while she is writing. Her knowledge of self allowed her to articulate how this makes her feel in a way someone without ADHD can understand. While some participants described difficulty maintaining focus, other participants demonstrated knowledge of self by describing their experiences with hyperfocusing. Hyperfocusing involves prolonged attention to detail and is associated with ADHD (Hupfeld, Abagis, & Shah, 2019). Some participants, such as Isabel, shared their experiences with hyperfocus, as it often demands extra time to complete exams and assignments. Isabel, explained, "Sometimes I get hyperfocused and detail-oriented. It takes me longer to do things." For Isabel, she especially notices that she hyperfocuses when she is working on math problems.

While several participants revealed their knowledge of self by discussing their experiences with focus and attention, other participants referred to challenges with processing speeds. For example, Cassie talked about what it is like to be a learner with slow processing speed and ADHD in undergraduate STEM courses.

> I'm less likely to speak up and participate in group activities because…I do have a disability where I think slower. I'm less likely to be as engaged in group activities as other classmates… Part of it is, I'm just sitting there processing what they're saying. But they're going so much faster than me.—Cassie

Cassie explained that she is more likely to be quiet in an interactive STEM classroom because she is listening and processing information at a different speed than her peers. Other participants demonstrated knowledge of self by explaining how different

processing speeds affect them while taking exams. For example, Megan talked about being a learner with dyslexia and ADHD, and how she uses extra time on exams to go through her thought process.

> I need to organize my thoughts, look at problems, see everything I'm given, write it all down. I feel like I go through a lot more steps than most people would need to answer the question…—Megan

Megan described how she uses a process to ensure she does not miss relevant information while she reads the exam. Other participants with a specific learning disability in reading explained that their processes can involve highlighting information in certain colors to help draw attention to important words in exam questions. Such a process is vital for Megan's success on the exam and requires time to fully complete.

Development of knowledge of self. All of our participants demonstrated knowledge of self, and some participants also explained how they developed this knowledge. For example, some participants described developing knowledge of themselves as a learner with a disability from previous experiences.

> I've had experiences where other people… constructively point [my weaknesses] out, and just myself internally just being like, "Hey, this is an area that I'm struggling in."—Mia

Mia shared that when she was first diagnosed with her disability in middle school, she worked with a reading tutor who was specially trained to help teach students with SLD in reading. Mia's tutor helped her identify weaknesses associated with her disability. The tutor's goal was to use this information to help Mia select methods to overcome those weaknesses so that she would be successful. Mia now uses self-reflection as a college student to help identify new weaknesses she was not aware of. This helps Mia to decide what action, if any, she needs to take to address the situation.

In contrast to Mia, many participants discussed that their official testing documentation informed their knowledge of self. For example, Oakley and Wyatt cited

their official testing documentation to explain their strengths and weaknesses as a learner with a disability. Oakley stated:

> The [doctors] explained…to me that the processing and verbal parts to my brain work at different speeds. So I can read a problem or if I was presented with a math problem or something, I could read it, understand it conceptually, even visualize it, but because the verbal part is not on the same par with the processing part, I don't process it correctly and I do the problem according to what I think it is, but that's not always [what the question asked].—Oakley

Oakley gleaned knowledge of self, in part, from her testing documentation. She used this knowledge of self to later communicate with her DRC coordinator and to defend her use of accommodations to peers who think accommodations are unfair. Similar to Oakley, Wyatt used his testing documentation to inform his knowledge of self. He explained that his short- and long-term memory, along with his processing speed, are at lower levels compared to other areas, such as reading comprehension, where he scored above average. For participants like Oakley and Wyatt, their testing documentation served as one way to develop knowledge of self.

Test's component: Knowledge of rights. Knowledge of rights in Test's conceptual framework of self-advocacy was defined as, "knowing one's rights as a citizen, as an individual with a disability, and as a student receiving services under federal law" (Test et al., 2005, p. 50). In our analysis, we considered any instance a participant mentioned that a law ensured their access to accommodations in college to be evidence of knowledge of rights. We found that only two participants, Mia and Archie, discussed laws concerning their accommodation use in college spontaneously, without prompting, while 23 participants did not. When we asked Archie what it is like for him to talk to instructors about his disability, he said:

> I'm not really afraid because I know I have legal protection…I'm assuming Section 504 of the Workers Compact of '73 would apply, considering that I got the same accommodation, the 504 stuff in high school.—Archie

Archie demonstrated knowledge of rights because he directly names one law, Section 504 of the Rehabilitation Act of 1973, which mandates universities and colleges provide access to accommodations for students with disabilities. Archie's knowledge of rights appeared to originate from his experiences in high school when he received accommodations under a Section 504 plan.

When we asked our participants about self-advocacy, most did not refer to laws. Instead, we found that our participants would say they know their instructors have to provide accommodations. Kendra, who worries about what her instructors will think of her when she talks to them about having ADHD, said that she uses this knowledge to help her prepare to talk to them. She said,

> It always makes me really nervous, but at some point, I'm like you know what, it's not up to them…They have to make that accommodation, regardless of their own personal opinions on the subject.—Kendra

Other participants, like River, cited university policy rather than any federal law. River noted, "It's university policy that professors have to accommodate people with disabilities." Both Kendra and River were aware that they have a right to accommodations, although they did not directly name federal law as the source of this right.

***Emergent component: Knowledge of accommodations and the process to obtain them*.** Our definition of knowledge of accommodations and the process to obtain them consists of two-parts, awareness of (1) accommodations that are available to a student with ADHD and/or SLD and (2) how the accommodation process in college works, including knowledge of the student role, the DRC coordinator role, and the instructor role in the process. We found that many of our participants were still developing a knowledge of their accommodations, and this influenced their self-advocacy. For example, Cassie explained that she has never requested a notetaking

accommodation, although she qualifies to receive it, because she is still developing knowledge of this accommodation.

> An accommodation that's an option is the notetaking [accommodation]. I just never had that in high school, so I think coming to college, I was like, I don't know what that is. I just opted out of that every semester for every class.—Cassie

At the end of the interview, the interviewer explained how the notetaking accommodation typically works for students. After hearing this explanation, Cassie stated that she would now seriously consider requesting the accommodation because she had a better understanding of how the accommodation would work for her. Other participants shared that they did not know they could request a certain type of accommodation in their undergraduate STEM courses until their DRC coordinator suggested it directly to them. One example of this was from Kendra, who reported that she did not know she could ask to audio-record lectures in her STEM courses instead of requesting a traditional notetaking accommodation. We found that many of our participants developed knowledge of accommodations through their DRC coordinator.

A majority of our participants demonstrated knowledge of the accommodation process when they explained to us how their accommodations worked from the start to the end of the semester. They described the roles and responsibilities of each party involved in the accommodation process in college, including the student, the DRC coordinator, and the STEM instructor. We considered this type of knowledge to be similar, yet distinct, to the sample subcomponents of leadership as defined by Test. Test specifically defined leadership as "awareness of the common needs and desires of others, working with others, group dynamics and responsibilities" (Test et al., 2005, p. 50). In Test's framework, leadership was not considered to be essential for self-advocacy. We considered a baseline knowledge of accommodations and the process to obtain them likely an essential component of self-advocacy for our participants. We saw

this type of knowledge to be an important component of self-advocacy that can be distinguished from leadership in our participants.

Emergent component: Knowledge of STEM learning contexts. We define knowledge of STEM learning contexts to be the awareness that accommodation needs are influenced by the learning environment experienced by students with ADHD/SLD in undergraduate STEM courses. This component of self-advocacy became salient during our analysis when many of our participants described their thought processes to determine what accommodations they wanted to request in a STEM course. Our participants explained that they consider the instructor expectations of students inherent to a particular learning environment when making accommodation decisions, and we term this thought process "task evaluation." We found evidence of ongoing task evaluation at a scale ranging from the entire STEM discipline to a single learning activity within a STEM course. We explain how our participants demonstrated their knowledge of STEM learning contexts within undergraduate STEM courses.

One participant, Wyatt, demonstrated knowledge of STEM learning contexts when he described how he decided to use his extended-time exam accommodation in one of his STEM courses. Within this particular STEM course, the lecture section of the course is 50 min in length and the laboratory section is at least 75 min in length. Wyatt shared that he first determined if the exams would be proctored in the lecture section of the course or in the lab section of the course before he signed up for extended-time exams at the DRC.

Other participants like Henry and Mia showed knowledge of STEM learning contexts when they described how they decide to use their available accommodations. For our participants, once they initially meet with their DRC coordinator, they have the freedom to select course-by-course what accommodation notification letters they will send to their STEM instructor through the online accommodation system. These are

accommodation decisions participants make on their own, unless they request a follow-up meeting with their DRC coordinator. Henry described his thought process in making this type of accommodation decision for his STEM courses,

> [I] figure out what the course is going to be. Is it going to be a lecture? Is it going to be group work? Is it going to be a lab? Is it actually a lab? Then see which of my accommodations actually apply…—Henry

At the time of this interview, Henry was early in his college career. Henry was still in the process of developing his knowledge of STEM learning contexts. He later explained that he would sometimes ask his STEM instructors if his accommodations would apply to STEM specific learning contexts, such as an organic chemistry laboratory section.

Our participants described other strategies besides talking to STEM instructors that they used to develop knowledge of STEM learning contexts. Isabel and Tyler shared that they will first attempt to complete a quiz or exam without their accommodations in an unfamiliar STEM learning context because they would prefer not to use their accommodations if they can earn a satisfactory grade without them. For participants, like Isabel and Tyler, they prioritized their own experience in unfamiliar STEM learning contexts. They did not seek out additional information about the learning context from their peers, teaching assistants, or instructors.

Several other participants shared with us that they did not know early in their STEM majors that they could request accommodations for assessments in lab sections, such as for a lab quiz or a lab practical. Kendra described her experience as a freshman in an undergraduate STEM course, where she ended up taking the lab practical without any accommodations, “I didn’t even know at the time that I could have gotten accommodations for [the lab practical].” Kendra explained that taking her lab practical without accommodation in her freshman year was extremely stressful and she felt

regretful when she later learned she could have requested them. Kendra's experience illustrates the importance of knowledge of STEM learning contexts.

Self-advocacy behaviors. In our model of self-advocacy, we consider the components, communication, and leadership to be examples of self-advocacy behaviors. We describe how our participants engage in these behaviors to characterize how students practice self-advocacy in their undergraduate STEM courses. We also introduce and describe a novel behavior we term "filling gaps". We see communication as the heart of self-advocacy and that it is required for leadership and filling gaps.

Test's component: Communication. In Test's conceptual framework, communication is designated as an essential component of self-advocacy. Communication for the purpose of self-advocacy involves "negotiation, assertiveness, and problem-solving in a variety of situations" (Test et al., 2005, p. 50). We sought to uncover the variety of situations our participants engage in communication for the purpose of self-advocacy. In this section, we describe situations involving communication with DRC coordinators and with STEM instructors. Our rationale for providing these examples of communication is to characterize the types of situations that warranted self-advocacy for our participants in the context of undergraduate STEM courses.

Communication with DRC coordinators. Once our participants registered with the DRC, they met with their assigned DRC coordinator to establish their accommodations. In the initial accommodation meeting, the participant and the coordinator agreed upon the accommodations the participant is eligible to request for the remainder of their college career at the university where data collection occurred (Figure 6.3). For many participants, this initial meeting was the only time they communicated face-to-face with their DRC coordinator because they found their initial accommodations to be sufficient. However, several participants reported ongoing communication with their

DRC coordinator to manage accommodation issues that developed after the initial accommodation meeting. We found that our participants communicated with their DRC coordinators about their extended-time exams, notetaking accommodations, and experiences with instructors.

Some participants communicated with their DRC coordinators to adjust the details of their extended-time exam accommodations. One example of participants adjusting their extended exams was given by Henry who communicated with his DRC coordinator to update the terms of his accommodations to better fit his needs as a student with an SLD in reading. He asked his DRC coordinator for an alternative format for his exam.

> It was during the first exam. I didn't do as well as I normally did previously in high school. When I went back and looked over the exam, I realized it's some of the reading mistakes I make, and the format of the exam was on the computer. Normally in high school, since everything was on paper, I could go back and highlight and underline and help myself focus. I wouldn't make as many reading mistakes. So then when I realized that was the problem, I went back to my DRC coordinator and I talked to her about it and then we got printed written exams. —Henry

Henry recognized that he is likely to perform better on exams if he reads the exam in a print format instead of on a computer screen. Henry successfully communicated with his coordinator to make this change to his exam accommodations.

Our participants also described self-advocating by communicating with their DRC coordinators, or the DRC office, when exam scheduling issues arise. The DRC at the university where data collection took place requires students taking exams at the DRC to schedule their exam 7 days in advance. Many of our participants shared instances where they missed the 7-day deadline. Some participants in this situation did not attempt to communicate with the DRC and decided to take the exam in class, without their accommodation(s). We found that a subset of our participants demonstrated self-

advocacy in this situation by communicating with the DRC to see if it was still possible for them to use their accommodations and take the exam at the DRC.

Besides extended-time exams, many of our participants qualified for a notetaking accommodation. At the institution where data collection occurred, the notetaking accommodation typically entailed the STEM instructor identifying a student in the class who agreed to upload a copy of their own notes to the DRC's online accommodation portal. The identity of the notetaker was usually unknown by the student requesting the accommodation. Once the notes were uploaded, the student using the accommodation could access the notes, and the notetaker was compensated $100 for their service. Our participants frequently reported to us that they have received low-quality notes from their DRC-paid notetaker. However, only one participant in our study, discussed issues about her notetaking accommodation with her DRC coordinator when she did not receive any notes.

> The one time I did [use a notetaker] I had issues. First of all, my first notetaker never sent me notes, so I just notified the DRC and they got me a new notetaker. Then that notetaker was very subpar…but I was doing well in the class so I never tried to find another one.—Megan

Megan only described communicating with the DRC about notetaking when she failed to receive any notes from her assigned notetaker. She did not communicate with the DRC to inform them that the notes she eventually received were of poor quality. Because several other participants had a similar experience with their notetaking accommodation, we asked participants why they chose not to communicate to their DRC coordinator when they received low-quality notes from their notetaker. Our participants expressed concern that if they reported the issue, the notetaker would no longer be paid $100 from the DRC. Issues with notetaking accommodations were prevalent in our data. However, situations where our participants communicated with their DRC coordinators about issues with notes were rare.

Communication with STEM instructors. In the following subsection, we detail how our participants described communication with their STEM instructors. We included these data because participants are not required to directly communicate with their STEM instructors about their disability or accommodations at the university where data collection occurred. In addition, all our participants use accommodations for invisible disabilities, so their instructors would not necessarily recognize them as a student using accommodations in their classrooms. We were interested in the experiences and perspectives of our participants: do they communicate directly with their STEM instructors about their disability and accommodations use? We also wanted to know what factors they considered in making the decision to talk directly to their STEM instructors about their disability and accommodation use.

Some participants found value in communicating with their STEM instructors about their accommodations. Isabel explained that she communicates with all her STEM instructors about her accommodations so she can gauge how familiar the instructor is with their role in the accommodation process.

> Some [STEM instructors] have a harder time accommodating than others…So, it's good to have that face-to-face contact [with STEM instructors] to communicate or get an understanding if they've had students who use accommodations before, if they know the process…—Isabel

Isabel explained that the instructor's familiarity with the accommodation process in college will determine how much follow-up communication she has with the instructor. This helps Isabel determine how much self-advocacy she will likely need to enact in a particular course, to ensure she receives her accommodations. A few of our participants, such as Mia and Eli, reported that they always discuss their accommodations and disclose what disability or disabilities they receive accommodations for with their STEM instructors. Mia tells all her STEM instructors that she has an SLD in reading because

she sees it as a means to make a personal connection and to inform the instructor so they can work together in the accommodation process if issues arise.

> I always discuss [my disability] with my professors…I feel like that's more courteous and it's also putting a face to the name and making it easier ultimately on both parties to recognize where we need to work together… Often, I'm just like, "I have dyslexia. It is what it is. I have these accommodations and if you have questions, then let me know."—Mia

Mia prefers to talk openly about her accommodations and disability with her STEM instructors. She operates under the assumption that the instructor wants to support her learning and accommodation use but thinks that the instructor may need more information than the official accommodation letter provides to do this successfully. For Mia, this conversation is an essential piece of her self-advocacy with an instructor.

Our participants also described situations when they communicated with their STEM instructors about their accommodations. Typically, these situations involved determining the logistics of a specific accommodation, such as extended-time exams, or finding a notetaker. Many participants, especially those in engineering majors, described communicating with their instructors to determine if they would take an extended-time exam at the DRC, or if the STEM instructor would proctor the extended-time exam in-house. Several of our participants shared that many of their engineering courses do not use traditional exams but, instead, require students to work in groups on projects that are submitted as an exam grade. Besides determining exam logistics, our participants also communicated with their STEM instructors to arrange accommodations for in-class quizzes and online exams. Claudia described how she recently communicated with a STEM instructor regarding pop quizzes,

> I went up to him and I said, "I'm struggling to finish these pop quizzes, this is stressful. I'm set up with extra time for my tests. Is it possible for there to be any sort of way to get extra time on these quizzes?" At first he said no, and I was like, "I'm not finishing these, I'm stressed out," and he said, "Okay, the best I can do is putting your paper down first and then picking yours up last," and I said, "I will do it, sounds good."—Claudia

Claudia later shared that this arrangement afforded her about 45 additional seconds on the quiz. Claudia felt satisfied with the solution. She successfully communicated for the purpose of self-advocacy by negotiating with her STEM instructor. Many participants described situations where communication with their STEM instructor was needed for the purpose of self-advocacy.

Test's component: Leadership. In Test's conceptual framework of self-advocacy, leadership was broadly defined as, "an awareness of the common needs and desires of others, working with others, group dynamics and responsibilities" (Test et al., 2005, p. 50). Examples of leadership could involve "working with others to speak up for their collective wants and needs through organization, community gatherings, and political forums" (Test et al., 2005, p. 50). Leadership was not considered to be essential for self-advocacy in Test's conceptual framework. We considered participants to show evidence of leadership when they discussed taking actions on the behalf of others, relating to issues of disability or accommodations. We only found a few examples of leadership, but the leadership described by our participants could be categorized into two types: taking action for others with diagnosed disabilities to overcome stigma and advocating for peers without formally diagnosed disabilities to be tested to receive academic accommodations. One example of leadership was from Oakley who showed leadership by engaging in a research project to find genetic markers for ADHD.

> I wanted to find a genetic marker to correlate with people who had been diagnosed with ADHD and I actually found one in a very small population size. But the whole reason I did that was because I wanted to reduce the stigma around ADHD.—Oakley

Oakley demonstrated leadership when she expressed that her motivation to conduct research was to reduce the stigma of ADHD for other people with ADHD. She demonstrated awareness that other people with ADHD wish that the condition was more

broadly accepted as a legitimate disability, validating the need for academic accommodations.

Emergent component: Filling gaps. We found that many of our participants described a novel collection of behaviors, associated with self-advocacy, that we call filling gaps. We define filling gaps as participant actions taken to overcome limitations in formal accommodations or instructional supports to ensure success as a learner with ADHD/SLD in undergraduate STEM courses. We see filling gaps as involving communication that extends beyond the bounds of the established accommodation and support systems that existed at the university where data collection occurred.

Many of our participants demonstrated that they recognized how, at times, their formal accommodations or instructional supports within a certain STEM course were not sufficient. For example, many of our participants reported receiving low-quality notes from their DRC-paid notetaker. While only one of our participants ever communicated with the DRC to make them aware of this issue, several of our participants describe filling the gap in this formal accommodation by establishing their own system to receive sufficient notes in a timely manner. One example of filling gaps comes from Mia who described how she set up a Google doc with her peers in her upper-division biology class to ensure she has access to a quality set of notes because if she missed information in class, one of her peers was likely to write it down, and vice-versa. This ensured that everyone in her peer group could access quality notes after class. Heath set up a similar system to take notes. He explained how developing his own note system is a form of self-advocacy,

> I do my own form of accommodating by having another support system that is not the DRC that I can fall back on.—Heath

Another prevalent example of filling gaps in our data comes from participants who do not feel they can ask their STEM instructors questions about class material

either after class or in office hours. Several of our participants expressed that they do not perceive their instructors to be approachable, so instead of going to office hours, they will seek out tutoring from a peer, or a third-party tutoring service. For example, Ryan shared that he has asked a peer in his upper-division STEM course to tutor him because there are no qualified tutors available at the university's office of academic enhancement and because he does not perceive his instructors to be approachable.

Our participants described filling gaps as a way they practice self-advocacy in their undergraduate STEM courses. We found that many participants may or may not disclose their disability status when they fill gaps. For example, they could ask their peers to take notes with them while their peers may or may not know they qualify for academic accommodations. We also asked our participants if they told their tutors about having a disability, and they said it never came up.

Emergent components: Beliefs influencing participant self-advocacy. In our analysis, we found that beliefs held by our participants influenced self-advocacy knowledge and self-advocacy behaviors. Agency and view of disability are the beliefs we found our participants to discuss when they described their self-advocacy. Each belief is detailed in the following sections.

Agency. We found that participants who strongly articulated a belief that they are the person responsible for their own accommodations and success in college tended to describe more components of self-advocacy. This belief is a form of agency, which is the belief that you are responsible for your own learning (Baxter Magolda, 2000). For example, Opal demonstrated agency when she explained how she "defends [herself] in a way" in a situation where her peers stated that the only reason Opal earned a better grade than them on an exam was because Opal qualifies for an extra time accommodation. Opal responded,

> I took it [into my] own hands, because I was struggling. I went and [asked] for help and figured that out for myself, so what's your problem with it? If you want extra time, go get tested, and go figure it out for yourself.—Opal

Other participants like Kendra and Henry also explained how they perceive themselves to be the person responsible for their own success and this influences how they engage in self-advocacy. Kendra described her perspective,

> The best thing…for me has been learning that if I need something, I have to learn how to do it myself. I know that if I don't go up to them and tell them, then I'm not going to get what I need.—Kendra

Kendra described how she knows she has to be the person to talk to her DRC coordinator or her STEM instructors if she needs an accommodation. Henry expounded on his perception of the student role in the accommodation process by stating, "If a problem arises, I go confront it, and I say I have this accommodation I would like to apply to this situation." Henry demonstrated agency by describing that he takes responsibility for his own accommodations and does not solely rely on his DRC coordinator to mediate situations with his STEM instructors. At times, for Henry, this was challenging because one of his STEM instructors stated they would prefer if Henry first contacted his DRC coordinator before speaking to them. Statements from Opal, Kendra, and Henry clearly illustrated that they see themselves as responsible for their own accommodations, and this idea was linked to their self-advocacy. These strong agentic statements were in contrast to some of our other participants, like Dana, who stated that she wished her DRC coordinator "would just send [her accommodation letters] to her instructors" without Dana having to initiate the request because Dana was prone to "procrastinating and forgetting." Dana did not appear to fully embrace her own role in the accommodation process and did not practice self-advocacy to the same extent as other participants.

View of disability. View of disability strongly influenced self-advocacy. Our participants described their own view of disability, and their perceptions of how STEM instructors and peers view disability and accommodation use in the context of

undergraduate STEM courses. Our participants reported a continuum of views regarding their own disability which ranged from negative to positive. Participants who tended to express a positive view of their disability also tended to describe more components of self-advocacy. This was exemplified by Mia, who showed multiple components of self-advocacy and who stated that she “is proud” of having dyslexia. Other participants like Opal explained that she does not see her disability “as a burden, or something that makes me lesser…it is just part of my chemical makeup.”

Another participant, Henry, shared that his knowledge of self informs his personal view of disability. He stated that he is aware that his SLD “changes the speed at which I intake and export information” but he does not “feel ashamed that I need [accommodations] because sometimes I think I’m smarter than people without accommodations because I had to work so hard to get to the same level of speed.” Henry explained that he thinks this extra work related to his SLD causes him to have a stronger knowledge base than some of his peers.

Participants who tended to see their disability in a positive manner described using accommodations, like Oakley, when she said, accommodations “level the playing field” between her and her peers without a diagnosed disability. Participants who felt their disability was shameful or embarrassing tended to describe feeling conflicted about using accommodations because they worried about what other people, like their STEM instructors and peers, would think about them if they found out. Aaron who tended to describe a negative view of his disability also explained that he worries about what his STEM instructors think of students who use accommodations. He explained that back when he would still sometimes use accommodations, he would meet with each STEM instructor and ask, “Do you think this makes me look like a lesser student?” Aaron would then determine how genuine his instructor’s response was to this question. Aaron

explained how one math instructor reassured him that it was okay for Aaron to use accommodations in his class,

> … he kind of said like with [my upper-division math course] time isn't a concern, because you can solve a problem for years, so I shouldn't be worried about it. So, he gave me… a concrete example of like why I shouldn't be worried.—Aaron

This interaction with his STEM instructor made Aaron feel comfortable to use accommodations in this STEM course. Conversely, Aaron shared another example of when his STEM instructor did not respond in a timely manner to his accommodation request. Aaron did not want to confront the instructor to ask why so he "cancelled" the request and decided to "take a new class."

Overall, many participants expressed that they perceive self-advocacy to be more challenging in STEM courses compared to other disciplines because they perceive their STEM instructors to think negatively about students with disabilities and accommodation use in their courses. Mia expressed her perception of STEM instructors' beliefs about students who use accommodations:

> A lot of times, professors are like, "STEM courses are for the smartest kids and you don't need accommodations if you're smart…" Versus like a non-STEM course, they're just like, "Oh yeah, I have worked with plenty of people who have accommodations. It's just another day."—Mia

We found one counterexample in Kendra who stated she felt that her STEM instructors would be more understanding of her disability, ADHD, because they were scientists and tended to be more "empirical." However, several of our participants perceived their STEM instructors to hold negative views of students who use accommodations in their courses, and consequently, self-advocacy in STEM could be more challenging to enact.

Discussion

We identified components of self-advocacy that are evident among 25 STEM majors through semi-structured interviews and qualitative analysis. We propose a model

of self-advocacy for students with ADHD/SLD in undergraduate STEM courses based on our participants' experiences (Figure 6.2). From our model of self-advocacy, we propose hypotheses regarding self-advocacy for students with ADHD/SLD in undergraduate STEM courses. Further testing of these hypotheses will determine if they apply to students in other contexts. In the following sections, we explain our hypotheses and situate them within existing literature. We also suggest implications for research and teaching if these hypotheses are supported by future research.

Hypothesis 1: Self-advocacy for students with ADHD/SLD in the context of undergraduate STEM courses requires novel forms of self-advocacy knowledge. We propose that additional forms of knowledge besides knowledge of self and knowledge of rights are involved in self-advocacy for students with ADHD/SLD in undergraduate STEM courses, namely, knowledge of accommodations and the process to obtain them, as well as knowledge of STEM learning contexts. We found knowledge of accommodations and the process to obtain them to be a stand-alone component of self-advocacy because this type of knowledge was distinct from knowledge of rights. Few participants directly named federal legislation that guides the accommodation process in college. However, many participants explained their knowledge of the accommodation process at the university where data collection occurred. We found several examples of how our participants developed knowledge of accommodations and the impact this knowledge or lack of this knowledge had on their self-advocacy. For example, Cassie told us that the main reason she decides not to use her notetaking accommodation in undergraduate STEM courses is because she did not use a notetaking accommodation in high school and she does not know how it works. In another study of college students with learning disabilities, students who had inaccurate information about accommodations and the process to obtain them tended to not disclose their disability status to the university and, as such, did not use

accommodations (Cole & Cawthon, 2015). Separating knowledge of rights from knowledge of accommodations and the process to obtain is logical (Vaccaro et al., 2015). Federal laws mandating access to accommodations for students with disabilities are the same across the country; however, the process by which students access these accommodations differs by institution. Thus, knowledge of accommodations and the process to obtain them at a student's home university is likely critical for practicing self-advocacy.

We propose knowledge of STEM learning contexts is a novel form of self-advocacy knowledge for students with ADHD/SLD in undergraduate STEM courses. Undergraduate STEM courses are known to possess unique barriers for students with disabilities. For example, STEM courses often encompass components besides traditional lecture-style classrooms including labs, fieldwork, small-group work, and design studios, which we refer to as "a STEM learning context" (Moon et al., 2012). Our data show that many of our participants consider the contexts of their STEM courses when making accommodations decisions and actively seek to develop this type of knowledge. This is evident in Henry when he describes evaluating the tasks in a given STEM course to determine if he will request formal accommodation for the course. For example, he met with his organic chemistry instructor to ask if his accommodations will apply to his organic chemistry lab quizzes and lab practical. These data suggest STEM instructors can play a role in helping students to develop knowledge of STEM learning contexts.

If hypothesis 1 is supported by future research, interventions to promote development of knowledge of accommodations and knowledge of STEM learning contexts would be appropriate. It would also call on STEM instructors to consider adopting practices in their courses to support student development of knowledge of STEM learning contexts. Instructors could consciously incorporate explanation of the

STEM learning contexts students will experience in their course using multiple means of representation, not only in an accessible course syllabus, but also through other avenues such as instructor talk, which is language an instructor uses to create the learning environment (Seidel, Reggi, Schinske, Burrus, & Tanner, 2015).

Hypothesis 2: Beliefs, such as agency and perceived view of disability, influence self-advocacy for students with ADHD/SLD in undergraduate STEM courses. We found that self-advocacy for our participants could be influenced by agency. In the context of our study, agency was defined as a sense of responsibility for your own learning as a student with ADHD/SLD. We found participants who demonstrated agency tended to describe more forms of self-advocacy. For example, Opal, Kendra, and Henry demonstrated agency when they explain a personal responsibility to ensure they can access the accommodations they need in an undergraduate STEM course. Our finding that self-advocacy is influenced by agency is consistent with what is known about self-advocacy. Self-advocacy is considered to be a component of self-determination (Test et al., 2005; Wehmeyer et al., 2003). Self-determination is a construct rooted in broader theories of human agency, and thus, self-advocacy is linked to agency (Walker et al., 2011).

Our data show that the self-advocacy of our participants was also influenced by view of disability. View of disability for our participants included the view of their own disability, and their perceptions of how other people, including STEM instructors and peers, view disability and accommodation use in undergraduate STEM courses. We found that participants who viewed their own disability in a positive manner tended to describe more components of self-advocacy. For example, Mia told us she is proud of her SLD in reading and she demonstrated evidence for nearly all a priori and emergent forms of self-advocacy. Similarly, a positive view of disability was found in another study

of college students with learning disabilities to be a factor related to deeper disclosure of disability to the university and college instructors (Cole & Cawthon, 2015).

In our study, participants who described substantial concerns about how others in their STEM courses viewed disability appeared to struggle to practice self-advocacy. This was evident in Aaron who would cancel his accommodation requests when a STEM instructor did not respond in a short period of time because he interpreted this to mean that his STEM instructor viewed disability and accommodation use negatively. It is important to underscore that this was Aaron's perception, which may or may not reflect the actual view of disability held by the STEM instructor. Our data show that participants' perceptions of how their peers and STEM instructors view disability and accommodation use impacted their self-advocacy.

We consider view of disability to be related to campus climate towards students with disabilities. Campus climate can be defined as "a measure of people's attitudes about, perceptions of, and experiences within a specified environment" (Ryder & Mitchell, 2013, p. 34). It has been suggested that students with disabilities often perceive campus climates to be less welcoming than students without disabilities (Harbour & Greenberg, 2017). Student perceptions of college faculty, in general, are that college faculty are willing to accommodate students with disabilities, but faculty are perceived to be skeptical about the legitimacy of ADHD as a disability necessitating academic accommodations (Stamp, Banerjee, & Brown, 2014; Yssel, Pak, & Beilke, 2016). Few studies examine the attitudes of STEM faculty and peers without disabilities towards students with disabilities in undergraduate STEM courses. In a small-scale study of five STEM faculty, participants indicated they are willing to accommodate students with disabilities in their courses (Love et al., 2014). Beyond this study, there is a dearth of literature regarding attitudes of STEM faculty towards students with invisible disabilities,

such as ADHD. In our study, many participants perceived STEM faculty as less receptive to their accommodation needs compared to faculty in other disciplines.

The notion that the culture of STEM may be less welcoming to students with learning disabilities compared to other disciplines is supported by a few previous studies. For example, researchers investigating students with learning disabilities in undergraduate STEM courses reported that they perceived their own research to be marginalized because STEM faculty and staff did not appear to consider students with learning disabilities to be capable of conducting STEM work (Thurston et al., 2017). Moreover, the use of accessible teaching approaches known to reduce barriers for students with disabilities in K-12 STEM education, called universal design for learning, is not widely adopted in undergraduate STEM courses (Schreffler et al., 2019). The fact that universal design for learning is known to be helpful for students with disabilities, yet is not frequently used in undergraduate STEM courses suggests the climate is not as welcoming as it could be to all students with disabilities.

Future studies examining how students with ADHD/SLD, as well as other disabilities, perceive undergraduate STEM courses and departments are needed. Our data suggest that students with ADHD/SLD form perceptions of how their STEM instructors and peers view disability and the use of accommodations in undergraduate STEM courses, sometimes without even any verbal exchanges at all. These perceptions of how disability is viewed have the potential to greatly influence the decision to use accommodations in a STEM course. We stress that these perceptions may or may not reflect the actual view of disability held by STEM instructors and peers, yet regardless these perceptions are likely at play when students decide whether or not to engage in self-advocacy. It is thus imperative that we understand how students with ADHD/SLD perceive the climate of their STEM courses so that we can take steps to make undergraduate STEM courses more welcoming and inclusive.

Hypothesis 3: Students with ADHD/SLD in undergraduate STEM courses engage in behaviors we call "filling gaps" to be successful in their undergraduate STEM courses. Our participants engaged in a novel set of behaviors we refer to as "filling gaps." Filling gaps involved our participants seeking out other people or resources to help them succeed in their undergraduate STEM courses. These behaviors involved going beyond officially sanctioned DRC accommodations or formal instructional supports. Examples of filling gaps came from Mia and Heath who described how they established their own notetaking systems with peers in a STEM course and from other participants, like Ryan, who discussed seeking out peer tutors. For some of our participants, filling gaps was a way they could access the supports they needed without having to necessarily disclose their disability status. Two studies examining the experiences of education majors with learning disabilities hint at the importance of informal supports during college for their participants (Couzens et al., 2015; Timmerman & Mulvihill, 2015). For example, one study reported that two of their participants, one participant who is a student with multiple disabilities, including ADHD and SLD, and another who is blind, described situations reminiscent of filling gaps in our study (Timmerman & Mulvihill, 2015). Their participants noted how friends or peers would occasionally help them by volunteering to read textbooks aloud or by providing copies of class lecture notes. However, both these participants noted that the willingness of their classmates to help may be because they are all special education majors and that this environment was likely to be more accepting of students who use accommodations, compared to other majors (Timmerman & Mulvihill, 2015).

If hypothesis 3 is supported by future research, it would connect self-advocacy to social capital. Social capital involves the resources that are afforded to and utilized by an individual through their connections to other people within a social network (Lin, 2001). A previous study of STEM majors with disabilities found self-reported gains in self-

advocacy skills after participation in a learning community that built social capital (Whitney, Langley-Turnbaugh, Lovewell, & Moeller, 2012). If filling gaps and self-advocacy are indeed connected, universities and STEM departments committed to the success of STEM majors with ADHD/SLD should pursue programming interventions that are likely to promote development of social capital. Interventions such as the formation of peer learning communities (e.g., Whitney et al., 2012) and opportunities for mentorship from graduate students, coupled with disability-related instruction from experts (e.g., Kreider et al., 2018) are examples of interventions that may help students access social capital to help fill gaps to enhance self-advocacy.

Considerations for transferability of our findings. Data were collected at one institution, which may limit the transferability of the findings to other settings. However, by limiting our data collection to one institution we were able to pursue clarifications for incongruities we encountered in our data (Stanton, Dye, & Johnson, 2019). For example, our participants referred to the DRC and the online accommodation system using many different names and we were able to clarify these terms. Our sample represents a convenience sample. All our participants were registered with the DRC. It is possible that our sample is missing some self-advocacy experiences of students with ADHD/SLD who are navigating college without formal accommodations. Yet the purpose of our study is to characterize the self-advocacy experiences of students with ADHD/SLD in undergraduate STEM courses. We reasoned that students registered with the DRC practice self-advocacy and would be willing to discuss their experiences with us. Our sample is likely enriched for self-advocacy.

Conclusion

In conclusion, the 25 STEM majors with ADHD/SLD in our study described their self-advocacy experiences in the context of undergraduate STEM courses. Based on our analysis of participants' experiences, we provide the first empirically derived model of

self-advocacy for students with ADHD/SLD in undergraduate STEM courses. In our model, we operationalized components of Test's original conceptual framework of self-advocacy by determining how our participants demonstrated knowledge of self, knowledge of rights, communication, and leadership in undergraduate STEM courses. We proposed additional components of self-advocacy knowledge and self-advocacy behaviors and identified beliefs which influenced self-advocacy in our participants. Together, these original and emergent components comprise an updated model of self-advocacy based on the experiences of our participants. Future testing of this model will permit development of a theoretical framework of self-advocacy for students with ADHD/SLD in undergraduate STEM courses. Such a theoretical framework can be used to develop valid and reliable measures of self-advocacy that, in turn, can be used to determine the effectiveness of interventions designed to promote and enhance self-advocacy for students. By promoting self-advocacy within students, we can help increase the retention rates of students with ADHD/SLD in undergraduate STEM courses and majors, which will lead to a more diverse and competitive STEM workforce.

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Supplemental File 6.1. Interview protocol

1. Tell me about yourself, what is your major and year in school?

2. Walk me through how the accommodation process works from the start of the semester to the end of the semester.

3. Think back to your first semester in college, tell me about your experience in learning how to request academic accommodations.

4. How did this experience compare to your experience with accommodations in high school?

5. Tell me about a time you decided not to use accommodations in a course or for a semester. Describe your thought process in making this decision.

6. What do you do when your accommodations are not working in a course? Who do you talk to?

7. What advice would you give to an incoming student about learning to request and use accommodations?

8. I want to talk with you about self-advocacy. Self-advocacy has different meanings to different people. I think of self-advocacy as speaking up to tell those around you about your disability to help them understand what accommodations you need to access the learning material or activities in class, including requesting accommodations from the DRC. What does self-advocacy mean to you?

9. Tell me how your disability affects you when you are in a STEM course.

10. What accommodations do you typically use in STEM courses?

11. How do you self-advocate in a STEM course?

12. How does self-advocating in a STEM course compare to self-advocating in a different type of course?

13. How do you decide to tell you instructor about your disability in a STEM course? Walk me through your thought process.

Possible prompts to follow-up with:
a) You mention _____, tell me more about that.
b) You mention _____, can you give me an example of that?
c) You mention_____, what was that like for you?

Note: We have omitted nine questions asked toward the end of the interview.

Supplemental Table 6.2. Components of self-advocacy codebook. Example data has been lightly edited for clarity. Brackets indicate words added, or long pauses during the interview. Ellipses indicate words removed for conciseness.

Major code	Subcode	Description and notes	Example data
Knowledge of self		Awareness of individual strengths, and weaknesses as a learner with a disability.	See subcodes below.
	Strengths	Apply subcode when a participant describes a strength they have as a student.	"... But I happen to be better at math."-Oakley "I always have been naturally better at math as opposed to English."-Opal
	Weakness	Apply subcode when a participant describes a weakness they have as a student.	"…I'm really bad at reading comprehension." -Megan
	Description of themselves as a learner with a disability	Apply subcode when a participant describes something about themselves as a learner with a disability, that is framed necessarily as a strength or a weakness, but an aspect of how they learn as a student with a disability.	"When the professor is lecturing, I need for them to repeat what they just said because I may have caught part of it, I may have been distracted and working on a problem and I'm not able to work on a problem and listen to them at the same time. -Isabel "I'm trying to hold on to [the instructor's] words, while also holding on to what I'm writing down. It makes me feel like I left something behind. You can feel when you leave for the airport and you feel like you're leaving something behind, you get there and it's like your I.D. That's what it feels like to me…With my notes, I feel like I haven't gathered everything." -Opal
Knowledge of rights		"Knowing one's rights as a citizen, as an individual with a disability, and as a student receiving services under federal law" (Test et al., 2005, p. 50) Note: Apply this code when a participant names a federal law specifically.	"I'm not really afraid because I know I have legal protection…I'm assuming Section 504 of the Workers Compact [Rehabilitation Act] of '73 would apply, considering that I got the same accommodation, the 504 stuff in high school." -Archie

Knowledge of STEM learning context		Awareness that accommodation needs are influenced by the learning environment experienced by students with ADHD and/or SLD in undergraduate STEM courses	"This past semester, I didn't use 'em [accommodations] as much as I previously did... In some classes like if…we took all of tests during our lab period, so you got two and a half hours to do it. There's no way I'm gonna go over two and half hours for a 15-question test."-Wyatt "[I] figure out what the course is going to be. Is it going to be a lecture? Is it going to be group work? Is it going to be a lab? Is it actually a lab? Then see which of my accommodations actually apply…" -Henry
Knowledge of accommodations		Awareness of: (1) Accommodations that are available to a student with ADHD and/or SLD, and (2) How the accommodation process in college works, including knowledge of the student role, the DRC coordinator role, and the instructor role in the process.	<u>Awareness of accommodations</u> "An accommodation that's an option is the notetaking [accommodation]. I just never had that in high school, so I think coming to college, I was like, I don't know what that is. I just opted out of that every semester for every class." - Cassie "I didn't even know at the time that I could have gotten accommodations for [the lab practical]." Kendra* **This example overlaps with knowledge of STEM learning contexts* <u>Awareness of accommodation process</u> "You request the [extended] time [accommodation], you can schedule all your exams in the beginning of the semester, and you can have it just ready to go, you don't have to worry about it…If you feel like you don't have the resources or one of your accommodations just isn't working for you, just speaking up, and if you don't feel comfortable talking to the teacher, you can bring it up to the DRC, I'm sure they're glad to contact the teacher directly."-Carter "So they do a service with the DRC where you can have ... people in your class, the teacher

Knowledge of accommodations (continued)			will make an announcement and they'll say, "Hey, by the way, we need a note-taker in this class. If you are coming to class on a regular basis and you take good notes you can get paid just for being here and sharing your notes." So I used a note-taker last semester and they were paid by the DRC and gave me my notes."- Claudia
Agency		An individual belief that an individual student with a disability is responsible for their own accommodations and success in college. Note: Agency is related to participant's knowledge of their own role as a student in the accommodation process.	"I took it [into my] own hands, because I was struggling. I went and [asked] for help and figured that out for myself, so what's your problem with it? If you want extra time, go get tested, and go figure it out for yourself." -Opal "The best thing…for me has been learning that if I need something, I have to learn how to do it myself. I know that if I don't go up to them and tell them, then I'm not going to get what I need." -Kendra "If a problem arises, I go confront it, and I say I have this accommodation I would like to apply to this situation. -Henry
View of disability		Individual participant's view of their own disability. Note: Code every instance a participant describes their view of disability during the interview. Examine totality of coded view of disability data for each participant to determine how participant tended to express the view of their own disability in the interview.	See subcodes below.

View of disability (continued)	Accepts or embraces disability	Participant explains that they accept or embrace their own disability.	"Let me just tell you. I don't care. I'm proud." There are definitely benefits that you can find about having dyslexia, but there are definitely negatives to it as well."-Mia "I don't see it as a burden, or something that makes me lesser, or less hard working or anything like that. It's just…your chemical makeup."-Opal "I know that my learning disabilities changes the speed at which I intake information and can export it, essentially, and my ability to do either one. My ability to misread or my ability to misspeak. My knowledge of the subject, though, is unaffected. I think ... So I don't feel ashamed that I need these things because sometimes I even think I'm smarter than the other people. Because I had to work so hard to get to the same speed-level, or standard, as other people, that it caused me to have to be almost better than them on the knowledge-base. I know it takes me longer to write out or to read information. So I had to speed up somewhere else. Which means I had to be more proficient with the knowledge, if that makes sense. If we were taking a multiple-choice exam, it could take me all ... it could take them 15 minutes and then take me an hour and I would get everything right..because I knew I had to step up and learn it more even though it took longer to physically read out the questions and then write out the responses... I feel no shame."-Henry **This example overlaps with knowledge of self*
	Conflicted about disability	Participant explains that they are conflicted about their disability.	<u>Talking about his view of people who use accommodations</u> "I don't really care what other people do, but I do think it's unfair for me personally. Actually, I don't even know if it's unfair. I'd like to see what my life would [long pause]] I mean, my life is

View of disability (continued)			fine. There's nothing wrong with me. I'd just be interested where I would be if I hadn't received them... Maybe things could be really bad. I don't know…It didn't used to bother me. For some reason it bothers me now, I guess."-Aaron
	Disability is negative	Participant explains that they view their own disability negatively.	"Technically, I have a disability. Even though I'm kind of embarrassed about it."-Ryan "Even though I was diagnosed with a disability, I really don't like to think I have it..."-Judd
Stigma of disability		Participant discusses their perception of disability in the context of undergraduate STEM courses. This includes how STEM instructors, and peers view disability and accommodation use in the context of undergraduate STEM courses. <u>Frequent examples are</u>: -ADHD over-diagnosed or not a real disability -People who use accommodations are not smart -Accommodation use is unfair <u>Note</u>: This is related to view of disability	"Sometimes I do tell them, talk to my friends and stuff. I think ... I don't think they really care. Like, you know? Just because like I said, people have their opinions about ADHD, so a lot of times when I mention it, it's always like a very snide comment on how ADHD is a made-up thing, and really kids just need to go outside, or you know? I don't know, it just seems very negatively viewed."-Dana "The stigma is primarily like people joke a lot about dyslexia…There is a negative stigma…[the] stigma seems like it's making people who have dyslexia out to be less intelligent than the average person just because their brains process information in a different way."-Mia <u>Asking instructor about their view of accommodation use</u>: "Do you think this makes me look like a lesser student?" -Aaron
Feeling/perception of using accommodations or having a disability		Participant describes how they felt about using accommodations, or their perceptions of what accommodation use is like for them in college.	"[Accommodations] level the playing field"-Oakley "Just at the beginning I guess I was nervous about asking, or telling a professor. I wasn't

Feeling/perception of using accommodations or having a disability (continued)			nervous about telling them I had the DRC, just about missing class and asking for something different."-Jake
Instructor supports self-advocacy		The instructor supports participant self-advocacy by being perceived as approachable, when the instructor affirms use of accommodations in their course, and when the instructor helps the student use their accommodations in the course.	<u>Instructor affirms accommodation use:</u> "He kind of said like with [my upper-division math course] time isn't a concern, because you can solve a problem for years, so I shouldn't be worried about it. So, he gave me… a concrete example of like why I shouldn't be worried." -Aaron
Communication		Communication for the purpose of self-advocacy involves "negotiation, assertiveness, and problem-solving in a variety of situations" (Test et al., 2005, p. 50). <u>Note</u>: This code includes stories participants tell about self-advocating, so it may be double-coded with self-advocacy with instructor or self-advocacy with DRC. Also relates to disclosure of disability codes.	"I have to consider professor to professor, how the best way to communicate with the professor. It is not particularly that I changed my form of advocacy, it's more that I recognize that each professor is going to respond in a different way or each professor is going to respond in a form of communication in a different way, which would be better. Like I said, my statistics professor really doesn't do face to face. I've just gotten that vibe from him, so it's I recognize that if I'm to advocate for myself, it's best if I do it over email. Whereas in ecology and biochemistry, the easiest way for me to advocate for myself is to be there in person and discuss with them in person, because that's just how they best communicate."-Mia
	Self-advocacy with instructor	Real-life example of participant self-advocating to a STEM instructor. <u>Note</u>: The instructor must know they use accommodations in their course to apply this code.	"I went up to him [a STEM instructor] and I said, 'I'm struggling to finish these pop quizzes, this is stressful. I'm set up with extra time for my tests. Is it possible for there to be any sort of way to get extra time on these quizzes?' At first he said no, and I was like, 'I'm not finishing these, I'm stressed out,' and he said, 'Okay, the best I can do is putting your paper down first and then picking yours up last,' and I said, 'I will do it, sounds good."-Claudia
	Self-advocacy with DRC	Real-life example of participant self-advocating with the DRC.	"It was during the first exam. I didn't do as well as I normally did previously in high school.

Communication (continued)		Note: This goes beyond just the general process of signing up for accommodations using the DRC portal each semester. Use this code when they describe going back to their coordinator to adjust existing accommodations, or to request new accommodations.	When I went back and looked over the exam, I realized it's some of the reading mistakes I make, and the format of the exam was on the computer. Normally in high school, since everything was on paper, I could go back and highlight and underline and help myself focus. I wouldn't make as many reading mistakes. So then when I realized that was the problem, I went back to my DRC coordinator and I talked to her about it and then we got printed written exams."–Henry
Disclosure of disability		Participant describes how much they tell their STEM instructors about their disability and their rationale for this level of disclosure.	See subcodes below.
	Level of disclosure	Participant explains how much they tell their STEM instructor about their disability, or accommodation use.	Interviewer: Do you typically tell them [STEM instructors] that you have dyslexia? Mia: Generally, I do. Obviously I'm not shy at all about having a disability…
	Why disclose?	Participant explains their rationale for disclosing their disability to their STEM instructors.	"I feel like if a teacher understands what disability I have, they can better comprehend what I may be needing from them in a course. Often, I'm just like, "I have dyslexia. It is what it is. I have these accommodations and if you have questions, you then let me know." Instead of just being like, "I have a disability. Let me leave this very vague. I have a disability and I have accommodations." To me, it just is easier if they know what I have so that they can work with me."-Mia
	Why not disclose?	Participant describes their rationale in deciding not to tell their STEM instructors they use accommodations, or why they do not tell them about their specific diagnosis.	Describing why she doesn't tell her STEM instructors more information than what is provided in the official accommodation letter. "I don't feel the need to tell the [instructor] because what are they really going to do with that information? They're gonna go on and teach their 300-person class the same way they're teaching it."-Opal

Participant definition of self-advocacy		Apply code when the participant says their personal definition of self-advocacy. Note: If the participant is unfamiliar with the exact term self-advocacy is, code the exchange between the interviewer and the participant.	"It means to me being able to verbalize and identify areas that I might not be strong in that I will need assistance in, and not being ashamed in needing the assistance. I think just being able to discuss, have a conversation with somebody about what your needs are…"-Mia "Just basically speaking up when you have an issue with something, even if the people around you don't necessarily have the same issue."-Oakley
Leadership		"An awareness of the common needs and desires of others, working with others, group dynamics and responsibilities" (Test et al., 2005, p. 50)	See subcodes below.
	Advocates for others with disabilities	Taking action for others with diagnosed disabilities to overcome stigma.	"I wanted to find a genetic marker to correlate with people who had been diagnosed with ADHD and I actually found one in a very small population size. But the whole reason I did that was because I wanted to reduce the stigma around ADHD." -Oakley
	Advocates for peers to be tested to receive accommodations	Taking action and advocating for peers without formally diagnosed disabilities to be tested to receive academic accommodations.	Informing/encouraging peer to be formally tested for ADHD: "My girlfriend felt like she was experiencing some symptoms [of ADHD] as well, especially after I did my [official testing]. I was telling her some of the stuff the [psychologist] said... they gave her a scholarship to pay for the [official] testing."-Carter
Filling gaps		Participants taking action to mitigate a perceived limitation in either their formal accommodations from the DRC, or a perceived limitation in the instructional practices used in a STEM course.	Developing own note-taking system "So, I have in my ecology class, I have a Google document that I take notes on, but they [other students in class] also will take notes on. So, if one of us misses something and another person will go ahead and just type it in or fill it in. So,

Filling gaps (continued)			that's been really beneficial, because she [the instructor] goes so fast. So, we can't always catch everything and so by having those other people be able to be there, then you're not missing anything. It's the same concept [as requesting a DRC notetaker].”-Mia “I do my own form of accommodating by having another support system that is not the DRC that I can fall back on.” -Heath Seeking out tutoring instead of going to office hours when instructor is not perceived as approachable “So in order to understand course material... I go to tutoring, both through the Department of Academic Enhancement [a place of free tutoring on-campus], and a third-party tutoring that I pay for if I need it. I just do what I need to do to do well in the class.”-Oakley

Note: Codes related to a subsequent study are redacted.

CHAPTER 7

INSIDE AND OUT: FACTORS THAT SUPPORT AND HINDER THE SELF-ADVOCACY OF UNDERGRADUATES WITH ADHD AND/OR SPECIFIC LEARNING DISABILITIES IN STEM[1]

[1] Pfeifer, M. A., Reiter, E.M., Cordero, J.J., Stanton, J.D. (2021). Inside and out: Factors that support and hinder the self-advocacy of undergraduates with ADHD and/or specific learning disabilities in STEM. *CBE—Life Sciences Education* 20.2 (2021): ar17. Reprinted here with permission of the publisher.

Abstract

Self-advocacy is linked to the success and retention of students with disabilities in college. Self-advocacy is defined as communicating individual wants, needs, and rights to determine and pursue required accommodations. While self-advocacy is linked to academic success, little is known about how students with disabilities in STEM practice self-advocacy. We previously developed a model of self-advocacy for STEM students with attention-deficit/hyperactivity disorder (ADHD) and/or specific learning disabilities (SLD). Here, we use this model to examine what factors support or hinder self-advocacy in undergraduate STEM courses. We conducted semi-structured interviews with 25 STEM majors with ADHD and/or SLD, and used qualitative approaches to analyze our data. We found internal factors, or factors within a participant, and external factors, the situations and people, described by our participants, which influenced self-advocacy. These factors often interacted and functioned as a support or barrier, depending on the individual and their unique experiences. We developed a model to understand how factors supported or hindered self-advocacy in STEM. Supporting factors contributed to a sense of comfort and security for our participants, and informed their perceptions that accommodation use was accepted in a STEM course. We share implications for research and teaching based on our results.

Introduction

Despite an overall increase in the number of students with disabilities enrolling in postsecondary education, students with disabilities remain underrepresented in science, technology, engineering, and mathematics (STEM) majors (National Science Foundation, 2019). The underrepresentation of students with disabilities in STEM majors is not due to a lack of interest in STEM. We know that students with disabilities are as likely as students without disabilities to initially pursue a STEM major, however, relatively few students will graduate with a STEM degree (Lee, 2011, 2014). The reasons relatively few students with disabilities graduate from STEM majors are not clear. Students with disabilities, regardless of major, encounter many barriers in college. By developing our understanding of these barriers, we can begin to address and mitigate the barriers students with disabilities experience, leading to increased representation of students with disabilities in STEM.

One of the most profound barriers students with disabilities in college encounter is the shift in legislation guiding the accommodation process (Eckes & Ochoa, 2005). In high school, educational laws such as the Individuals with Disabilities Education Act mandate that school personnel identify and accommodate students with disabilities (Smith, 2001). The goal of these educational laws is academic success of students with disabilities. In college, two civil rights laws, the Americans with Disabilities Act and Section 504 of the Rehabilitation Act of 1973 require students themselves to take sole responsibility for their own accommodations (Eckes & Ochoa, 2005). The purpose of these civil rights laws is equal access to educational opportunities. The differences in these laws becomes important because for many students with disabilities, college is the first time they have been responsible for their own accommodations, which can lead to difficulty in accessing and utilizing them (Getzel & Thoma, 2008; Hadley, 2007). Understanding the factors that promote or hinder students from using their

accommodations is needed to inform development of university policies, classroom pedagogies, and other practices that support retention of students with disabilities in STEM.

Self-Advocacy. While accessing and using accommodations in college can be challenging for students with disabilities, many students with disabilities access and use accommodations effectively in their courses. Accessing and using accommodations in college is related to self-advocacy (C. Dunn, Rabren, Taylor, & Dotson, 2012; Getzel & Thoma, 2008; Hadley, 2007; Pfeifer, Reiter, Hendrickson & Stanton, 2020). Self-advocacy is defined as "the ability to assertively state wants, needs and rights, determine and pursue needed supports" and to obtain and evaluate the needed support with the ultimate goal of conducting affairs independently (Martin & Marshall, 1995; Izzo & Lamb, 2002, p. 6). Self-advocacy is linked to higher GPAs, increased graduation rates, and is considered to be essential in the overall success of a student with a disability in college (Getzel & Thoma, 2008; Hadley, 2007; Janiga & Costenbader, 2002; Kinney & Eakman, 2017; Kreider et al., 2018; Lombardi, Gerdes, & Murray, 2011). Enhancing self-advocacy is a promising way to reduce attrition of students with disabilities from STEM majors considering the link between self-advocacy and success (C. Dunn et al., 2012; Lee, 2011).

Current research indicates that STEM courses can be challenging places to practice self-advocacy. STEM courses possess specific barriers in terms of content, including the informational materials required or instructional approaches used to participate or understand topics taught in a STEM course, and climate, including the quality and the nature of interactions in the course, for students with disabilities (C. Dunn et al., 2012; Hedrick, Dizen, Collins, Evans, & Grayson, 2010; Isaacson, Srinivasan, & Lloyd, 2011; Isaacson & Michaels, 2015; Moon, Todd, Morton, & Ivey, 2012; Ofiesh, 2007; Tuosto et al., 2020). For example, a recent systematic literature review found the

adoption of universal design for learning, a principle touted to be one of the best ways to ensure accessible course content, is minimal in college STEM courses (Schreffler, Vasquez Iii, Chini, & James, 2019). Furthermore, STEM students with disabilities may be less likely than their counterparts in other majors to use accommodations in their courses, and accommodation use varies by type of disability (Lee, 2011, 2014; Newman et al., 2011). For instance, students with learning disabilities are less likely than students with other types of disabilities to use accommodations in their courses (Newman et al., 2011). The mechanisms contributing to these phenomena are not yet fully characterized. However, it is proposed that students in STEM majors experience more barriers accessing accommodations than their non-STEM counterparts (Lee, 2011; Lee, 2014). Thus, undergraduate STEM courses likely represent a context in which students with learning disabilities experience issues practicing self-advocacy in the face of many factors that can function as barriers to learning and inclusion.

Although self-advocacy is recognized as important for success in college, our understanding of self-advocacy is still developing. Self-advocacy was originally derived from self-determination theory for people with disabilities (Test, Fowler, Wood, Brewer, & Eddy, 2005; Wehmeyer, Abery, Mithaug, & Stancliffe, 2003). A conceptual framework for self-advocacy was developed, which demarcated self-advocacy as a separate construct from self-determination (Test et al., 2005). The original self-advocacy framework was developed based on meta-analysis of existing research related to self-advocacy at that time, along with the input of self-advocacy stakeholders. Research included in the analysis varied in terms of context and participant characteristics. Once this framework was developed, it was not empirically tested to determine if, and to what degree the framework explained the self-advocacy experiences of college students with disabilities. Many of the subsequent studies of self-advocacy in college students with disabilities utilized this framework without first determining if the framework applied to their target

populations. This is problematic because experiences of disability are not universal. For example, the experience of someone with a physical disability is much different than the experience of an individual with an invisible, or non-apparent disability (Daly-Cano, Vaccaro, & Newman, 2015; Vaccaro, Kimball, Wells, & Ostiguy, 2015). Additionally, the climate a student with a disability encounters in college is known to influence their perceptions of acceptance, which likely influences self-advocacy (Harbour & Greenberg, 2017; Hedrick et al., 2010; Stodden, Brown, & Roberts, 2011). In sum, existing self-advocacy research may be missing or overemphasizing aspects of self-advocacy that are not relevant to particular groups of college students with disabilities in certain academic contexts, such as STEM.

Because self-advocacy is considered to be essential in the success and retention of college students with disabilities, we sought to study self-advocacy within the context of undergraduate STEM courses. We previously conducted an empirical study to test and revise the existing conceptual model of self-advocacy, based on the experiences of STEM majors with attention-deficit/hyperactivity disorder (ADHD) and/or specific learning disorders, also referred to as specific learning disabilities (Pfeifer et al., 2020). We decided to study self-advocacy in this group of students because ADHD and SLD are two examples of a non-apparent disabilities, they are both common in college students, they often co-occur, and they share many similar features though they are distinct disability types (Budd, Fichten, Jorgensen, Havel, & Flanagan, 2016; DuPaul, Gormley, & Laracy, 2013; Pham & Riviere, 2015; Raue, Education, Statistics, & Lewis, 2011; Wolf, 2001). ADHD is divided into two major subtypes, predominantly inattentive and predominantly hyperactive/impulsive (American Psychiatric Association, 2013). A feature of ADHD predominantly inattentive is experiencing difficulty in remaining focused throughout daily life, while a feature of ADHD predominantly hyperactive/impulsive is extreme restlessness that may appear as intrusive behaviors, e.g., excessive talking

(American Psychiatric Association, 2013). SLD are made of three major subtypes, impairment in reading (dyslexia), impairment in written expression (dysgraphia), and impairment in mathematics (dyscalculia) (American Psychiatric Association, 2013).

From our previous in-depth qualitative analysis, we found that self-advocacy for students with ADHD and/or SLD (ADHD/SLD) in STEM was more complex than posited in the original self-advocacy framework (Pfeifer et al., 2020). In our previous study, we revised the original framework to develop our model of self-advocacy. We use this model to define self-advocacy in our current study (Figure 7.1). Both studies analyze data collected from the same participants. In this study, we examine how the contextual factors of our participants influence the components of self-advocacy from our model.

In our model, self-advocacy is comprised of self-advocacy knowledge, self-advocacy beliefs, and self-advocacy behaviors. Self-advocacy knowledge involves knowledge of self, rights, accommodations and the process to obtain them, as well as STEM learning contexts. Self-advocacy beliefs include view of disability and agency, the belief that a student with a disability is responsible for their own accommodations and success in college. Self-advocacy behaviors encompass communication, which is required for self-advocacy, filling gaps, and leadership. Filling gaps are the actions students take to mitigate a perceived limitation in either their formal accommodations, or in the instructional practices used in a STEM course. Each part of our model of self-advocacy is defined in Table 7.1. In our model, we see accommodation use as one possible manifestation of self-advocacy, which can enhance the academic success of students with ADHD/SLD leading to increased retention in STEM majors.

Table 7.1. Definitions of self-advocacy components from our model of self-advocacy for students with ADHD and/or SLD (ADHD/SLD) in undergraduate STEM courses. Communication is bolded because it is required for self-advocacy. [a] Indicates a definition from Test et al., (2005). [b] Indicates a definition from Pfeifer et al., (2020).

Self-advocacy component	Definition
Knowledge of self [a]	Awareness of individual strengths and weaknesses as a learner with a disability.
Knowledge of rights [a]	"Knowing one's rights as a citizen, as an individual with a disability, and as a student receiving services under federal law" (Test et al., 2005, p. 50).
Knowledge of STEM learning contexts [b]	Awareness of the learning environment experienced by students with ADHD/SLD in undergraduate STEM courses, which influences accommodations needs. STEM learning contexts discussed by our participants include: STEM lecture courses, laboratory courses, laboratory sections of a STEM course, discussion or recitation sections of STEM courses, online STEM courses, independent research experiences in academic labs, and internships with local STEM companies.
Knowledge of accommodations [b]	Awareness of: (1) accommodations that are available to a student with ADHD/SLD, and (2) how the accommodation process in college works, including knowledge of the student role, the DRC coordinator role, and the instructor role.
Communication [a]	Communication for the purpose of self-advocacy involves "negotiation, assertiveness, and problem-solving in a variety of situations" (Test et al., 2005, p. 50).
Leadership [b]	Taking action for others with diagnosed disabilities to overcome stigma, and advocating for peers without formally diagnosed disabilities to be tested to receive accommodations.
Filling gaps [b]	Taking action to mitigate a perceived limitation in either formal accommodations, or in the instructional practices used in a STEM course.
View of disability [b]	Individual student view of their own disability and their perceptions of how STEM instructors and peers view disability and accommodation use in the context of undergraduate STEM courses.
Agency [b]	Belief that a student with a disability is responsible for their own accommodations and success in college.

Theoretical Framework. Our study is also guided by a broader theoretical framework, the social model of disability (Berghs, Atkin, Graham, Hatton, & Thomas, 2016; Haegele & Hodge, 2016). We selected this framework because it offers a clear conceptualization of disability and how social contexts, such as undergraduate STEM courses, contribute to the formation of disability. The social model of disability separates impairment from disability. Impairments are biological differences, such as ADHD/SLD. Disability is the hardship that arises within a context due to societal expectations of an individual with an impairment. The social model of disability posits that an impairment does not equate to disability unless a societal expectation makes the impairment tangible. For example, a student with ADHD/SLD may not experience their impairment as a disability until they encounter an expectation in their STEM course that makes their impairment evident. One example of such an expectation could be completing a written exam within a relatively limited amount of time. If this type of expectation causes hardship, the student now experiences disability. From the perspective of the social model, a biological difference does not need to be "cured" to address disability, rather changes to the social context can be made. The other reason we used the social model of disability is because self-advocacy can mediate the relationship between impairment, disability, and the social context (Goodley, 1997). That is, an individual with an impairment can engage in self-advocacy to improve their own conditions within a social context, and mitigate hardship due to disability. Thus, the social model of disability empowers individuals with impairments to practice self-advocacy.

Throughout our paper, we use person-first language, which purposefully emphasizes an individual and not their disability towards preserving human dignity (D. S. Dunn & Andrews, 2015). We acknowledge that person-first language is not always the preferred terminology of all individuals with disabilities (D. S. Dunn & Andrews, 2015; Sinclair, 2013). As reviewed in D.S. Dunn & Andrews (2015) some people feel that using

person-first language emphasizes disability as a negative aspect of human experience. While other people prefer identity-first language because they do not view disability to be shameful and embrace this characteristic as part of themselves, among other reasons. We use person-first language here for two reasons: (1) person-first language was used by most of our participants when discussing their own disability, and (2) because person-first language remains the preferred style guideline by many professional associations, such as the American Psychiatric Association.

Current Study. In our current study, we expand on our prior work by characterizing the factors that support or hinder self-advocacy for 25 students with ADHD/SLD who were STEM majors. By conducting this research, we aim to enhance the self-advocacy experiences of students with ADHD/SLD in undergraduate STEM courses as a mechanism for retaining of students with disabilities in STEM. In our current study, we investigated the following research question: What factors influenced the self-advocacy of our participants in undergraduate STEM courses?

Methods

Context of study. This study was conducted at a public university in the southeastern United States with highest research activity. Our study was approved for exempt status by the University of Georgia Institutional Review Board (STUDY00004663). All participants in our study were STEM majors who were registered with the university's Disability Resource Center (DRC), and eligible to receive services for either ADHD and/or SLD (ADHD/SLD) as their primary or secondary condition. Given the similar nature of ADHD and SLD (see Introduction) we reasoned these groups of students would have similar self-advocacy experiences. We recruited students who were STEM majors, as opposed to life science majors specifically. Although, life science majors outnumber other STEM majors at the institution where data collection took place. This work is a component of a larger study about self-advocacy of students with

ADHD/SLD in undergraduate STEM courses. For additional analysis of these data, please see Pfeifer et al., 2020.

Overview of the accommodation process for participants. The accommodation process at the institution where data collection took place is an important part of this study's context. Students submitted official documentation of their disability to the DRC to be reviewed and approved. Students were then assigned to a specific DRC coordinator, and asked to schedule an initial accommodation meeting. In the initial accommodation meeting, students and their DRC coordinator agreed to the accommodations the student would be eligible to request in their courses, and the DRC coordinator explained how the student will request accommodations using an online accommodation system. All official accommodation letters were sent to instructors through the online accommodation system, once the student selected the accommodation(s) they would use in a particular course. Instructors must acknowledge receipt of the letters. Students were only required to meet with their DRC coordinator once during their college career, unless the student initiated further meetings or communication.

Data collection. Data were collected using semi-structured interviews and a short demographic survey. Participant demographics are summarized in Table 7.2. Participants were recruited in partnership with the university's DRC to preserve confidentiality of all registered students in Fall 2018 and Spring 2019. We previously described our detailed methods, including participant recruitment, and development of our interview protocol (Pfeifer et al., 2020; Supplemental File 7.1). DRC coordinators forwarded a standard recruitment email to all eligible participants on their caseloads and students interested in participating in the

Table 7.2. Summary of participant demographic information. This table is modified from our previous publication Pfeifer et al., (2020), a *Springer* publication.

Participants (n=25)	**Number (%)**
Gender	
Female	11 (44%)
Male	14 (56%)
Race	
White	23 (92%)
Black or African American	2 (8%)
STEM major	
Life Sciences	13 (52%)
Engineering	7 (28%)
Physical Science	2 (8%)
Mathematics	2 (8%)
Computer Science	1 (4%)
Year in college	
First year	3 (12%)
Second year	3 (12%)
Third year	8 (32%)
Fourth year	4 (16%)
Fifth year	5 (20%)
Sixth year +	2 (8%)
Participant diagnoses	
ADHD	15 (60%)
Specific Learning Disability	5 (20%)
ADHD and Specific Learning Disability	5 (20%)
Time of Official Diagnosis	
College	8 (32%)
Before College	17 (68%)
Type of High School Attended	
Public	14 (56%)
Private	11 (44%)
Other	
Transfer students	6 (24%)
First-generation students	2 (8%)
Pell grant recipients	5 (20%)

study then contacted the research team directly. We used this approach because we reasoned students currently registered with the DRC would be more likely to engage in self-advocacy at the time of data collection. We see registering with the DRC as a prerequisite for use of accommodations in STEM courses, which is one prominent way a student demonstrates self-advocacy (Figure 7.1). However, use of accommodations is

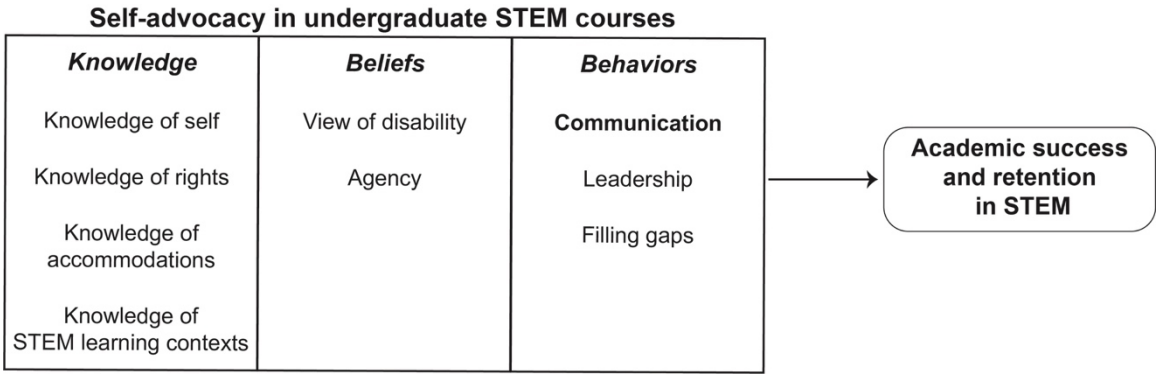

Figure 7.1. Our guiding model of self-advocacy for students with ADHD and/or SLD in undergraduate STEM courses. Each component of the self-advocacy model is defined in Table 7.1. These components are aspects contributing to self-advocacy in undergraduate STEM courses. Knowledge, beliefs, and behaviors are not intended to be linear, i.e., it is not yet clear if knowledge leads to beliefs which leads to behaviors. Communication is bolded because it is essential for self-advocacy. One possible product of self-advocacy is accommodation use in a STEM course. Self-advocacy likely enhances academic success and retention of students with ADHD/SLD in STEM majors (arrow and rounded-edge box). Figure 7.1 modified from Pfeifer et al., (2020).

not the only manifestation of self-advocacy and it is possible that students with ADHD/SLD not registered with the DRC also possess self-advocacy, although more research is needed to better understand those unique experiences.

Qualitative data analysis. Data were analyzed using MaxQDA 2018 by a diverse research team. Our team consisted of one or more researchers who was/were a STEM major with ADHD/SLD, a researcher with 5 years previous work experience in a DRC at a different university, and a current undergraduate STEM instructor. Coder identities and roles are left anonymous in an effort to preserve confidentiality. We embarked upon our analysis by open coding, also called initial coding (Saldaña, 2015). The goal of our open-coding process was to consider the entirety of our data, and begin identifying the nuances and processes related to self-advocacy. Individual researchers open coded a subset of the interviews, wrote analytic memos following each interview, and then met to discuss emergent ideas as a team. We identified five interviews (i.e., 20% of our data) that represented the range of our data to begin development of our codebook.

Codebook development and subsequent analysis employed the constant comparison method to ensure rigor in our coding (Charmaz, 2006; Fram, 2013). Our deductive codes originated from Test's framework of self-advocacy: knowledge of self, knowledge of rights, communication, and leadership (Test et al., 2005). We developed inductive, or emergent, codes based on the experiences of our participants. Three members of our research team proposed codes after reading the five interviews representing the range of our data. We refined these codes through discussion and careful consideration of which proposed codes aligned to our research questions. We further refined these codes by analyzing another subset of the interviews, and meeting to add, remove, or redefine our existing codes. We then coded interviews individually, and then met as a team to discuss how each researcher applied the codes. Through these iterations, our codebook stabilized. Two researchers then coded all 25 interviews using our stabilized codebook (available in Supplemental File 7.2). The researchers met after coding sets of three to four interviews to discuss coding and to resolve any coding differences. In these meetings, coding differences were resolved, and data were re-coded as needed to code to consensus.

We examined first-cycle codes during second-cycle. We relied on pattern and axial coding to identify themes within our data. Pattern coding involves organizing similar data into themes, and axial coding involves identifying code attributes and determining how these attributes relate (Saldaña, 2015). During our second-cycle coding process, one researcher took the lead in proposing second-cycle codes to the other researchers. We discussed these second-cycle codes, and resolved any disagreements. Feedback on our emergent results was gathered from our research team, and refinements were made to encompass all our perspectives. From our analysis, we identified the factors supported or hindered self-advocacy for students with ADHD/SLD in undergraduate

STEM courses. We organized these factors into a model to explain how self-advocacy is affected in undergraduate STEM courses.

Trustworthiness of study. We sought to establish the trustworthiness of our study by using several techniques. We deliver detailed methods and provide our codebook in Supplemental File 7.2., highlighting our data analysis procedures so that readers may assess our processes (Krefting, 1991; Richards & Hemphill, 2018; Tracy, 2010). Throughout our study all researchers utilized research journals to provide an audit trail of our decisions, and engaged in self-reflexivity by writing analytic memos as a check for individual researcher bias (Johnson, 1997; Richards & Hemphill, 2018; Saldaña, 2015). One particular strength of our study is the use of multiple researchers coding to consensus as a form of triangulation (Olson, McAllister, Grinnell, Gehrke Walters, & Appunn, 2016; Richards & Hemphill, 2018; Tracy, 2010). Coding to consensus by a diverse research team is a rigorous approach for analyzing complex constructs, such as self-advocacy (Olson et al., 2016; Pfeifer et al., 2020; Richards & Hemphill, 2018; Stanton, Dye, & Johnson, 2019). Importantly, our research team consisted of one or more members who was/were a STEM major with ADHD/SLD, which provided essential expertise into the lived experiences of our participants during first and second-cycle coding (Vaccaro et al., 2015). Finally, we provide our readers with a consideration for transferability of our findings (Krefting, 1991).

Results

We identified factors that functioned as a support or as a barrier to the self-advocacy of our participants based on their experiences in undergraduate STEM courses. We considered self-advocacy to be supported when our participants described factors that encouraged or reinforced their self-advocacy behaviors. Conversely, self-advocacy was hindered when our participants described factors that discouraged or thwarted their self-advocacy behaviors or accommodation use. In our analysis, self-

advocacy behaviors included communication, filling gaps, and leadership. We view accommodation use as one possible product of self-advocacy. Our participants described factors within themselves that influenced their own self-advocacy. We call these **internal factors**. Participants also shared with us how the situations and people they encountered as a student with ADHD/SLD in undergraduate STEM courses influenced their self-advocacy. We refer to these situations as **external factors**. Our data revealed how internal and external factors interact, and how these factors and interactions affected the self-advocacy of our participants.

Internal factors. First, we describe how internal factors functioned as a support or as a barrier to the self-advocacy of our participants. Internal factors included: self-advocacy knowledge, self-advocacy beliefs, and identity. We describe data demonstrating how self-advocacy knowledge and self-advocacy beliefs support or hinder self-advocacy. We close our internal factors section by sharing data which illuminate the complexity of individual identity, and how this identity influenced the self-advocacy of our participants. All of our participants are represented by pseudonyms. Quotes have been lightly edited for clarity. Ellipses represent language removed from the participant's quote for brevity.

Self-advocacy knowledge. Self-advocacy knowledge is comprised of knowledge of self, knowledge of rights, knowledge of accommodations, and knowledge of STEM learning contexts (Figure 7.1; Pfeifer et al., 2020). We define STEM learning contexts as the various learning environments an undergraduate student encounters during their college career as a STEM major. Our participants discussed their self-advocacy experiences in STEM lecture courses, laboratory courses, laboratory sections of a STEM course, discussion or recitation sections of a STEM course, online STEM courses, independent research experiences in academic labs, and internships with local STEM companies. In our previous study, we found that self-advocacy knowledge varied

between our participants (Pfeifer et al., 2020). In this study, we sought to understand how self-advocacy knowledge supported or hindered self-advocacy beliefs and self-advocacy behaviors in undergraduate STEM courses.

We found that self-advocacy knowledge supported other aspects of self-advocacy when our participants displayed what we termed sufficient self-advocacy knowledge. Participants demonstrated sufficient self-advocacy knowledge when they explained how their knowledge of self, knowledge of accommodations, and knowledge of STEM learning contexts influenced their accommodation decisions, or decisions to discuss an accommodation issue with a STEM instructor. One participant, Mia, explained how her self-advocacy knowledge supports her self-advocacy, “I'm aware of what [accommodations] I need and I'm aware of where this disability affects me. So, why not [communicate that to my STEM instructors]?” Many participants displayed sufficient self-advocacy knowledge, and this knowledge supported their self-advocacy. When participants demonstrated sufficient self-advocacy knowledge, they were aware of how to procure accommodations in a variety of STEM learning contexts, including the laboratory section of a STEM course, and to troubleshoot accommodation issues that occurred during the course of the semester. They were also aware that if they found an accommodation to no longer meet their learning needs, that they could communicate with their DRC coordinator to explore adjusting the ineffective accommodation. Although many of our participants demonstrated sufficient self-advocacy knowledge, some of our participants were still developing this knowledge.

We found examples of when insufficient self-advocacy knowledge hindered our participants’ self-advocacy. Insufficient self-advocacy knowledge occurred when participants described that they were not aware that they could request adjustments to their accommodations if they found an accommodation inadequate. We also identified instances when participants held inaccurate ideas about the accommodation process,

which hindered their self-advocacy. One example of this came from Megan who shared that she did not know how much information about her disability was included in the official notification letter sent to her STEM instructors by the DRC on her behalf.

> "I wish I knew what the DRC was sending [my STEM instructors] because I guess I've always assumed that they were being informed of what my disability is, and I would rather that be how it works…."

Megan's assumption that her STEM instructors already knew what her disability was hindered her self-advocacy because it caused miscommunication when she met one-on-one with her instructors, which could lead to confusion. Official accommodation letters sent to instructors do not disclose disability diagnoses. Megan described a time she misread an exam question, and wanted to meet with her STEM instructor to talk about why she missed points on the exam. Because Megan thought her instructor knew she had dyslexia from the official notification letter, she assumed they would understand why she misunderstood the question on the exam. Thus, Megan's insufficient self-advocacy knowledge hindered her self-advocacy.

Some participants also reported other inaccurate ideas about accommodations. Participants told us that accommodations were not available in online courses, accommodations were not available in summer courses, and that accommodations were not available at smaller, two-year colleges. These were our participant's perceptions and we do not know why our participants held these inaccurate ideas. We did inform these participants that students with ADHD/SLD are legally entitled to accommodations in these instances at the end of the interview. Additionally, a few participants told us that they thought that the only way they could request accommodations was to directly disclose their exact disability diagnosis(es) to a STEM instructor in a one-on-one meeting. Several participants reported that they currently thought or, at one time earlier in their college careers, had thought accommodations were not available in the lab

section of a STEM course. The inaccurate idea that accommodations were not available in lab sections of STEM courses was common in students who recently start college, and participants who recently started using accommodations for the first time in college.

During our analysis, it became apparent that self-advocacy knowledge was often tied to self-advocacy beliefs. Participants with sufficient self-advocacy knowledge tended to display beliefs about themselves that positively influenced self-advocacy, and participants still developing their self-advocacy knowledge tended to display beliefs that did not support their self-advocacy. We explain how self-advocacy beliefs functioned as both a support and a barrier for our participants in the next section.

Self-advocacy beliefs. Self-advocacy beliefs are comprised of view of disability and agency. We define agency as a participant belief that they are responsible for their own accommodations and success in college. Participants who tended to view their disability in a positive manner and demonstrate agency engaged in more self-advocacy, as we previously reported (Pfeifer et al., 2020). These participants appeared to be more willing to seek information about their disability or accommodations when they encountered a problem, which likely supported their development of self-advocacy knowledge. None of our participants displayed a positive view of disability with low agency.

Participants who tended to view their disability negatively or in a conflicted manner, appeared to struggle to practice self-advocacy. Ryan explained that in high school he felt his disability was a “threat to being normal…with my peers,” and that he mainly used accommodations in college because his mom recommended it. He stated, “technically, I have a disability, even though I’m kind of embarrassed about it.” Ryan was a participant who appeared to still be developing sufficient self-advocacy knowledge because he held many inaccurate ideas about the accommodation process. Other participants, like Aaron and Judd, also tended to view their own disability negatively,

while several others appeared to be conflicted about their disability, and this view could also make self-advocacy challenging.

Participants who did not display agency appeared to struggle to engage in self-advocacy behaviors in their STEM courses. This was most evident in Dana, a sixth-year student, who was registered with the DRC, but had never used accommodations in college. She stated that it took her several semesters to register with the DRC because she “kept forgetting.” She further stated, “So yeah, ask the ADHD kid to go get accommodations. You know, my mom used to just do it [for me] when I was in high school.” Dana appeared to still be developing her agency, and was an example of someone who experienced major challenges in the transition from high school to college in terms of accommodations. Dana candidly stated that she had struggled in college because of her decision to not use accommodations. She expressed that she wished she would have used accommodations sooner. Dana also held many inaccurate ideas about accommodations and the process to obtain them in college. Although Dana was still developing her knowledge of accommodations, she did display some self-advocacy behaviors, such as filling gaps, when she would ask her close friends to tutor her in her engineering courses.

Our data showed how self-advocacy knowledge and beliefs functioned as both a support and a barrier depending upon the participant. We became curious as to what other internal factors influenced self-advocacy. We found that the identities of our participants were complex, and that the facets of their identity affected their self-advocacy. We explain how the internal factors of identity related to the self-advocacy of our participants in the next section.

Identity. Participants reported that the intersectional nature of their identity (i.e., belonging to multiple groups traditionally underrepresented in STEM in terms of disability, gender and race), could sometimes hinder their sense of comfort to engage in

self-advocacy behaviors within their STEM courses. We acknowledge that we did not design any interview questions to determine how the intersectional nature of identity can also function as a support to self-advocacy. We hypothesize that these dimensions of identity can also function as a support, depending upon the context. We include these ideas because they emerged from our data and they point to the need for instructors to consider the intersectional nature of student identity in their teaching.

The intersectionality of disability, gender, and race in undergraduate STEM courses. Some of our participants described how their perceptions of exclusion from STEM were exacerbated by the intersectional nature of their identity. Two participants, Cassie and Dana, shared that identifying as female in a male-dominated STEM field hindered their self-advocacy. When we asked Cassie, who is a female Physics major, what factors prevent her from communicating for the purpose of self-advocacy, she responded, “Just in general I get intimidated by specifically older men in an authoritative position, which is the majority of my professors.” Cassie explained that she already found it challenging to talk about her disability and accommodation use in general, and this discomfort was amplified because she is also female. For Cassie it was more challenging to talk to her STEM instructors because they are mostly men. Another participant, Dana, explained that as a female Engineering major, she does not want her male peers to know she uses accommodations because she thinks they will see it as a weakness. “Guys… they're so judgmental in engineering. They think every girl's dumb and they treat you as such.” Dana expounded that one reason she has never used accommodations in college, although she is registered with the DRC, is because she does not want her peers to find out she has ADHD.

One participant, Carter, shared that the intersectional nature of his identity can complicate his self-advocacy. Carter, an African American male student with ADHD at a

predominantly white university, reported that he perceives his peers to think less of him. When we asked him why, he stated,

> "Being African American…is very, very, very hard in undergraduate STEM courses because these people, they already think less of you regardless…Then for you to be going and getting accommodations…that just puts the icing on the cake."-Carter

Carter spoke to the intricacy of his identity. He felt that peers already thought less of him because of his racial identity, and this negative feeling is magnified because he also has ADHD, and uses accommodations in his STEM courses. Carter revealed later in the interview that he rarely, if ever, talks about having ADHD and using accommodations with his peers.

We found the internal factors described by our participants to be complex, and often interconnected. Participants explained to us how the situations they encountered as a student with ADHD/SLD influenced their own self-advocacy knowledge, self-advocacy beliefs, and contributed to the formation and understanding of their own identity within a STEM context. In the following section, we demonstrate how external factors supported or hindered self-advocacy based on the experiences of our participants.

External factors. Our participants shared a myriad of situations and interactions with people, which we call external factors, that encouraged, discouraged, or in extreme cases prevented self-advocacy behaviors and accommodation use in their undergraduate STEM courses. External factors included other individuals, the logistics of accommodation implementation, classroom environment, and the norms and values of the STEM discipline. Throughout this section, we present data demonstrating how an external factor functioned as a support or as a barrier to the self-advocacy of our participants. We emphasize that these data are from the point-of-view of the participants,

and that our participants' perceptions may or may not reflect the reality of the situation. Yet these perceptions influenced self-advocacy. We begin by examining how other individuals supported or hindered self-advocacy.

Other individuals. During the interview, we asked participants who, if anyone, helped them with accommodations in college. Our participants named several individuals, including peers, family, DRC coordinators, and other professionals who helped them with accommodations in college. When we asked our participants to describe the type of help these individuals provided, we found our participants described two major forms of self-advocacy support: (1) information and advice related to self-advocacy, and (2) emotional support. Information and advice helped participants develop self-advocacy knowledge, and gain skills to more effectively practice self-advocacy in their undergraduate STEM courses. Emotional support encouraged positive self-advocacy beliefs, and promoted positive perceptions of identity. Although other individuals supported the self-advocacy of our participants, many participants discussed situations in which other individuals hindered their self-advocacy. For instance, some participants explained how negative comments from peers functioned as a barrier to their self-advocacy. We explain in the following sections how peers supported or hindered the self-advocacy of our participants. Because families and other professionals were not directly involved in self-advocacy experiences within an undergraduate STEM course, i.e., they were not physically present in the classroom, we report these results in Supplemental File 7.3.

Peers as a support. Participants in our study described how their peers support them in college as a student with ADHD/SLD. In our analysis, we found that peers supported the self-advocacy of our participants by engaging in meaningful conversations about disability and accommodation use. These types of conversations helped our participants feel comfortable discussing their disability or accommodation use with other

people. For example, Tyler described how his friends helped him find humor in a potentially unpleasant situation with an ignorant peer. “Well, what happened was this kid didn’t know what dyslexia was and he thought it was colorblindness. So, now, my friends…if I spell something wrong, they’ll be like, “Oh you’re so colorblind”. So we usually joke about it.” Here, Tyler, felt a sense of comradery with his friends because they shared an inside joke. We interpreted this as a sign he felt comfortable talking to his friends about his specific learning disability in reading.

We also found that other participants, like Jake, shared that their peers responded positively when they disclosed their disability, “As soon as they found out, they were all asking me questions and getting involved.” Jake described that many of his friends are Human Development majors, and they wanted to know more about his experiences as a student with ADHD. Other participants, like Ryan, explained that he felt like he needed to hide his disability from most of his peers. However, the one time he did talk to his friends about it, he described it as “comforting” and a “great feeling” because they “supported me.” These examples underscored that peers supported self-advocacy by making our participants feel like they can talk openly about having a disability.

Our participants also shared more specific examples of how their peers supported their self-advocacy. For some participants, their peers encouraged them to use accommodations in their STEM courses because they could see that the participant would benefit from using them. One of our participants, Kendra, described a time when her peers supported her self-advocacy directly, by helping her figure out who she should communicate with when she forgot to schedule a final exam at the DRC her freshman year of college.

> “The night before I was panicking. The day of I was panicking, freaking out, and my friends helped me figure out what to do. They were like, ‘Here, you should go and talk to the DRC office, even if you can't get your accommodations. And then

> if you do get them, that's great. And if you don't get them, then you should go to your instructor.'"-Kendra

Kendra's friends supported her self-advocacy because they helped her make a plan to deal with an accommodation issue. Interestingly, we found that our participants especially valued the support of peers who have the same disability and accommodations. Kirsten expressed that she appreciates being part of an undergraduate research lab because many of her peers also have ADHD. She stated,

> "There's actually a small group of us that all have ADHD…we're all on the same medication, and we all do the same things, so we talk and we vent to each other a lot about any frustrations we have…It's kind of nice to have that there."-Kirsten

Kirsten found that her peers with ADHD understand what it is like to have ADHD as a student who is a STEM major, and that this peer group is a support of her self-advocacy. Heath reported receiving support for self-advocacy from his roommate who also has ADHD. Heath and his roommate reminded each other to schedule exams at the DRC, and preferred to study together because they can both study for several hours at a time without taking any breaks. Other participants, like Opal, reported that she studies in a group with two other people who also take extended time exams at the DRC. Opal stated that she feels better about going to the DRC to take her exams because she can go with her peers. She explained that having friends who also use accommodations is a support for self-advocacy because it helps her overcome negative comments from other peers who do not believe using accommodations is fair. "I mean, those negative comments are always around, but it's not bothersome because I have two friends who also go to the DRC to take exams." Opal felt more comfortable to use her own accommodations because her friends use accommodations too, and this supports her self-advocacy. While our participants shared many examples of how their peers supported their self-advocacy, there were instances when peers hindered self-advocacy.

Peers as a barrier. Several participants described interactions with their peers that left them feeling that their peers questioned their use of accommodations in their STEM courses, or did not understand their disability. Kirsten stated, “I don't think [most of my peers] take people that test at the DRC particularly seriously, which is sometimes frustrating. I just regularly get called “lucky” for having [accommodations]...I'm like that's not really how it works.” Another participant, Oakley, described when a peer in her STEM course implied that using extra time on exams gave Oakley an unfair advantage.

> “She said [students who use accommodations] are not on the same playing field as everyone else [because they use accommodations]. I said, ‘No, I actually have this diagnosed thing. Here's a report on it.’ And she was like, ‘Well, yeah, a lot of people get diagnosed with ADHD.’"-Oakley

Opal had a similar experience as Oakley. Opal explained that her peer even went as far as discrediting the grade Opal earned on an exam because Opal used extra time. Opal’s peer stated to a group of classmates, “Oh, she gets extra time. No wonder she got a better grade than everyone." These types of negative comments made participants reluctant to discuss their disability or accommodation use openly with their peers.

For many participants, inadvertently revealing their disability or their accommodation use to their peers was a substantial concern. For two participants, Dana and Aaron, this concern was so elevated, they declined to use accommodations in their STEM courses at the time of the interview. Dana, an Engineering major who has never used accommodations in college, shared why she worries about her peers knowing she uses accommodations in her STEM courses,

> “My peers might be my co-workers…I could be in a job with them, and they know my habits…You realize you not only have to impress the professor, you realize you have to impress your peers, too, because they're watching you more so than the professors are.”-Dana

Within Dana's major, upper-division students will often be placed together at internships with local companies and these internships often lead to future employment. Dana explained that she does not want her peers to know that she qualifies for accommodations, because she thinks if they know it would prevent her from finding a job. Another participant, Aaron, who at one point felt comfortable using his accommodations, shared that he now felt self-conscious in his upper-division math classes. This unpleasant feeling influenced his decision to opt out of accommodations in these courses.

> "I experienced like shame, not directly, but just… internally from my peers. Because once you get into like a higher-level math class, you start seeing the same people again, and I always felt self-conscious of not being there in class on the day of the exam."-Aaron

Aaron further explained that one of his peers previously convinced him that using accommodations was unfair, and now he thinks all his peers believe using accommodations is unfair, even if they do not say that directly to Aaron. This perception of peer disapproval is a barrier for Aaron because he stopped using accommodations in his STEM courses.

Some of our participants described defending the use of accommodations to their peers who say accommodations are unfair. Carter stated, "They can think what they want, but I'm still going to do what I need to be a better student." Carter and some other participants responded to negative peer attitudes regarding disability and accommodation use with resilience. This resilience was tied to positive self-advocacy beliefs. Besides peers, the logistics of accommodation implementation influenced the self-advocacy of our participants.

Logistics of accommodation implementation. During the interview, we asked participants to describe a time they decided not to use accommodations in a STEM

course, and to share their rationale for their decision. These data revealed that the way an accommodation is administered influenced the accommodation decisions of our participants. We begin by sharing how accommodation implementation supported the self-advocacy of our participants, and transition into instances in which self-advocacy was hindered.

Logistics of accommodation implementation as a support. The way in which accommodations were implemented supported the self-advocacy of our participants. For instance, a proportion of our participants began using accommodations in college at a time when their university was still utilizing a paper-based system for accommodations. In this system, after the initial accommodation meeting with their DRC coordinator students were required to bring their accommodation notification letters to their STEM instructors in-person. For these participants, they acknowledged that the former paper system “forced” them out of their comfort zone because they had to talk to their STEM instructors. However, they reported that they feel much less stress using the current online accommodation system because they have the choice of face-to-face communication with their STEM instructors. Participants who did not experience the paper-based system also reported that the online accommodation system encourages their accommodation use because it does not require them to talk to their STEM instructors in-person. This is one example of how the logistics of accommodation implementation was reported by our participants to support their self-advocacy. While there were ways in which accommodation implementation functioned as a support, our participants also explained how the logistics of an accommodation hindered their self-advocacy.

Logistics of accommodation implementation as a barrier. We found that the details of how an accommodation was implemented hindered the self-advocacy of some participants. The cost of diagnostic testing to initially register with the DRC was a

logistical barrier encountered by some participants in our study. However, these participants overcame this barrier with help from their families and, in some cases, scholarships. In the following section, we focus on aspects of the accommodation process after initial registration with the DRC that functioned as a barrier.

We found that our participants discussed how the logistics of their notetaking and extended time accommodations compromised their sense of confidentiality. Some participants explained that they forego use of their notetaking accommodations because of the logistics in finding a peer notetaker. In these cases, participants often have to remind their STEM instructors to make an announcement to the class to identify a peer who would provide a copy of their notes to the DRC for participant access. Many of our participants were concerned that the STEM instructor would reveal their identity in the class announcement, or later to the peer who agreed to provide notes. These participants often expressed great concern about their peers finding out they use accommodations in their STEM courses. A perceived loss of confidentiality, or the potential for loss of confidentiality, also influenced participants taking extended time exams at the DRC. Several participants described opting out of their extended time accommodations to ensure their peers do not notice their absence from the classroom on exam day.

Besides issues of confidentiality, our participants explained how other logistical aspects of extended time accommodations hindered their self-advocacy. Some participants explained that they decided not to use accommodations on exam day because they perceived a disparity in the information they could access from the instructor during the exam. For instance, several participants explained that when they take an exam at the DRC, they sometimes do not have all the information they need to complete the exam. One example of this was during a Chemistry exam, the exam provided to the DRC did not have a periodic table because the instructor planned to

project a copy of the periodic table in the classroom. Our participants further reported that they would opt of out of their exam accommodations so they could ask the instructor questions in-person in the classroom on exam day.

These data establish that our participants considered how accommodations were implemented, and the details of this implementation process influenced their self-advocacy. We also found that our participants were especially perceptive of the classroom environment in their STEM courses. In the following section, we describe how the classroom environment of an individual STEM course supported or hindered our participants' self-advocacy.

Classroom environment. Our participants reported that their self-advocacy could be supported or hindered by the classroom environment in a single STEM course. We found that our participants' perceptions of the classroom environment were substantially influenced by STEM instructors, and the policies STEM instructors chose to put in place in their classrooms. In this section, we present data showing how STEM instructors functioned as a support or a barrier to the self-advocacy of our participants.

STEM instructors as a support. In our study, participants shared their perceptions of their STEM instructors, and described interactions they have had with their STEM instructors that positively influenced their self-advocacy. We found that participants designated STEM instructors to be supportive of self-advocacy when they perceived their STEM instructor to be open to listening to their students. Our participants also shared that STEM instructors supported their self-advocacy by directly encouraging accommodation use in their courses. For a few participants, their STEM instructors were the person who first encouraged them to use accommodations in college. Jake explained that his Calculus instructor was the person who connected him to the DRC first because "she realized that during the problems on the exam I was getting distracted." His STEM instructor directly supported his self-advocacy by informing Jake

accommodations were available to him in college, and by helping him start the accommodation process with the DRC. Other participants besides Jake also described instances when their STEM instructors walked them over to the DRC to help them start the accommodation process early in their college careers.

A few participants shared that their STEM instructors supported their self-advocacy by directly affirming use of accommodations in their course. Kendra, shared that STEM instructors supported her self-advocacy by inviting her to contact them if any accommodation issues arise during the semester. For instance, Kendra, reported that some STEM instructors will say, “If there's anything you need, for me to help you, please just let me know." Kendra continued,

> “I've only had a teacher say that to me a couple of times, but it's always kind of relieving…It's just nice to hear, okay yeah, they'll help me out…I had a teacher do that recently, and it was like oh my gosh, thank you so much. You don't understand what this means.” -Kendra

Kendra, and other participants like Aaron, felt extremely concerned about their instructor’s perception of them. Aaron explained that one of his STEM instructors encouraged his self-advocacy by assuring Aaron that his use of accommodations did not make him a “lesser student.” By encouraging students to use accommodations in their course, and to contact the instructor if an accommodation issue occurred, instructors supported self-advocacy.

We found our participants especially appreciated when their STEM instructors planned to provide accommodations for in-class quizzes. For example, Opal described that in one of her STEM courses, her instructor had already approved her accommodation request for extended time exams proctored at the DRC. When the STEM instructor proctored an in-class quiz, he communicated with Opal to explain that he already had a plan in place for her to take in-class quizzes in a way that allowed Opal

to take the quiz in-class and still use extended time in a confidential manner. Opal found this helpful because she did not have to ask him again for extra time on the in-class quizzes, as she sometimes does in other courses.

Our participants also shared that when their STEM instructors follow-up with them about their accommodations it supports their self-advocacy because it shows that the instructor cares about their success in the course. For example, some participants said that when their instructors ask them (in a way that preserves their confidentiality) about issues they may have experienced when taking an extended-time exam at the DRC it supported their self-advocacy. One participant, Mia, reported a unique way one of her former STEM instructors helped her develop self-advocacy. Mia shared that early in her college career, one of her STEM instructors initiated a conversation about an upcoming exam Mia would take at the DRC. The STEM instructor asked Mia if she had any questions for the instructor about the upcoming exam because Mia would take the exam off-site at the DRC, and the instructor would not physically be there to answer questions about the exam. This conversation prompted Mia to ask her instructor about the details she needed to know when she took the exam, like if she needed a Scantron, or a formula sheet. It also helped Mia and the instructor develop a plan to address any questions Mia may have while taking the exam at the DRC. Mia credited this instructor as the person who taught her how to better self-advocate in an undergraduate STEM course.

The experience Mia described taught her to ask her future STEM instructors questions about the exam logistics. She found this practice to be helpful because she was better prepared to take her exam at the DRC. She also reported that it helped her instructors remember she is taking the exam away from the class, without access to announcements or resources they share with the class extemporaneously on exam day. Many STEM instructors supported the self-advocacy of our participants in their courses.

Yet this was not always the case. The next section describes how STEM instructors hindered self-advocacy.

STEM instructors as a barrier. Participants shared experiences when their STEM instructors either inadvertently or blatantly discouraged accommodation use in their courses. Several participants perceived self-advocacy to be more challenging to enact in their STEM courses because of their STEM instructors. Eli, a student with an SLD in reading, reported that his English instructors know more about dyslexia than his STEM instructors. He explained,

> "English instructors realize…the processing speed for people with dyslexia is just slower. So everything just takes more time. It's not just reading and writing that is harder. Getting through everything just takes longer … I feel like the English instructors are more accustomed to having to deal with accommodations so they just know more about it. They know more than the STEM instructors."-Eli

Eli shared that he feels like he has to explain more to his STEM instructors than his English instructors and this requires more self-advocacy. Aaron echoed this sentiment, "STEM instructors are stereotypically colder…you have to do more advocacy, depending on the teacher." Although many of our participants view their STEM instructors as less likely to provide accommodations willingly than instructors in other disciplines, one participant disagreed. Kendra felt that her STEM instructors were "logical and very empirical" so they would understand that the symptoms of ADHD warrant use of accommodations, as noted in our previous paper (Pfeifer et al., 2020). Our participants also shared specific examples of how their STEM instructors hindered their self-advocacy. We describe how STEM instructors likely inadvertently hindered self-advocacy in the next section.

STEM instructors inadvertently hinder self-advocacy. Our participants described specific incidences when their STEM instructors inadvertently discouraged

their self-advocacy. We briefly describe these instances here. We saw that STEM instructors can unintentionally hinder self-advocacy in their choice of language when interacting with students. Kendra shared,

> "My instructor was like, 'Ha, you're so crazy, I'm so happy you're not one of those people on medication and stuff.' …When your instructors say stuff like that, you just have to know that you have gone through a pretty rigorous process of getting evaluated for ADHD and just be confident in what you have, but also in yourself."-Kendra

Kendra explained that she had an amicable relationship with this STEM instructor up to this point, but when her instructor implied that you must be "crazy" if you take medication, she felt less inclined to talk with instructors in the future about her disability and accommodations. For Kendra and other participants, instances like this could be considered examples of disability microaggressions, although none of our participants used the term microaggression to describe these occurrences (Keller & Galgay, 2010).

Our participants also explained that the comments instructors make about the amount of time students should be spending on an exam hindered their self-advocacy. Jake described that he decided not to use extended time on exams in an Engineering class because, "we were taught to go through problems fast and efficient and if you didn't know them you wouldn't figure them out. So, I didn't use accommodations." It is likely that Jake's Engineering instructor did not want to directly discourage Jake from using extended time for his exams when he said students should go through problems quickly. Yet Jake interpreted this language to mean that he should not use his extended-time accommodation.

Another participant, Aaron, shared that he was in a similar situation where his STEM instructor would make general prescriptive statements about how much time students should take to answer questions on the exam, "I had one instructor that was

saying I should know how to do the problems before I come in to do the exam…the instructor said that if you know how to do the problems then it shouldn't take you that much time to complete the exam." Aaron found it very frustrating when instructors tell the entire class how much time a single problem should take to complete. It is possible that the instructors in these cases described by Jake and Aaron were prescribing time guidelines for the perceived benefit of their students. However, it is not clear if these instructors were considering the experiences of all their students, including students who use extended time accommodations into account, when they made these announcements to the class.

We found other factors of the classroom environment that hindered self-advocacy. For instance, instructors may inadvertently hinder self-advocacy by adopting "anti-technology" policies in the classroom. Eli, a student who qualifies to record lectures on his laptop in class, shared that he didn't want to "push the boundary" with his STEM instructor. He knows that technically he could record lectures, but he doesn't want to because, "It's felt kind of awkward being the only one in the room with my laptop up, even though it's probably something I should be doing." Eli explained that he feels he loses his confidentiality when he is the only student using a laptop in the class.

Besides these specific examples of how STEM instructors inadvertently hindered self-advocacy, our participants also described how STEM instructors unfamiliar with their own responsibilities as an instructor in the accommodation process hindered their self-advocacy. Cassie reported, "My math instructor my freshman year, he wasn't being responsive to my accommodation requests. I think he was…new to teaching, so I think he was just confused about what to do." Many of our participants had experiences similar to Cassie with STEM instructors who lacked knowledge of their role as an instructor in the accommodation process. Often times these instances were difficult for our participants to navigate because it was early in their college careers when the

participant was still learning how the accommodation process works in college. Lack of knowledge regarding the accommodation process from both the STEM instructor and the participant made self-advocacy more challenging.

We found that several participants did not initiate communication with their STEM instructors for the purpose of self-advocacy because they did not want to burden the instructor. Henry said that when he is in a large-enrollment STEM course he feels less likely to communicate with his instructor about accommodation issues because he realizes the instructor is responding to the needs of many students. He stated,

> "I feel like if I have an accommodation issue, I just have to deal with it myself because…everyone has a lot of issues and everyone is emailing the professor about something. Sometimes I feel less compelled to speak up for myself because of it."-Henry

Henry explained that he considers the instructor's time before he communicates about an accommodation issue. Other participants, like Dana, reported a similar sentiment. She stated that she does not make accommodation requests, in part, because she does not want to create extra work for her STEM instructor. Dana stated throughout her interview that she sees her instructors are very busy, and not able to prioritize teaching due to demanding research agendas. Her perception was that her instructors do not have time to attend to her accommodation needs because they prioritize conducting research over teaching. Many of our participants discussed how STEM instructors are inadvertently discouraging them from using accommodations or how their perceptions of their STEM instructors hinder their self-advocacy. We found in rare occasions that some STEM instructors blatantly discouraged the use of accommodations in their courses, which hindered our participants' self-advocacy.

STEM instructors discourage use of accommodations. Our participants shared that some STEM instructors discouraged accommodation use in their course,

which hindered our participants' self-advocacy. One example of how STEM instructors discouraged use of accommodations was by violating the privacy of our participants. This often occurred to our participants when their STEM instructors would reveal to the entire class that they were the student needing a notetaking accommodation in the course, or by discussing extended time accommodations with a participant in front of their peers. When STEM instructors violated the privacy of our participants, it made our participants wary of requesting accommodations in other courses in the future because they worried that the next instructor would also disclose their disability status to their peers.

Additionally, STEM instructors blatantly discouraged accommodation use by telling our participants their accommodations would be difficult to implement in their STEM course. One example of a STEM instructor blatantly discouraging use of accommodations came from Henry. Henry, a first-year student, arranged to meet with his Chemistry instructor to ask if he could use accommodations in the lab section of the course. We asked Henry if he planned to formally request accommodations for the lab, he replied, "None of my accommodations will…my Chemistry instructor said they wouldn't work out very well." Here, Henry was practicing self-advocacy. He wanted to learn about the STEM learning context from his Chemistry instructor to determine how he could use his extra-time accommodation for the in-class quizzes at the beginning of lab each week. The conversation with his Chemistry instructor directly discouraged Henry from making a formal accommodation request.

One of the most extreme examples of how a STEM instructor blatantly discouraged accommodation use, and thus hindered self-advocacy, was reported by Mia. Mia explained that at a previous college where she transferred from, her Genetics instructor "really pressured me to take the exam in class, without my extended time accommodation…and that resulted in a really, really low score." Mia reported that her

Genetics instructor did this because he did not consider dyslexia to be a “real disability.” Fortunately, Mia communicated with her DRC coordinator and reported the incident to the DRC. The DRC reminded the STEM instructor that Mia is legally entitled to these accommodations. With the help of the DRC, she was able to access accommodations for subsequent exams in the course.

Finally, our participants reported that their STEM instructors can be negligent of their responsibilities as an instructor in the accommodation process. For example, Opal shared that she encountered difficulty using her accommodations because one of her STEM instructors still had not approved her accommodation request in the online accommodation system for online quizzes. When STEM instructors failed to respond to accommodation requests and follow-up emails from our participants in a timely manner, it left many participants wondering what the lack of response meant. Because we interviewed only students and not instructors, we were unable to determine if instructor negligence was an example of inadvertent or blatant discouragement of accommodation use in their courses. Regardless of intent, it hindered Opal’s self-advocacy. This type of negligence was reported by other participants, as well.

Norms and values of STEM as a discipline. In this section we present data showing how our participants’ perceptions of the norms and values of STEM as a discipline influenced their self-advocacy. We found these data to often connect to internal factors influencing self-advocacy. We first explain how some participants perceived self-advocacy to be a way to show their STEM instructors that they are good students. They viewed STEM as a discipline to value students who worked hard to succeed, and this perception supported their self-advocacy. We then explain how other participants perceived their disability or disabilities to be negatively viewed in the context of STEM as a discipline, and how this perception hindered their self-advocacy.

I am a good student if I self-advocate. For some participants, they see self-advocacy as a way to demonstrate to their STEM instructors that they are a good student who is engaged in the learning process. One example of this perception comes from Claudia. She felt that talking to her instructors about ADHD and accommodation use was a way to show them she is invested in her own learning as a student. Claudia shared that she engages in self-advocacy behaviors because, “it shows that I'm an active student… I seem less like a bad student.” Other participants expounded on this perception by explaining that they want to practice self-advocacy with their instructors because they think their instructors will approve of their effort to succeed in a STEM course. Carter explained that he is comfortable self-advocating with his STEM instructors because,

> “I feel like at the end of the day, especially if my STEM instructors know that I'm receiving these accommodations…and I'm doing well, they will see that I am overcoming [adversity], like outside things that I can't control. I feel like…they'll see you. Like this kid really knows what they're doing. They're really trying their hardest.”-Carter

Carter continued to say that he perceives self-advocacy to be positively viewed by his STEM instructors because it means he is trying to help himself succeed in their course. Not all of our participants articulated this view of self-advocacy. Many of our participants shared that their perceptions of the norms and values of STEM as a discipline hindered their self-advocacy because they felt like people with ADHD/SLD were viewed negatively within the discipline, or that they were not as valuable as individuals because they differed from the typical student.

Perceptions of individuals with ADHD/SLD in STEM. Our participants described their perceptions of STEM courses as students with ADHD/SLD, and how their perceptions of what personal characteristics are valued in a STEM course

functioned as a barrier to their self-advocacy. Dana described that she feels like she has to defend her decision to pursue a STEM major as a student with ADHD. “When you have ADHD in STEM it's hard, people kind of look at you like why are you even doing it? Like it's just hard for you. You might as well find something easier.” Dana’s quote illustrated her perception that other people in STEM do not think she can succeed in STEM as a student with ADHD. Another participant, Cassie, elaborated that she felt like she is an outsider to STEM because her brain works differently than her peers.

> “With the atmosphere of STEM courses and the STEM field in general, being like [able to] think on your feet, be quick. Have the answer pop out of your head when you look at a graph. In my head, if I can't do that, I'm not as good as these other people.”-Cassie

Cassie described that she perceives the ability to quickly answer a question to be highly valued in her STEM courses. She felt that she is not equal to her peers if she takes a longer amount of time to produce the same correct answer.

Participants also explained how they perceive others in their STEM courses to think about people with their disabilities. For example, many participants with ADHD stated that their instructors and peers do not think it is fair for them to use accommodations because ADHD is not considered to be a “real disability.” This perception made communicating for the purpose of self-advocacy challenging. For instance, Isabel stated that her peers do not think it is fair for her to use accommodations because “it’s just ADHD.” Kendra revealed to us that she prefers to tell people she “just has a learning disability” instead of sharing that she is diagnosed with ADHD because she perceives others to view ADHD negatively.

Participants with a specific learning disability in reading described how the stigma of dyslexia is manifested in STEM courses. Megan shared that people think having dyslexia means you are just bad at spelling, but they do not realize there can be more to

having dyslexia. She further explained that this reductionist view of dyslexia makes her less likely to communicate with others about her disability because, “it's frustrating when I would say something about having dyslexia and people don't understand at all…” Another participant with a specific learning disability in reading, Mia, shared that she perceives dyslexia to be tied to stigma, but that this stigma is not isolated to STEM courses exclusively. Mia reported, “People with dyslexia are seen as less intelligent than the average person just because their brain processes the same information in a different way.” Several of our participants reported that they felt different from their peers in a STEM course, and not well understood by those around them. These feelings made communicating for the purpose of self-advocacy intimidating and difficult.

The nature of STEM courses and its influence on self-advocacy. In an effort to further contextualize the factors that influenced the self-advocacy of our participants in undergraduate STEM courses, we asked our participants, “How does self-advocating in a STEM course compare to self-advocating in a different type of course?” Rarely did participants report that there was no difference between self-advocating in an undergraduate STEM course versus a non-STEM course. Participants explained several aspects of undergraduate STEM courses that pose challenges to them in terms of their self-advocacy. We include these data as a separate section to describe the unique self-advocacy experiences students with ADHD/SLD encounter in undergraduate STEM courses. We note that these data overlap with many of the factors influencing self-advocacy.

Several participants shared that the technical nature of STEM content will require them to use their accommodations in order to be successful in the course. Judd shared his perception of practicing self-advocacy in an undergraduate STEM course as a learner with a disability,

> “I think that STEM is probably the hardest to adapt to as having a learning disability…[STEM] is just rigorous, requires a lot of time, a lot of critical thinking, a lot of stuff that, on your psych eval, you were told you were missing. So it's kind of like, since you're missing this, you're probably not going to make it in STEM.”

Judd further explained that he is determined to succeed in his STEM courses, even if he views others to think he is not likely to graduate as a STEM major because of his disability.

For many participants, the main accommodations they use are exam accommodations (e.g., extended time in a less distractable environment). Because STEM courses tend to use exams as the major form of student assessment, several participants explained they are more likely to use accommodations in STEM compared to non-STEM courses. Oakley stated, “Math and science, it's a lot of practicing different types of problems in different settings, different scenarios and I feel like in non-STEM courses, it's more discussion based and more writing papers.” Opal reiterated this point. She further explained how the detail-oriented nature of STEM content requires her to use extra time on her exams because of her disability. She stated,

> “With math and science, you have so many steps in between where you have to go back and check the equation, and then check that you gathered the right numbers from the table, and then check to see that you have the right states of matter, and then check to see if you're copying the long decimal from the equation above the same, and the equation below, and you're typing it right into your calculator…and you need to make sure again that you're typing it right. I don't find the need to double check myself so often with non-STEM courses, whereas in STEM, I need that extra time. In a non-STEM course…I usually finish on time. With my STEM courses, I realize holy cow, I need these 45 extra minutes [on my exam].”-Opal

Finally, our participants explained that the numerous learning contexts that they encounter as STEM majors makes self-advocacy challenging. For instance, many participants shared that they did not realize that they could request accommodations in a lab section of a STEM course. A few participants stated they needed accommodations for in-lab quizzes. Most of our participants explained that they only needed accommodations for summative assessments in STEM lab sections, such as for lab practicals. When participants did use accommodations for lab practicals, they sometimes found that their confidentiality was compromised because the rest of the class could see that they continued working after the rest of the class was asked to stop. One example of this comes from Carter who used his extended-time accommodation for his anatomy lab practical.

> “I remember when the lab practical was over in my anatomy class, [the teaching assistants] were like, ‘Cool everybody go,’ and people looked at me like, ‘Hey how come he's not leaving?"

Carter continued to say, “People kinda look at you, they set you away from the pack if you will, and they think of it as weakness, or like you're stupid, but it's okay.” Carter’s positive self-advocacy beliefs appeared to help him practice self-advocacy in this situation. The totality of our data regarding self-advocacy experiences in undergraduate STEM courses allowed us to develop a model of factors influencing the self-advocacy of our participants.

A model of factors influencing the self-advocacy of our participants. Based on our participants’ experiences, we generated a model of the factors that influenced their self-advocacy behaviors within undergraduate STEM courses (Figure 7.2). The purpose of this model is to define the factors that influence self-advocacy in an effort to begin characterizing mechanisms which affect self-advocacy behaviors in undergraduate STEM courses. We found that the internal and external factors discussed

by our participants could either support or hinder self-advocacy. Determining if a factor functioned as a support or barrier depended upon the participant and their unique experiences. The factors also frequently interacted. For instance, our participants discussed how they gained self-advocacy knowledge (an internal factor) from their own previous experiences with the logistics of accommodations (an external factor) and from other individuals (another external factor). The internal factors, self-advocacy beliefs and identity, were affected by external factors for some of our participants. We could also see in our data that the internal factors, self-advocacy beliefs and identity of a participant, influenced their perceptions of external factors. For example, participants

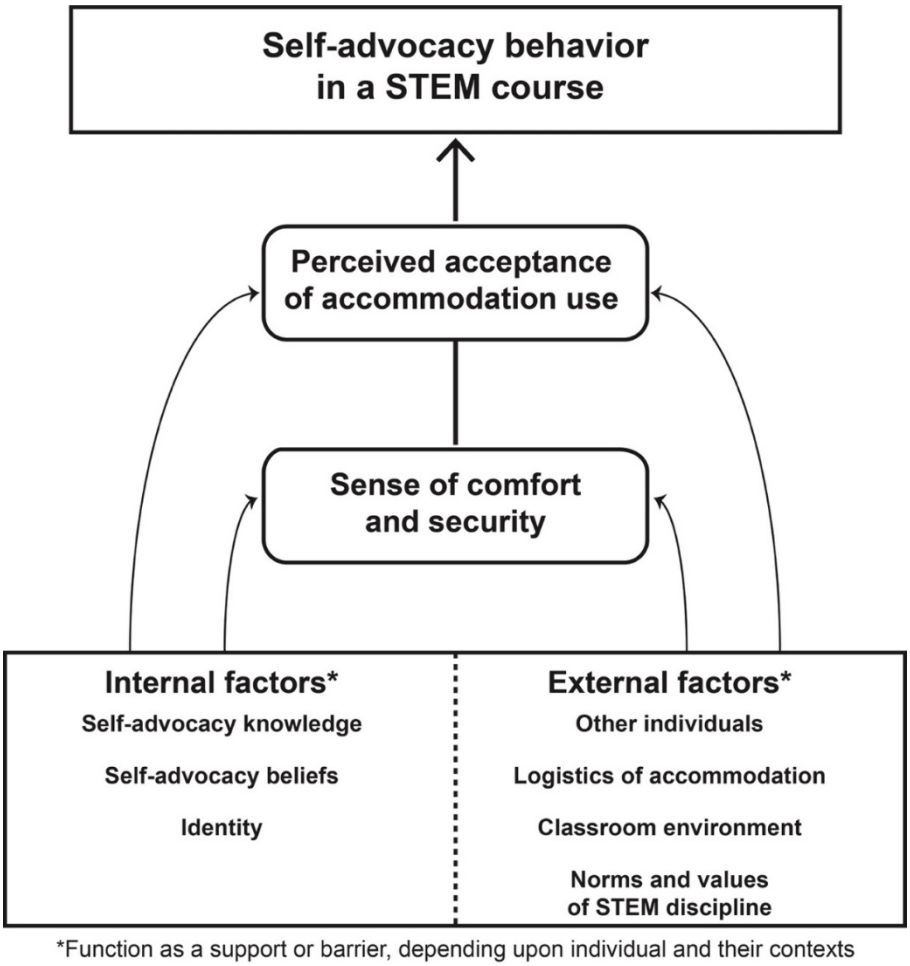

Figure 7.2. Our emergent model describing how factors influence the self-advocacy of our participants in the context of undergraduate STEM courses. Square-edged boxes represent findings from our data, while round-edged boxes represent components we propose to influence self-advocacy behaviors in a STEM course.

Figure 7.2. (continued) Our emergent model describing how factors influence the self-advocacy of our participants in the context of undergraduate STEM courses. Square-edged boxes represent findings from our data, while round-edged boxes represent components we propose to influence self-advocacy behaviors in a STEM course. Internal factors are aspects of self-advocacy within our participants. External factors are aspects that influence self-advocacy outside the participant. Internal and external factors often interact (dashed line). Factors function as a support or as a barrier (arched lines) depending upon the individual participant and their experiences. The lines are intended to be multidirectional. Factors contribute to, or diminish a sense of comfort and security, and inform our participants' perceptions that accommodation use is accepted within a STEM course. Sense of comfort and security and a perceived acceptance of accommodation use function together (straight, bolded arrow) to support self-advocacy behaviors in STEM courses.

who tended to see their own disability in a negative manner tended to perceive their peers as a barrier to self-advocacy because they tended to assume that a majority of their peers would view accommodation use negatively. Internal and external factors, as well as the interactions between factors, affected (1) a participant's sense of comfort and security as a student with ADHD/SLD, and (2) their perception that accommodation use is accepted within a particular context. Both a sense of comfort and a perception that accommodation use is accepted in a STEM course promoted self-advocacy behaviors. Conversely, when participants did not feel comfortable or secure as a student with ADHD/SLD, or when they did not perceive accommodation use to be accepted in an undergraduate STEM course, self-advocacy behaviors were diminished. Our model of the factors influencing self-advocacy suggests directions for future research and provides implications for teaching undergraduate STEM courses.

Discussion

In our study, we used an in-depth qualitative approach to characterize the factors that supported or hindered our participants' self-advocacy behaviors in undergraduate STEM courses. Our model illustrates that internal and external factors work in concert to affect a sense of comfort and security as a student with ADHD/SLD and the perceived acceptance of accommodation use in a STEM course, which in turn influences self-

advocacy behaviors. We situate the results of our study within the literature while discussing implications of our results for research and teaching.

Implications for research. The major contribution of our research is a deeper understanding of the complexity of practicing self-advocacy in undergraduate STEM courses. Our study suggests that the social model of disability does not fully capture the intricacy of this experience. At the surface level, the social model of disability argues that an individual with an impairment can address the hardship of societal expectations, and thus disability, through self-advocacy (Goodley, 1997). However, in our view, the social model of disability does not fully account for the effect of the context upon an individual which makes them more or less likely to engage in self-advocacy in the first-place. In this study we found participants to vary in self-advocacy, although they were all experiencing similar societal contexts, undergraduate STEM courses. The social model of disability tends to overlook the internal factors, such as identity, which can influence the self-advocacy of our participants. The intersectional nature of identity influences the experiences of a student in the context of undergraduate STEM (e.g., Ireland et al., 2018), and affects many educational constructs such as sense of belonging in STEM (e.g., Rodriguez & Blaney, 2020). Future self-advocacy research may be better served by other theoretical frameworks, such as Tinto's model of student retention, that more robustly attend to the role of identity, and other internal factors, in self-advocacy (Tinto, 1993).

Our results show that factors must be considered within a context to determine if they are functioning as a support or barrier to self-advocacy. For example, participants described their STEM instructors as a support to their self-advocacy when the STEM instructor follows up with them about their accommodations. However, the context of this conversation is important. If this conversation occurs in front of peers it can be perceived as violating privacy, and therefore a barrier to self-advocacy. In some ways, finding that

context influences self-advocacy is not surprising because as our study and others demonstrate, the experience of disability is highly-individualized (e.g. Mullins & Preyde, 2013). Nonetheless, viewing context as a contributor to the formation of a support or a barrier is valuable. The model generated by our analysis can be used to inform future research in undergraduate STEM, and to test what supports and barriers exist in other educational settings. Our model also establishes the importance of studying self-advocacy and accommodation use in a highly contextualized manner, such as within the STEM discipline, to fully characterize the underlying processes affecting self-advocacy.

Some of the factors identified by our analysis are already known to influence students with disabilities in college. For instance, we identified that students who were still developing their self-advocacy knowledge struggled to engage in self-advocacy behaviors within their STEM courses (Pfeifer et al., 2020). Two studies of college students with learning disabilities found participants possessed varying levels of knowledge in relation to their own learning disabilities, which influenced accommodation use and self-advocacy (Cawthon & Cole, 2010, Cole & Cawthon, 2015). Another study found that other individuals, such as educators and family members, supported the development of self-advocacy in K–12 settings (Daly-Cano et al., 2015). Our results demonstrated that the support of educators and family does not end in high school. Many of our participants reported that the information and advice provided by their STEM instructors and families continued to support their self-advocacy in college.

We identified additional factors besides self-advocacy knowledge and other individuals that influenced our participants' self-advocacy. One of our participants, Dana, described her DRC coordinator as a barrier to her self-advocacy because she perceived the accommodation process to be prescriptive (Supplemental File 7.3). Dana described feeling forced to choose her accommodations from a list based on her diagnosis, not from her own experiences and needs as an individual. The notion that accommodations

are prescriptive, or a “menu of services” that can be “selected on a case-by-case basis” is suggested to contribute to student perceptions of ineffective accommodations (Kurth & Mellard, 2006; Richard, 1995). This result exemplifies how self-advocacy can be influenced by both other individuals and the systems embedded within higher education. It also highlights the importance of conducting self-advocacy research in a manner which preserves the accommodation context of a university or college within the analysis because this context matters in the experience of the participant. Finally, our results suggest that STEM majors with ADHD/SLD may be more likely to use accommodations in their STEM courses compared to other disciplines, however, more research is needed to determine if this result is found in other settings.

Implications for teaching. In our study, the classroom environment influenced the self-advocacy of our participants. Because classroom environments, in part, are controlled by STEM instructors, we considered the actions of STEM instructors to be related to the classroom environment. Some participants shared examples of how STEM instructors supported their self-advocacy. For example, a Calculus instructor supported the self-advocacy of one of our participants, Jake, by helping him realize he may be experiencing issues completing his exams due to ADHD, and connected him with the DRC to establish accommodations. However, we found that many participants perceived their STEM instructors to generate barriers to self-advocacy. We summarize these perceived barriers and explain how these barriers hinder self-advocacy. We also provide supports that can overcome barriers to self-advocacy in undergraduate STEM courses (Table 7.3).

Our participants reported that they perceived some of their STEM instructors to be uninformed about their experiences, and that some STEM instructors use language or enact classroom policies that discouraged their use of accommodations. Examples of this type of language included STEM instructors joking about “being dyslexic” or “being

Table 7.3. Perceived self-advocacy barriers generated by STEM instructors and recommended practices to support self-advocacy in STEM courses.

Barrier perceived by student	Hinders	Supports to self-advocacy that can overcome barriers
Instructor is uninformed about the experiences of students with ADHD/SLD in STEM course.	Sense of comfort and security	Consider disability a facet of student diversity. Seek opportunities to learn about student experiences.
Instructor is uninformed about the instructor's role in the accommodation process.	Perceived acceptance of accommodation use	Visit your campus DRC's website, seek professional development opportunities offered by the DRC, and communicate with colleagues in your department to learn what is expected of instructors.
Instructor fails to respond to accommodation requests or emails about issues in a timely manner.	Perceived acceptance of accommodation use	Communicate to students how long you typically take to respond to accommodation requests or emails. If you have a question about the accommodation requested, communicate with the student and their DRC coordinator as soon as possible.
Instructor discusses accommodation or accommodation issues openly in front of peers.	Sense of comfort and security	Take your cue from the student. If they initiate a conversation in front of their peers with you, it is likely they feel comfortable talking about accommodations in that situation. If they do not initiate a conversation in front of their peers, communicate with student over email, or offer to schedule a meeting with student to talk in-person about accommodations or issues. In our study, students reported they did not feel comfortable talking about accommodations with their instructor if they thought a peer could hear them talking with the instructor.
Instructor tells student their accommodations would be "difficult" to implement in a lab, or other STEM contexts.	Both perceived acceptance of accommodation use and sense of comfort and security	Explain to student that you are willing to help them access their accommodations in a lab, even if it may be difficult for you to figure out how to do this at first. Discuss best options to implement an accommodation with the student and their DRC coordinator. Communicate with the DRC coordinator to ask questions you have about the accommodation. The DRC can often help find a workable solution for you and the student.
Instructor actively discourages student from using their exam accommodations.	Both	Students are legally entitled to use their accommodations. If you feel a requested accommodation fundamentally alters the nature of the course, you can communicate this to the

		DRC, and work with the DRC to find a solution.
Instructors use dismissive or hurtful language. For example, making jokes about "being ADHD" or "being dyslexic" in front of the class.	Both	Consider the experiences of all students in your classroom before making these statements. Remember that using disability terms when they don't apply can be a barrier to students.
Instructor makes statements prescribing the amount of time it "should" take a student to complete an exam question, or the entire exam.	Both	Avoid making general prescriptive statements about the time a student "should take" to complete an exam, or a question on the exam. If you do make these types of statements, qualify them. Explain that some students may take longer or shorter time, and that is also acceptable.
Instructor adopts "anti-technology" policies in their classroom.	Both	Explicitly state to the class and in your syllabus that technology for accommodation purposes in an exception to this rule, and that you fully support the use of accommodations in your course.
Instructor fails to develop a plan to proctor in-class quizzes, so that students using extended time accommodations can use their accommodations in a confidential manner.	Both	Consider how students using extra time will complete the quiz without missing class instruction. Communicate options with student to see what they may prefer. Some possible solutions include: (1) Proctoring the quiz online prior to class. (2) Proctoring the quiz at the end of class, so students can either take the quiz at the DRC, or stay after class to complete the quiz with extra time.
Instructor fails to provide equal access to information on exam day to students taking the exam at the DRC.	Both	Consider the exam day experiences of students testing at the DRC. Ask yourself these questions, to develop policies that ensure equal access to information: (1) Did I include all necessary information (e.g. formula sheet, periodic table, etc.) to take the test? (2) Do I make announcements to the class that students testing at the DRC would not have access to? (3) Do I answer student questions about the exam when the test in-class, but not at the DRC? How could I ensure both groups of students can ask questions?

ADHD," or implying that a student must be "crazy" if they take medication. We also found that when STEM instructors made general prescriptive announcements to the class about the amount of time a task "should" take to complete, or adopt "anti-technology" policies in their courses, the self-advocacy of our participants was hindered. Participants reported examples of hurtful or dismissive language used by STEM instructors that was reminiscent of negatively phrased instructor talk found to dismantle the student/instructor relationship, disestablish the classroom culture, compromise pedagogical choices, and share personal judgement (Harrison et al., 2019). Such language and certain classroom policies represent cues from the instructor that diminish a student's sense of comfort and security, and inform their perceptions that accommodation use is not fully accepted in a STEM course.

Within our study, we found that some participants perceived themselves to be excluded from their STEM courses. For some participants, their feelings of exclusion from their STEM courses could be amplified by the interaction of their disability identity with other facets of identity, such as gender and race. These findings are related to issues of inclusivity within undergraduate STEM courses. We encourage STEM instructors to consider disability as another aspect of student diversity (Ben-Moshe & Magaña, 2014; Vaccaro et al., 2015). Considering disability in this manner can help instructors enhance inclusivity within their own courses by developing their own self-awareness and fostering empathy for students with ADHD/SLD, students with other types of disabilities, and students with other facets of diversity (Dewsbury & Brame, 2019).

Seeking opportunities to learn more about students with ADHD/SLD and other disabilities is likely to enhance the inclusiveness of a STEM course. STEM instructors can demonstrate to students with ADHD/SLD that they are willing to learn about their

unique experiences by inviting students to discuss disability or accommodation issues with them in a private meeting at the start of the course. Many of our participants shared that they do not feel comfortable talking to their STEM instructors during open-door office hours because they worry about losing confidentiality. We emphasize that STEM instructors should invite their students, but not insist, they meet with them because it is the student's right to choose to engage in follow-up communication with their STEM instructor. In our own teaching experience, making a short announcement at the start of the course stating that we fully support the use of accommodations in our course has increased the number of students who use their accommodations. We hypothesize that when STEM instructors make these types of announcements at the start of the course, it demonstrates to students with ADHD/SLD that this instructor is someone who will support their self-advocacy. It may help students feel more comfortable to engage in communication for the purpose of self-advocacy, and clarify to students that classroom practices or policies are not in place to discourage accommodation use.

We found that participants perceived STEM instructors to hinder their self-advocacy when a STEM instructor lacked knowledge or neglected their own role as an instructor in the accommodation process. An instructor's lack of awareness about the accommodation process made it challenging to practice self-advocacy because the student was often still developing their own self-advocacy knowledge. One study of college faculty indicated faculty have general and limited knowledge about laws related to disability in higher education that drive the accommodation process in postsecondary settings (Villarreal III, 2002). Two recent studies suggest that STEM instructors want to support student use of accommodations but they feel unprepared to do so, in part, because they feel they lack the necessary knowledge (Gokool-Baurhoo & Asghar, 2019; Love et al., 2014). In one interview study conducted with five STEM faculty, participants reported that they gained their knowledge of accommodations and disabilities through

experience “on the job,” and that one participant had “no formal educational opportunities working with students with disabilities” (Love et al., 2014, p. 33). This lack of preparation is troubling given the major influence STEM instructors had on the self-advocacy of our participants.

Our data and other studies show that self-advocacy involves negotiation of power structures inherent to instructor-student relationships (Charlton, 2010; Trammell, 2009). For example, Henry attempted to negotiate against a power differential when he met with his Chemistry instructor to ask about using his extended time accommodation on the quizzes in the lab section of the course. The instructor implied to Henry that his accommodations could be difficult to implement, so Henry did not request them because he did not want to complicate his relationship with the instructor. In this instance, Henry may have benefited from informing his DRC coordinator about the situation, so that his DRC coordinator could explain to the instructor that Henry should be able to use accommodations on lab quizzes. Because we did not interview STEM instructors we can only speculate if the Chemistry instructor in this case was fully aware of the power the instructor holds in the accommodation process. While the process to obtain accommodations may differ depending upon the university or college, in general, the instructor must at least acknowledge the accommodation requested for each student in their course. If the instructor feels that the accommodation “fundamentally alters” the nature of their course, it is within the right of the instructor to communicate with their DRC to explore other accommodation options. If the Chemistry instructor felt the extended time accommodation on lab quizzes accommodation would be difficult to implement, it would have been better to discuss the issue with Henry and his DRC coordinator to find a workable solution for all parties involved. It is important instructors are aware of the power they hold in the classroom and within the accommodation

process. Being more aware of this power may help STEM instructors be better supporters of self-advocacy.

In our study, our participants reported that some STEM instructors excelled as supporters of self-advocacy, while other STEM instructors did not. It is clear from our study that concerted efforts are needed to better prepare STEM instructors to be effective supporters of self-advocacy and to create classroom environments conducive to self-advocacy. It is likely that enhancing student self-advocacy by itself is necessary, but not sufficient, to promote the retention of students with ADHD/SLD and other disabilities in STEM. We are more likely to retain students with disabilities in STEM by simultaneously enhancing student self-advocacy and STEM instructor knowledge.

Limitations. Data were collected from 25 individuals at a single university who were currently registered to receive services from a DRC for ADHD/SLD. A majority of our participants were white, Life Sciences majors, and reported having only ADHD. We examined the self-advocacy experiences of STEM majors broadly, as opposed to Life Science majors exclusively. Because our participants were already registered and willing to participate in our study, many of our participants likely represent individuals with developing or well-developed self-advocacy. Our data do not encompass the perspectives of individuals with ADHD/SLD who have never registered with their campus DRC, and this difference in experience may or may not include the factors influencing self-advocacy that were identified in this study. Additionally, our participants currently requested their accommodations using an online accommodation system. This practice eliminates the requirement of students taking a physical copy of their accommodation letters to their instructors, as occurs at some universities and colleges. We expect the details of how students access their accommodations will influence their self-advocacy and their perceptions of what supports and hinders self-advocacy in undergraduate

STEM courses. Future research is needed to understand if and how the factors identified in our study apply to other students with ADHD/SLD in different contexts.

Conclusion

Our study is part of an emergent body of research regarding the experiences of students with disabilities in undergraduate STEM contexts (Braun, Gormally & Clarke, 2017; Gin, Guerrero, Cooper & Brownell, 2020; Majocha, Davenport, Braun, & Gormally, 2018; McCall, Shew, Simmons, Paretti, & McNair, 2020; Pfeifer et al., 2020). Across these studies, a pattern appears. Students with disabilities report perceptions of exclusion from STEM. We encourage all STEM instructors to consider disability as a feature of student diversity. Our aim in disseminating the factors that influenced the self-advocacy of our participants is to bring attention to the ways STEM instructors can support or hinder the self-advocacy of their students. We call on STEM instructors to deeply consider the language and practices they use within their own courses, and to take action to support the self-advocacy of their students. Supporting the self-advocacy of students with ADHD/SLD is likely to encourage accommodation use in STEM courses, and this, in turn, will promote retention of students with ADHD/SLD, and other disabilities in undergraduate STEM majors.

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Supplemental File 7.1. Interview protocol[2]

1. Tell me about yourself, what is your major and year in school?

2. Walk me through how the accommodation process works from the start of the semester to the end of the semester.

3. Think back to your first semester in college. Tell me about your experience in learning how to request academic accommodations.

4. How did this experience compare to your experience with accommodations in high school?

5. Who, if anyone, helps you with accommodations in college? What kind of help do they provide?

6. Tell me about a time you decided not to use accommodations in a course or a semester. Describe your thought process in making this decision.

7. What do you do when your accommodations are not working in a course? Who do you talk to?

8. What advice would you give to an incoming student about learning to request and use accommodations?

9. *I want to talk with you about self-advocacy. Self-advocacy has different meanings to different people. I think of self-advocacy as speaking up to tell those around you about your disability to help them understand what accommodations you need to access the learning material or activities in class, including requesting accommodations from the DRC.* What does self-advocacy mean to you?

10. In your email, you mentioned that you have taken a STEM course to meet the Science and/or Quantitative Reasoning Core Curriculum requirement. Which course(s) did you take?

11. Tell me how your disability affects you when you are in a STEM course.

12. What accommodations do you typically use in STEM courses?

13. How do you self-advocate in a STEM course?

14. How does self-advocating in a STEM course compare to self-advocating in a different type of course?

15. How do you decide to tell you instructor about your disability in a STEM course? Walk me through your thought process.

[2] Note: Six interview questions were omitted because they were used for data collection in a different study.

Supplemental File 7.2. Factors influencing self-advocacy codebook. Example data has been lightly edited for clarity. Brackets indicate words added, or long pauses during the interview. Ellipses indicate words removed for conciseness. Codes related to development of the model of self-advocacy are provided in Pfeifer et al., 2020. *Asterisks indicate codes we previously published, but also include here because of their importance in informing our understanding of the factors that influence self-advocacy.

Major code	Subcode	Description and notes	Example data
Practicing self-advocacy before coming to college		Participant describes how they practiced self-advocacy before starting college.	See subcodes below.
	Attending accommodation meetings in high school	Participant describes attending their own accommodations meetings in high school. Note: These are typically called IEP meetings or 504 meetings. IEP stands for Individualized Education Plan and 504 refers to Section 504 of the Rehabilitation Act of 1973.	"…As soon as I got into the [IEP] meeting, I got used to it." -Ryan
	Asking teachers for extra time to finish tests in high school	Participant recalls having to work with teachers individually to get extra time on tests in high school. Note: Official accommodations may or may not be in place.	"…if I needed a few extra minutes, the [high school teachers] were always willing to let me stay." -Oakley
	At a previous college	Participant describes how they practiced self-advocacy at another college before transferring to current institution.	"So when it came time to take my first test, [my STEM instructor] did not allow me to set it up with the testing services. He really pressured me to take it in class and I was like, and that resulted in a really, really low score. Because of that, I discussed with their disability office what had happened. They had asked me if I wanted to take it further and file a complaint. I didn't, because he still was in control of my grades. Like I said, he's an interesting man, so I didn't know what the results of that would have been…I just was like, "Just so that you guys are aware." So after that, they I think sent him an email that they didn't tell him that I talked to them. I think he got an email just saying, "I think it's required by law for you to provide accommodations for your students." After

Practicing self-advocacy before coming to college (continued)			that, I was able to use my accommodations, but it's definitely an interesting experience." -Mia Note: This participant quote is double-coded with STEM instructor as a barrier, subcode actively discriminates
Did not practice self-advocacy before college		Participant explains that they did not practice self-advocacy before coming to college.	Talking about what her mom did for her in K-12 classrooms. "..If she saw I was struggling, she would go talk to the teachers."-Dana
Recognizing a need for official accommodations		Participant shares a story about how they recognized they needed accommodations. This can be in high school or college.	"I was getting really frustrated with my grades not reflecting what I'm capable of. And after enough of that I was like I want to do something about this." -Cassie Listening back over recorded class session. "When I went back and listened to it, and I missed over 30 minutes of class just from zoning our or wondering what somebody's talking about or something like that." -Wyatt
Asking for help before officially registered with the DRC		Participant shares a story of reaching out for help when they recognized a need for accommodations. Note: Possible examples include working with a counselor, or working with an instructor who encouraged them to get tested or connected to the DRC, etc.	Receiving a DRC referral from another campus student support office. "I just came to [college], took a couple math tests, was like, I need more time. I talked to the CRC person about it. They were like, talk to the DRC. I talked to them and got it figured out. I guess more to your point, I didn't know that the DRC existed…"-Cassie Note: The CRC is the Collegiate Recovery Center.
Learning about disability		Participant describes what it was like for them when they first learned they had a disability, or how they felt reviewing their official testing paperwork. Note: This includes both official and unofficial means to learn about disability, i.e., receiving a formal diagnosis or an experience that taught them about themselves as a learner with a disability.	Receiving formal diagnosis. "Then I went to a psychologist…And he was like, 'Oh yeah, this is ADHD. For sure.' I was really relieved, because I was like oh, it'll be a lot easier to fix this [than changing all the stress I have] …I thought I was just gonna get medicated and I would be back and better than ever, but it's not really that way, at all." -Kendra Reading official testing report**.** They came up with this report that really accurately describes how I struggle in a lot of different academic areas and just like how my brain functions generally…there

Learning about disability (continued)			were observations that I notice about myself and they had in this report without me telling [them] at all." -River
DRC teaches how to request accommodations		Participant talks about learning how to request accommodations in college through the DRC.	Talking about his first DRC meeting. "…they were just like, this is what you need, this is what we can offer you. They would tell me one of the accommodations, like actually it might be a little bit better if you did this, notetakers are awesome but sometimes they don't take very good notes. I'd rather you record lectures and stuff like that…"-Carter
Being taught by an older adult how to self-advocate		Participant describes how their parents or other trusted adult helped them learn to practice self-advocacy when they were younger. Note: Most of the time this will be their parents, but could be a teacher, tutor, etc.	"Yeah, so my parents were very big advocates on assisting me with setting up accommodations [in K-12] …They were definitely very big on helping me set-up a 504 plan and making sure I was talking and communicating with my teachers for what I needed." -Mia
Parents "push" student to sign up with DRC		Participant describes their parents being a "major" factor or "forcing" them to sign up for accommodations in college.	"My mom recommended that I go take testing accommodations…because technically I have a disability." -Ryan
Planning for accommodation use each semester		Participant describes how they generally approach their accommodations for the semester.	"I primarily use all of my accommodations."-Mia
	Description of how accommodation letters are sent to professors	Participant describes how they generally approach their accommodations for the semester. Note: If they say they have trouble planning for the semester, code this as knowledge of self. Possible examples include they send out accommodation requests to every instructor, they pick and choose classes, or they wait until they take the	"I go through all of my syllabuses and find all the dates of my exams and papers and stuff, and I'll write them all down in my agenda. Then I'll go onto the DRC login portal that I have saved in a favorite on my computer so I can find it easily, and I will then just go through and get the letters of approval for each class…"-Heath

Planning for accommodation use each semester (continued)		first exam. Don't use this code for lack of plan.	
	Description of strategy for scheduling exams	Participant explains how they schedule their extended time exams, can be at DRC or with instructors.	"Sometimes at the beginning of the semester. I'll go in and submit all of my tests as soon as I get my syllabus, which usually works out well." -Eli After getting the letters of approval from each class, it takes about a day for them to come back, then I schedule all of the exams. I just go one class, start to finish. Next class, start to finish. Start to finish, start to finish, start to finish. All the exams, all of the finals. Then just go through the semester…" -Heath
	Using accommodations besides extended time exams	Participant describes how they plan and use accommodations besides extended time exams. <u>Note</u>: Possible examples are notetaking accommodations, scantron accommodations, alternative textbooks, etc.	I have a note taking accommodation, but I don't really use it, because I feel like if I had somebody taking notes for me I would never show up to class. I know, that's not the case for other people, but also I don't really trust other people's notes as much as I trust my own, because I know what I need to have written down. I like everything super written down. And I'm worried that I might miss [something]." -Kendra
	"Better to have more than less accommodations"	Participant says that they plan to use all their accommodations because it's better to have more and not need them, then to ask later.	"It's better to have more accommodations and then just not use them to have less accommodations and be struggling."-Mia
Support network provides emotional support		Participant describes some sort of supporting individual or network of individuals that help them feel comfortable discussing their disability or accommodation issues.	"I had friends that were getting accommodations here too…"-Opal
Support network fosters self-advocacy		Participant describes how someone helps or helped them practice self-advocacy, including learning about available accommodations, or practicing self-advocacy.	"I still discuss it with my parents…"-Mia "The day of I was panicking, freaking out, and my friends helped me figure out what to do."-Kendra

STEM instructor supports self-advocacy*		The instructor supports participant self-advocacy by being perceived as approachable, when the instructor affirms use of accommodations in their course, and when the instructor helps the student use their accommodations in the course.	Instructor affirms accommodation use: "He kind of said like with [my upper-division math course] time isn't a concern, because you can solve a problem for years, so I shouldn't be worried about it. So, he gave me… a concrete example of like why I shouldn't be worried." -Aaron
STEM instructor as a barrier			
	Not perceived as open	Participant perceives their instructor to be judgmental of their accommodation use.	"He seems kind of judgmental, so the more judgmental the more uncomfortable I feel."-Aaron
	Not informed about disabilities or accommodations	Participant describes a time when their instructors were not informed about how accommodations work at the location data collection occurred.	"I think it was his first time teaching, and he was like, 'Oh, yeah, I don't really know anything about that...'"- Kendra
	Neglectful of student	Participant describes that their instructors do not respond to their accommodation requests in a timely manner or do not consider them in the design of the classroom. Note: Examples include instructors not responding to official DRC notification letters or emails about accommodations, instructors using pop quizzes at the start of class with no considerations in place for those using exam accommodations, etc.	"That's what's frustrating, is some of them never submit the agreement [acknowledge accommodation letter sent from the DRC]." -Opal
	Actively discriminates	Participant describes a time when their instructor denies them accommodations, or has a negative attitude or action towards the student because they use accommodations.	"…he denied me my accommodations." -Mia
	Violates privacy	Participant describes a time when their instructor violated their privacy by telling others or implying to others that the participant uses accommodations.	"When I take a test at the DRC, a lot of times the [instructor] has to pick it up or they'll give it to the [instructor]. Sometimes I have to drop off the test, depends on what the [instructor] puts down for how they want me to return it or if they're gonna

STEM instructor as a barrier (continued)			pick it up. This [instructor] I guess did the option where he picks it up, and he would forget. I think we had three tests, and each time he would pass back tests he would say, 'Oh Megan I haven't had a chance to go to the DRC to get your test, sorry I always forget.' And this would be in front of the whole class, and I was uncomfortable with that." -Megan
	Doesn't want to burden instructor	Participant perceives accommodations as a burden for their instructor, so they do not pursue them or ask their instructor for help if problems with accommodations arise.	Because you're basically telling them I need extra time and I don't know how they feel about that. Because that's extra work for them, I know that. Especially if they don't have [teaching assistants]."-Dana
Peers as barrier in a STEM course		Participant describes their peers as a barrier to their self-advocacy, includes experiences with a close friend or roommate. Note: May also be double coded with stigma of disability.	Close friend thinks accommodations are unfair. "So it was actually my roommate my freshman and sophomore year. We took a lot of the same courses…she made this comment like people who just get accommodations to get extra time, they don't really earn that grade." -Oakley Peers as unsupportive. "I always feel like I have to defend myself in a way..."Oh, she gets extra time" and that to the class, "No wonder she got a better grade than everyone."-Opal Responding to peers with self-advocacy. "Yeah, she did. She took it and with her own hands, because she was struggling, to go and ask for help and figure that out for herself, so what's your problem with it? If you want extra time, go get tested and go figure it out for yourself, but don't just sit around and bag on someone about that." -Opal
Logistics of accommodations prevent utilization		Participant describes how the logistics of their accommodation act as a barrier to their self-advocacy or use of accommodations. Note: Only use major code if unable to decide on subcode.	See subcodes below.

Logistics of accommodations prevent utilization (continued)	Avoiding exam day reveal to peers	Participant does not want to take their exams at the DRC because they don't want their peers to notice they are gone from the classroom on the day of the exam.	Deciding not to use extra time. "…maybe sometimes people [peers] will like text me, 'where were you during the exam?' Then it's like I have to explain, so that makes me self-conscious." -Aaron
	Doesn't want to talk about accommodations	Participant does not want to talk to their instructors or a peer about accommodations, so they do not request or use their accommodations.	Talking about stopping use of accommodations. "I would only take the accommodation if the email processed and I didn't have to talk to anyone about it."-Aaron
	Wants to ask questions during the exam	Participant does not like to test at the DRC because they cannot ask instructors questions on the exam.	Taking an exam at the DRC. "So I was sitting in the DRC, I was like ... I mean, I can ask the DRC to ask a question for me, but if the teacher's not available by email or phone, then there's nothing they can do. So that's kinda rough." -Kendra
	ADHD medication issue	Participant avoids taking their ADHD medication for class or exams because of the side effects of the medication.	Describing how scheduling extended time exams affects medication schedule. "Next week, I have my Genetics and Organic Chemistry test(s), one after the other. I take [ADHD] medications to help myself focus, but since the O chemistry people don't want me to have taken the test before anyone else has started taking the test, the earliest I can take it is 4:45 PM, which would mean I would have to take more medication later in the day, which would inhibit my ability to sleep well that night, and most likely affect my performance on my Genetics test the next day…I got [my Organic Chemistry test] moved as early as I could so I would not be up until 4:00 in the morning." -Henry
DRC as barrier		Participant says that their DRC coordinator was not helpful for them. Note: Also use this code when an inaccurate idea of the accommodation process rises to the level of a barrier.	"That's why it was weird talking to him, because I felt like he was just treating me like a little kid." -Dana
Intersectionality		Participant describes how other identities influence their feeling of belonging in STEM and willingness to	"…because guys, oh my…they're so judgmental in engineering. They think every girl's dumb and they treat you as such. So I think it is kind of like a don't show fear…I mean not fear, but you know don't let

Intersectionality (continued)		talk about disability and pursue academic accommodations.	them know you're weak or something." -Dana "Especially with black men, there's a lot of hyper masculinity and mental health or being sad or expression emotion or concern for yourself is seen as weakness." -Carter …I guess because women are less represented in the field...it's just an intimidating environment in general. It's just the way I was raised and stuff, just fighting through a lot of that and trying to get over gender stereotypes and whatnot."-Cassie
Opting out of accommodations		Participant describes their reasoning for not using academic accommodations.	"An accommodation that is an option is the note-taking thing. I just never had that in high school, so I think coming to college, I was like, I don't know what this is. I just opted out of that every semester for every class…" -Cassie
Clear career aspiration		Participant describes a clear STEM or STEM related career aspiration.	"I have thought up until this year that I wanted to get my PhD in genetics and do research for a living, but now I've decided that I actually want to go to medical school." -Oakley
Stigma of disability*		Participant discusses their perception of disability in the context of undergraduate STEM courses. This includes how STEM instructors, and peers view disability and accommodation use in the context of undergraduate STEM courses. <u>Possible examples are</u>: -ADHD over-diagnosed or not a real disability -People who use accommodations are not smart -Accommodation use is unfair	"Sometimes I do tell them, talk to my friends and stuff. I think ... I don't think they really care. Like, you know? Just because like I said, people have their opinions about ADHD, so a lot of times when I mention it, it's always like a very snide comment on how ADHD is a made-up thing, and really kids just need to go outside, or you know? I don't know, it just seems very negatively viewed." -Dana "The stigma is primarily like people joke a lot about dyslexia…There is a negative stigma…[the] stigma seems like it's making people who have dyslexia out to be less intelligent than the average person just because their brains process information in a different way."-Mia

Stigma of disability* (continued)			Asking instructor about their view of accommodation use. “Do you think this makes me look like a lesser student?” -Aaron
Feeling/perception of using accommodations or having a disability*		Participant describes how they felt about using accommodations, or their perceptions of what accommodation use is like in college.	“[Accommodations] level the playing field”-Oakley “Just at the beginning I guess I was nervous about asking, or telling a professor. I wasn't nervous about telling them I had the DRC, just about missing class and asking for something different.”-Jake

Note: Codes related to a subsequent study are redacted.

Supplemental File 7.3. Other individuals influence self-advocacy

Family as a support

We found numerous examples of how families supported the self-advocacy of our participants. Many of our participants reported that several of their immediate family members also have a disability, and this helped our participants develop their own self-advocacy. Tamrin elaborated,

> "Everyone in my family has a learning disability…all of my sisters went to college here so they've been through it, so it was easier for me because I knew what to expect…My sisters knew about the notetaking accommodation and the audio books so they told me to ask for them...They just knew everything that was available, so I knew more of what to ask for."-Tamrin

Tamrin's family served as a support for her self-advocacy because their previous experiences with the accommodation process informed her accommodation decisions in college. Some of our participants were the only person in their family to have a diagnosed disability. In these cases, families often provided general emotional support that helped self-advocacy. Other participants like Mia explained that their family supported their self-advocacy by providing advice regarding accommodation issues. Mia stated,

> "I still discuss it with my parents, but I'm the primary decision maker about my accommodations…I'll talk to my parents and be like, 'Hey, these ones are available. Do you think that these would be beneficial?' I use them as a back-up resource just as a confirmation, because they know me."-Mia

Mia shared that she talks about accommodation issues she experiences with her parents because they know Mia's strengths and weaknesses as a learner with a disability. Mia valued their input because they help her to feel confident in her accommodation-related decisions.

For many of our participants, their parents played an important role in helping them initially establish accommodations in college, and this was a support for their self-advocacy. We found that several participants credit their parents as the sole reason they pursued accommodations at the start of their college career. Eli shared that his parents told him that he needed to register with the DRC because he needed to keep his grades up in college. Eli stated, “My parents said, ‘If your grades fall, then we’re going to hammer you.’ That evidently got me into the door at the DRC.” Two more of our participants explained that their mothers encouraged them to register with the DRC. Judd and Ryan shared that they felt reluctant to register with the DRC at first. Judd noted this was because the word “disability” was in the name of the DRC. Judd and Ryan credit their mothers as the primary reason they use accommodations in college.

Another participant, Henry, reported that his mom helped him plan for his initial accommodation meeting by helping Henry write a list of accommodations he wanted to request in the meeting. Henry also explained that his mom attended the meeting with him to help him communicate with his DRC coordinator. Henry stated,

> “We had it planned out. I would explain as much as I could. Then she'd have everything that we wrote out on my accommodation list, and if I missed anything, she helped cover those accommodation requests.”-Henry

It is important to note, that Henry had to sign a waiver granting the DRC permission to discuss his accommodations with his mom, due to the Family Educational Rights and Privacy Act (FERPA), which protects the privacy of student educational records once students turn 18. In this example, Henry’s mom directly supported his self-advocacy by helping him develop a plan for his initial accommodation meeting. Henry’s mom further supported his self-advocacy by attending the meeting with him, and ensuring all his accommodation needs were discussed. Family served as a vital support of self-

advocacy for many of our participants. But this was not the case for some participants, whose families hindered their self-advocacy.

Family as a barrier

A few of our participants described how their families functioned as a barrier to their self-advocacy. In some cases, families prevented participants from accessing accommodations, failed to instruct students how to self-advocate in high school, and appeared to lack empathy. One example of families preventing access to accommodations comes from Hunter. Hunter reported that he showed symptoms of ADHD in high school. He asked his parents if he could be tested for ADHD to receive accommodations, but he was not allowed to be tested because his "[parents] didn't believe in it." Hunter explained that his parents did not view ADHD to be a real disability, and that without accommodations he struggled academically in high school. Hunter, however, was able to access accommodations in college. He was formally tested for ADHD one month after starting college, and began using accommodations soon after. Dana was another participant who described how her family hindered her self-advocacy. Dana shared that throughout elementary, middle, and high school her mom intervened on her behalf whenever Dana encountered a problem related to ADHD. Dana reported, "... [my mom] would step in most of the time if she saw there was a need to help. So I think that's why I kind of struggle a little bit [with self-advocacy in college]." Dana felt her self-advocacy was hindered, partly, because her mom did not provide opportunities for Dana to learn how to self-advocate in high school.

Another participant, Kendra, explained that her family sometimes hindered her self-advocacy in college because Kendra perceived her parents not to understand her experiences as a college student with ADHD. Kendra elaborated,

> "I've tried to talk to [my parents] before, especially freshman year… a lot of times they don't really understand how I could let myself get in certain situations in the

first place. They're like, ‘Why would you even do that? How can you get yourself into a situation where you have three papers that are overdue?’"

For Kendra, she felt reluctant to communicate with her parents about self-advocacy. She had to find other individuals, like her friends or academic coach, to communicate with about self-advocacy and accommodation issues because she felt her parents tended to lack empathy. Besides families, our participants also described how professionals within the university supported or hindered their self-advocacy.

Professionals as a support

We now explain how professionals supported the self-advocacy of our participants. Almost all participants described their DRC coordinators as a support for their self-advocacy. Besides DRC coordinators, both Wyatt and Kendra found professionals with similar disabilities to their own to be supportive. For Wyatt, who was diagnosed with ADHD in college, he became motivated to practice self-advocacy by an academic counselor who also had ADHD. His counselor helped him see that, “Having ADHD is not a big deal. It’s just something you’re gonna have to live with and learn how to deal with.” Wyatt shared that this conversation helped him feel comfortable to talk to his STEM instructors about accommodations because that is how he “deals” with ADHD.

Another participant, Kendra, explained that she feels more comfortable talking to her academic coach than her parents about accommodation issues because her academic coach has the same disability. In our study, Kendra was the only participant who reported meeting with an academic coach each week. Kendra explained that she was able to access an academic coach on her own, not through any services or offices associated with the university at the time data collection occurred. Kendra shared that her academic coach would help her develop a plan each week to complete coursework, as well as, make suggestions on how to manage her accommodations. Kendra stated that her academic coach, “also has ADHD. So, she understands from that point of view.”

Kendra explained that her academic coach helped her navigate the accommodation process by determining when and how to communicate with her STEM instructors, and her DRC coordinator when Kendra experienced an accommodation-related issue.

Professionals as a barrier

Although many of our participants found their DRC coordinator to be supportive of their self-advocacy, one participant did not. Dana explained that she did not find her DRC coordinator to be helpful in her initial accommodation meeting, which hindered her self-advocacy. This perception of her DRC coordinator influenced her decision not to use accommodations in her STEM courses. Dana described talking to her DRC coordinator was like, “talking to an answering machine…it gives you your options and you got to pick one.” Dana felt as though she had to choose her accommodations from a list, without much, if any, personal input about her needs and wants as an individual. This experience in her initial accommodation meeting discouraged her from communicating with her DRC coordinator, and from using accommodations in her courses.

CHAPTER 8

CONCLUSIONS

My dissertation research is comprised of two research areas that reflect my dual training in fungal cellular biology and STEM education research. The major findings and contributions of my bench research project are outlined in the first half of this conclusions chapter. In a similar manner, the major findings and contributions of my STEM education research project are presented in the second half of this conclusions chapter. Some of the unique benefits associated with completing a split dissertation are discussed in the closing section.

Bench Research

In my bench research project, I investigated mechanisms of nuclear migration within the rice blast fungus, *Magnaporthe oryzae*, during initial rice cell invasion and colonization. In Chapter 2, I present a literature review that was published in *Mycology* to summarize existing research related to nuclear migration in the rice blast fungus (Pfeifer & Khang, 2018). Within the literature review, I described the early morphological and cellular events in rice blast infection, the mitotic spectrum in fungi, evidence of intermediate mitosis in *M. oryzae*, and current knowledge of nuclear constriction and the possible mechanisms permitting extreme nuclear migration events during rice cell invasion and proliferation by *M. oryzae*. Completing this literature review allowed me to identify gaps in the literature and to develop the research questions that I investigated in the subsequent chapters of my dissertation. Chapters 3-5 of my dissertation each focus on a specific developmental stage of *M. oryzae* rice infection.

Chapter 3 of my dissertation was published in *Fungal Genetics and Biology* (Pfeifer & Khang, 2021). This chapter examined the state of the nuclear envelope during appressorium development, and the timing of nuclear migration through the germ tube. Previous research concluded that the outer nuclear membrane of the rice blast fungus remained intact during mitosis, and that a single nucleus migrated through the germ tube of a developing appressorium in a post-mitotic manner (Saunders et al., 2010b). Both the inner nuclear membrane and core nucleoporins had not yet been studied in *M. oryzae*. I used super-resolution structural illumination microscopy to determine the arrangement of the inner nuclear membrane and core nucleoporins within interphase and mitosis. In my study, I fluorescently labeled a protein within the inner nuclear membrane (Src1-GFP) and a core nucleoporin (Nup84-tdTomato). In interphase nuclei, Src1-GFP and Nup84-tdTomato co-localized, as expected. During mitosis, Src1-GFP was no longer detectable, and Nup84-tdTomato localized at the polar edges of the dividing nucleus and near the spindle pole bodies of the spindle. Because Src1-GFP fluorescence was relatively weak during mitosis, we cannot rule out that the inner nuclear membrane was intact, but the fluorescence signal was not bright enough to detect during mitotic nuclear migration through the germ tube. Moreover, our results clarified within the literature that nuclear migration occurs during mitosis and not post-mitotically as previously reported. Clarifying the timing of nuclear migration through the germ tube is biologically important for reasons discussed below (*See Contributions of Bench Research Project*).

Chapter 4 of my dissertation, also published in *Fungal Genetics and Biology*, focused on a later stage of rice cell infection, cell-to-cell movement (Pfeifer, Jones & Khang, 2019). At this stage, *M. oryzae* invasive hyphae (IH) seek pit fields where plasmodesmata are located to form IH pegs. IH pegs are narrow (~0.5 μm) (Jones et al.,

2016; Kankanala et al., 2007; Sakulkoo et al., 2018). A previous study showed that a single nucleus becomes highly-elongated as it migrates through the IH peg and that this extreme nuclear migration event likely occurs during mitosis (Jones et al., 2016). Yet the contributions of the mitotic spindle during nuclear migration through the IH peg were not defined. In this study, we followed the localization of microtubules (MTs) labeled with GFP relative to nuclei (histone-H1-tdTomato) using live-cell confocal microscopy. We found that during interphase in IH, MTs form a cage around the nucleus. This suggests that microtubule organizing centers (MTOCs) are not associated with the nucleus during interphase. We investigated the role of the spindle in mediating nuclear migration through the IH peg and observed that the mitotic spindle propelled a single nucleus through the IH peg. Remarkably, many of these spindles adopted a striking angle during movement through the IH peg. The drastic angle we observed was not previously reported within the literature. We presume that the spindle can become drastically angled because the IH themselves can become highly angled to facilitate cell-to-cell movement through plasmodesmata (Kankanala et al., 2007; Sakulkoo et al., 2018). This study was important because it shows that, like other *M. oryzae* developmental stages, nuclear migration through the IH peg occurs during mitosis.

The major effort of my bench research project is presented in Chapter 5 of my dissertation, submitted to *mBIO*. Here, I investigated the role of the mitotic spindle during extreme nuclear migration through the penetration peg, and I identified and began characterizing kinesin-5 and kinesin-14 motor proteins in *M. oryzae*. In this study, I used live-cell confocal microscopy to observe subcellular dynamics and RT-qPCR to quantify gene expression levels. Studying nuclear migration through the penetration peg is technically challenging for at least three reasons. First, the nucleus within the appressorium and the mitotic spindle are considered to be more sensitive to imaging

stress relative to nuclei and spindles within IH (our own observations). Second, visualizing fluorescent reporter strains can be challenging due to the highly melanized nature of the appressorium (Howard and Ferrari, 1989). Third, the dividing nucleus typically migrates over 20 μm in the z-dimension from the appressorium located on top of the rice leaf to the primary hypha located within the first-invaded rice cell in less than five minutes (Jenkinson et al., 2017). Thus, researchers must possess well-developed confocal microscopy skills to capture nuclear migration through the penetration peg.

Based on previous studies (Jenkinson et al., 2017; Shipman et al., 2017), I hypothesized that the mitotic spindle mediated nuclear migration through the penetration peg. I observed that nuclear migration through the penetration peg and found that, indeed, the spindle was involved. I also observed that the spindle within the appressorium rotates quickly to become aligned to the axis of the penetration peg and that the spindle pole body bound to the migrating daughter nucleus was the first part of the spindle to emerge from the penetration peg. Consistent with a previous study, the migrating nucleus was highly elongated during movement through the penetration peg (Jenkinson et al., 2017). The dynamics of the spindle suggested that a mechanism of spindle guidance points the spindle to the appressorial pore, where the penetration peg is located. Other cytoskeletons such as F-actins and septins are localized at the appressorial pore (Dagdas et al., 2012).

Because the spindle mediated nuclear migration through the penetration peg, I hypothesized that genetically perturbing the spindle would impair nuclear migration. In the *M. oryzae* field, inducible promoters are not widely available. To overcome this limitation, I generated a novel inducible overexpression (OE) system. This system uses the *Bas4* gene promoter (p). Bas4 is a secreted effector protein that presumably modulates the host plant's immune response (Khang et al., 2010; Mosquera et al.,

2009). *Bas4* gene expression is highly induced upon plant penetration (Mosquera et al., 2009). I generated MoKin5 and MoKin14 OE strains and quantified gene expression levels to validate the inducible overexpression system. The resulting strains carrying the *Bas4p-MoKin5* and *Bas4p-MoKin14* constructs showed that MoKin5 and MoKin14 were significantly upregulated in IH, respectively.

I then conducted live-cell imaging of the MoKin5 and MoKin14 OE strains. Overexpression of MoKin5 and MoKin14 caused substantial defects in nuclear positioning and morphology, IH development, and disease lesion development. By analyzing the spindles in both the MoKin5 and MoKin14 OE strains, I found that MoKin5 is likely generating an outward force on MTs within the spindle, as well as acting to promote MT polymerization (Chen et al., 2019). Moreover, we determined the localization of MoKin5-RFP expressed by its native promoter and found that it accumulates within the nucleus during interphase and at the spindle pole bodies during mitosis. The MoKin14 OE strain displayed arrested growth at the primary hyphal stage of development and monopolar spindles within the appressoria. One unique MoKin14 OE strain that developed more extensive IH revealed that spindles experience rounds of collapse when the spindle is less than ~5 μm. Taken together, MoKin14 likely generates an inward force on MTs within the spindle. These data further suggest MoKin14 is likely more important in early mitosis compared to late mitosis.

Contributions of Bench Research Project

The totality of my bench studies provides direct evidence that nuclear migration through the germ tube, nuclear migration through the penetration peg, and nuclear migration through the IH peg is mediated by the mitotic spindle. This knowledge is valuable because it exemplifies the intricate link between development and cell cycle progression within *M. oryzae.* For instance, other developmental stages such as

appressorium formation and maturation are known to be cell cycle dependent (Osés-Ruiz et al., 2017; Saunders et al., 2010a). My dissertation research establishes important groundwork for future studies of extreme nuclear migration in the blast fungus, in which a mitotic nucleus moves through a relatively small fungal structure, such as a penetration or IH peg. In studies of nuclear migration in higher eukaryotes, DNA and nuclear envelope repair mechanisms are required for successful nuclear migration through constricted spaces (Denais et al., 2016; Raab et al., 2016). Importantly, these nuclear migration events occur during interphase. Testing if similar repair mechanisms are required in *M. oryzae* may be complicated by the fact that in this species, nuclear migration occurs during mitosis in which some of this conserved cellular machinery is likely already mobilized. My studies show that the spindle is involved in nuclear migration through the penetration and IH pegs, yet we do not understand the mechanisms that guide the spindle precisely to these small infection structures. We also do not know if and how the spindle connects to other cytoskeletons present at these structures. Investigating possible mechanisms of spindle guidance to the penetration and IH pegs are important future directions.

My studies further identified previously uncharacterized proteins in *M. oryzae*. The structure of the nuclear envelope was not fully defined. I identified a component of the inner nuclear membrane (Src1) and a core nucleoporin (Nup84). I also identified and began to characterize kinesin motor proteins, MoKin5 and MoKin14. The subcellular localization of MoKin5-RFP was determined. The subcellular localization of MoKin14 remains unknown. Our results suggest that it is likely MoKin5 and MoKin14 exhibit canonical functions. MoKin5 likely exerts an outward force and acts to promote MT polymerization, while MoKin14 likely exerts an inward force upon the spindle. However, precise *in vitro* experiments are needed to confirm the directionality of these motor

proteins along MTs. Additionally, double knockout experiments are needed to conclude that MoKin5 and MoKin14 are both required for formation and elongation of the spindle.

Finally, my bench research suggests that overexpressing kinesin motor proteins is an effective way to thwart development of *M. oryzae* IH and blast disease lesions on rice. There is potential to apply this knowledge to develop novel biotechnological approaches to treat and prevent rice blast disease. In theory, *Bas4p*-kinesin motor protein constructs could be engineered and expressed by a mycovirus specific to *M. oryzae.* The mycovirus could be allowed to infect the fungus, and if the construct is properly expressed within the genome of *M. oryzae*, the fungus would fail to develop IH once it penetrated into plant tissue. Although this technology does not yet exist, it is an exciting prospect to consider in the future.

While my bench studies focused solely on the blast fungus, other plant pathogenic fungi develop similar infection structures. *Colletotrichum* species form appressoria and penetration hyphae to invade plant cells (Nesher et al., 2008), and *Fusarium graminearum* develops appressorium-like structures[1] during invasion and IH pegs at points of hyphal constriction during colonization of wheat and barley (Jansen et al., 2005; Qiu et al., 2019). Future research will demonstrate if the spindle mediates extreme nuclear migration in these fungi.

STEM Education Research

[1] These appressorium-like structures are also called compound or lobate appressoria, or infection cushions. Currently, it is unknown how mechanistically similar these appressorium-like structures are to the appressoria of *M. oyrzae.* For instance, generation of turgor pressure and the action of conserved signaling pathways, such as the cAMP and PMK1 pathways, are not yet known to be required for the formation of functional *F. graminearum* appressorium-like structures (Qiu et al., 2019).

In my STEM education research project, I characterized the self-advocacy experiences of undergraduate students with ADHD and/or specific learning disabilities (also referred to as specific learning disorders). Self-advocacy is defined as “the ability to assertively state wants, needs and rights, determine and pursue needed supports” and to obtain and evaluate the needed support with the ultimate goal of conducting affairs independently (Martin and Marshall, 1995; Izzo and Lamb, 2002; p. 6). Self-advocacy is linked to academic success for college students with disabilities (Fleming et al., 2017; Lombardi et al., 2011). Self-advocacy is related to requesting and using academic accommodations. A conceptual framework of self-advocacy was developed by Test et al., (2005) through a meta-analysis of existing studies related to self-advocacy. However, few, if any, subsequent studies empirically tested if this framework applied to their research participants. This is problematic because the existing self-advocacy framework may or may not encompass all the components of self-advocacy that apply to specific groups of students. The existing self-advocacy framework may also emphasize components of self-advocacy that are not relevant for particular groups of students. For instance, it was not clear if the existing framework explained the varied experiences of students with certain types disabilities, (e.g., ADHD versus a physical disability), or if the framework accounted for the self-advocacy experiences of students within certain academic disciplines, such as STEM.

Students with disabilities are underrepresented in STEM (National Science Foundation, 2019). While students with disabilities are equally likely to pursue a STEM major, relatively few will graduate with a STEM degree (Lee, 2011, 2014). Existing research also suggests that students with disabilities in STEM are less likely to use accommodations compared to students with disabilities in other majors (Lee, 2011, 2014). STEM courses are known to possess many barriers to students with disabilities

(Moon et al., 2012). Known barriers include inaccessible content, unwelcoming environments, and faculty who self-report being underprepared to support students with disabilities in their courses (Isaacson et al., 2011; Isaacson and Michaels, 2015; Love et al., 2014; Tuosto et al., 2020). Thus, undergraduate STEM courses are likely challenging places for students with disabilities to practice self-advocacy.

We conducted an empirical study to characterize the experiences of students with ADHD and/or SLD in undergraduate STEM courses (Pfeifer, Reiter, Hendrickson & Stanton, 2020; Pfeifer, Reiter, Cordero & Stanton, 2021). We reasoned that students with ADHD and/or SLD (ADHD/SLD) would share similar self-advocacy experiences. Thus, we partnered with the Disability Resource Center (DRC) at the University of Georgia to recruit participants in a manner that preserved student confidentiality. All participants were registered with the DRC and received services for either ADHD/SLD as a primary or secondary condition. Each participant was a STEM major and had completed at least one semester of undergraduate coursework.

Research interviews were conducted with 25 STEM majors with ADHD/SLD. Resulting data were analyzed by at least one or more researchers who was, or were, a STEM major with ADHD/SLD. We ensured rigor in our analysis using several techniques, including coding to consensus. Coding to consensus means that each researcher agreed with how the codes were applied and the themes that emerged from our iterative analytic process. From this project, two papers resulted. The first paper published in the *International Journal of STEM Education* (*See Chapter 6*) used content analysis to determine how Test's conceptual model of self-advocacy applied to STEM students with ADHD/SLD (Pfeifer et al., 2020). We found that self-advocacy for our participants was more complex than posited by Test's original model. Self-advocacy required more than knowledge of self, knowledge of rights, communication, and

leadership. Novel self-advocacy components emerged from our study. These components included: knowledge of STEM learning contexts, knowledge of accommodations and the process to obtain them, agency, view of disability, and filling gaps. Using these novel components and Test's original components, we developed a revised model of self-advocacy for STEM students with ADHD/SLD. In our model, self-advocacy is comprised broadly of self-advocacy knowledge, beliefs, and behaviors. Self-advocacy knowledge includes knowledge of self, knowledge of rights, knowledge of accommodations, knowledge of STEM learning contexts. Self-advocacy beliefs include agency and view of disability. Self-advocacy behaviors are communication, filling gaps, and leadership. (*See Table 7.1 on page 214 for a description of each component).*

The richness of our interview data warranted additional analysis to fully understand the complexity of self-advocacy for our participants. We conducted a second analysis of the interview data. The goal of this resulting study (*See Chapter 7*) was to utilize our revised conceptual model of self-advocacy to characterize the factors influencing the self-advocacy of our participants (Pfeifer et al., 2021). In this study, highlighted recently by the Editors-in-Chief of *CBE-Life Sciences Education*, we found that self-advocacy was influenced by internal and external factors. Internal factors were aspects within an individual participant and included self-advocacy knowledge, self-advocacy beliefs, and identity. We found that some participants held what we termed sufficient self-advocacy knowledge, and positive views of their own disability, which positively affected self-advocacy. Other participants were still developing their self-advocacy knowledge and tended to hold negative views of their own disability, which hindered self-advocacy. We also found that a participant's identity influenced their self-advocacy. For instance, participants who are also underrepresented by race and gender within STEM explained that self-advocacy was more challenging for them. We did not

design interview questions to assess how race and gender may also support self-advocacy. It is possible that for some individuals this is a self-advocacy support.

Several external factors influencing self-advocacy were identified in our study. External factors included other individuals, logistics of accommodation implementation, classroom environment, and the norms and values of the STEM discipline. Other individuals were peers, family, and professionals. Logistics of accommodations referred to the way in which academic accommodations were arranged or administered. Classroom environment included the actions of STEM instructors and the policies STEM instructors enforced in their courses. The norms and values of the STEM discipline involved participant perceptions of what instructors valued within STEM students, and how people with ADHD/SLD are viewed broadly within STEM disciplines. We found that internal and external factors frequently interacted to support or hinder an individual's self-advocacy. When self-advocacy is supported, a student perceives a sense of comfort and security as a student with ADHD/SLD, and that accommodation use is accepted within a STEM context (*See Figure 7.2 on page 250*). A sense of comfort and security, along with perceptions that accommodation use is accepted, leads to self-advocacy behaviors within a STEM context.

Contributions of STEM Education Research Project

Self-advocacy is considered essential for the academic success of college students with disabilities (Fleming et al., 2017; Getzel and Thoma, 2008; Hadley, 2007; Janiga and Costenbader, 2002; Lombardi et al., 2011). Yet some self-advocacy studies use a conceptual framework of self-advocacy that was not fully validated for their intended participants (e.g., Kinney and Eakman, 2017). Additionally, few studies investigate how students with disabilities practice self-advocacy in their everyday lives (Daly-Cano et al., 2015; Walker and Test, 2011). In my first STEM education research

chapter, the original self-advocacy conceptual framework was empirically tested (Pfeifer et al., 2020). Our study found that self-advocacy for STEM undergraduates with ADHD/SLD was more nuanced than laid out in the original framework. From an in-depth qualitative analysis, we revised the self-advocacy conceptual framework to better explain the self-advocacy experiences of our participants. The revised framework offers a clear conceptualization of all the components of self-advocacy. Our hope is that self-advocacy stakeholders will utilize this information to learn about ways they can promote their own self-advocacy (as an individual), or by developing practices, policies, or programing tailored to enhance self-advocacy in STEM (as a STEM instructor, a university staff member, or as an administrator). Developing the revised conceptual framework of self-advocacy constitutes an important theoretical contribution to the self-advocacy field, and to the STEM education research community. Future studies of self-advocacy can use this conceptual framework as a starting point when examining self-advocacy in STEM. For instance, we utilized the revised framework in the second analysis of our research interview data.

In my second STEM education research chapter, we described the internal and external factors that influenced the self-advocacy of our participants (Pfeifer et al., 2021). We found that these factors are complex and often interact. The resulting model of how factors support self-advocacy can be tested in other contexts. We conducted our study at a single university. Future research should examine if these factors exist at other institutions and how these factors support or hinder self-advocacy for STEM students with ADHD/SLD. Another important future direction is to elucidate if these factors are relevant to students with other disability types. The future research described above will establish how broadly the findings of our study apply to all STEM students with disabilities.

Our results shed light on important teaching considerations for STEM instructors. One of the major findings of our self-advocacy research is that STEM instructors can influence a student's willingness to engage in self-advocacy within a STEM course. Participants in our study described how they appraise their STEM instructor's view of disability and accommodation use by students, and that certain actions or policies enacted by STEM instructors directly support or hinder individual self-advocacy. We generated a table of the self-advocacy barriers STEM instructors created for participants in our study (*See Table 7.3 on page 255-256*). For each barrier, we provide a recommended support to enhance self-advocacy. Our goal in disseminating this information is that STEM instructors can reflect on ways they can support their students' self-advocacy in the classroom. We hypothesize that while enhancing student self-advocacy is necessary to enhance retention of students with disabilities in STEM, it is not sufficient. We must also do a better job preparing STEM instructors to support the self-advocacy of their students.

Together our self-advocacy studies support the conclusion that engaging in self-advocacy for STEM undergraduates with ADHD/SLD is challenging. Nonetheless, many participants with ADHD/SLD in our study effectively developed the knowledge, demonstrated the attitudes, and engaged in behaviors to self-advocate in their STEM courses. Overall, self-advocacy may be enriched in our sample because all the participants agreed to participate in a study where they knew they would be asked to discuss their self-advocacy experiences in STEM courses. We do not know if our revised conceptual model of self-advocacy and the factors influencing self-advocacy also apply to students with ADHD/SLD who are not currently registered with their campus DRC. Currently, the number of studies regarding students with disabilities in STEM is limited. We hope that STEM education researchers will more frequently consider this population

of students in future work. In the future, we plan to continue testing the conceptual model of self-advocacy and the model of factors influencing self-advocacy at different institution types with more students. The findings of these studies may be translated to develop instruments to measure self-advocacy and interventions that promote self-advocacy, ultimately enhancing the retention of students with ADHD/SLD in undergraduate STEM courses.

Closing

While the content of my bench and STEM education research did not directly overlap, I discovered that in both projects I used similar mental processes to ensure the overall success of my research studies. For instance, I found that I could apply the concepts of experimental controls from my bench research project to the qualitative data analysis process in my STEM education research project. Specifically, I began to see coding to consensus as a means to control individual researcher bias. I also found that applying principles of qualitative data analysis enhanced the efficiency of scoring microscopy images in my bench research project. For example, in both projects, I learned that it is best to begin analyzing ~20% of the data. By first focusing on this subset, researchers become sufficiently familiar with the data. In this initial exposure, it is easier to recognize the fine details that distinguish each piece of the data without becoming overwhelmed. This familiarity enables researchers to more readily identify relevant patterns or phenotypes. Then, in iterations, the remaining data can be analyzed in a consistent and fitting manner. This is the type of iterative approach I used to develop an initial codebook in my STEM education research project and how I developed the rubrics I used to score micrographs in my bench research project. Besides these examples of how I transferred knowledge between my research projects, more tacit examples of knowledge, skills, and behaviors exist. I gained experience working in two

different research labs across two academic departments. This unique experience provided many excellent learning opportunities in both conducting research and collaborating with my colleagues. I am grateful for the opportunity to develop distinct content knowledge and skillsets. I am fortunate to have gained an intimate awareness of how discoveries in both fungal cellular biology and in STEM education research are made. Although I have learned so much, I recognize there is still more to learn.

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APPENDIX A

NECESSARY AND SUFFICIENT? SOLVING THE MYSTERY OF THE MITOCHONDRIAL PYRUVATE TRANSPORTER [1]

[1] Pfeifer MA, Stanton JD. (2020). Necessary and sufficient? Solving the mystery of the mitochondrial pyruvate transporter. *CourseSource*. https://doi.org/10.24918/cs.2020.11. Reprinted here with permission of the publisher. Supplemental materials available on *CourseSource*.

Abstract

While there are several available lessons for teaching introductory biology students about diffusion, facilitated diffusion, and active transport, fewer materials exist to support upper-division students' understanding of the proteins that mediate these forms of transport. In the 1970s, mitochondrial pyruvate carrier (MPC) proteins were predicted to import pyruvate from the cytoplasm into mitochondria for cellular respiration. Yet it was not until 2012 that the identity of the proteins responsible for this transport was confirmed in two seminal publications. In this Lesson, students will use their background knowledge of transport mechanisms to analyze data from those papers to determine which of the predicted MPC proteins are actually part of the mitochondrial pyruvate transporter. Student will also learn how scientists test whether a protein is necessary and sufficient. The Lesson is written in the style of process-oriented guided inquiry learning (POGIL). POGIL is a teaching approach that requires students to work collaboratively in small groups to answer a set of questions based on scientific data. Questions in the POGIL activity, called the problem set, are structured so that each question leads to the next, helping to guide students to a deeper understanding of the content. During this Lesson, the instructor acts as a facilitator to guide student learning. Multiple forms of assessment are included within the Lesson, allowing instructors to assess learning gains. This Lesson has been used multiple times by over 10 faculty in an upper-division Cell Biology course and can also be used in other upper-division biology courses.

Scientific Teaching Context

Learning Goals

From Cell Biology Learning Framework:

- Membrane Structure & Function: How do solutes and other materials move across membranes?
- Methods and Tools of Cell Biology: How do the methods and tools of cell biology enable and limit our understanding of the cell?

From the Biochemistry and Molecular Biology Framework:

- How does the nucleotide sequence of the gene lead to biological function?
- How are a variety of experimental and computational approaches used to observe and quantitatively measure the structure, dynamics and function of biological macromolecules?
- What is the scientific process?

Specific Learning Goals

- Students will understand how scientists identify proteins responsible for transport across membranes.
- Students will know how to analyze data from primary literature to draw conclusions about transport mechanisms within the cell.

Learning Objectives

After completing the Lesson, students will be able to:

- Differentiate between types of transport across membranes (diffusion, facilitated diffusion, and active transport)
- Determine if proteins are necessary or sufficient for transport of pyruvate across a membrane based on experimental data
- Interpret data obtained from pyruvate transport mutants
- Design an experiment to test a specific hypothesis related to transport across membranes

Introduction

The cell's ability to selectively transport molecules across its membranes is critical for its survival. Whether transport of molecules occurs across the cell membrane or the membranes of organelles, regulation of molecular gradients is important for proper cellular function. Thus, life science students need a broad understanding of transport mechanisms such as diffusion, facilitated diffusion, and active transport, as well as the proteins that mediate these mechanisms.

Origin and Rationale for the Lesson. Lessons exist for helping introductory biology students understand the basics of membrane transport. These lessons rely on role playing (1-3), computer simulations (4), case studies (5), and analysis of classic papers in the primary literature (6). Our lesson differs from existing lessons in three major ways. First, our lesson was specifically developed for upper-division biology students rather than introductory biology students. We wanted students in our upper-division Cell Biology course to use their basic understanding of membrane transport concepts to gain a deeper understanding of the mechanisms involved. Second, we designed our lesson so that students would learn how scientists identify the proteins that are responsible for transport of a molecule using biochemical and genetic experiments. We also wanted students to understand how scientists use results from more than one organism to confirm the identity of transport proteins. Third, we created a lesson that draws on an approach for small-group learning called Process-Oriented Guided Inquiry Learning (POGIL) (7).

We used two seminal studies published back-to-back in *Science* that revealed the identity of the long unknown mitochondrial pyruvate carrier (MPC), a transporter that brings pyruvate into the mitochondrial matrix from the cytoplasm (8, 9). We selected key figures from the papers to give students practice analyzing real data while drawing on their pre-requisite knowledge from biochemistry and genetics. The studies from the

papers serve as models to help students understand how to design experiments for confirming whether other proteins serve as transporters for particular molecules. We created a Lesson in the spirit of POGIL, similar to our previous POGIL-inspired Lesson on protein localization methods (10).

Background

Intended Audience. The Lesson is intended for senior-level life science majors who are taking an upper-division Cell Biology course. This Lesson has been used in an upper-division Cell Biology course at a large research university in classes ranging from 40 to 135 students. Depending on the background of the students, this Lesson can also be used in an upper-division Biochemistry or Genetics course.

Required Learning Time. The Lesson is designed for 75-minute class session. We have also taught this Lesson in a 50-minute class session with some modifications (see *Suggestions for Possible Adaptations*).

Pre-requisite Student Knowledge.

Background Content Knowledge.

- Introductory Biology: mitochondria structure and function, cellular respiration, role of pyruvate, differences between prokaryotic and eukaryotic cells, differences in cellular transport mechanisms (i.e., diffusion, facilitated diffusion, and active transport)
- Genetics: conserved genes, wild-type, single mutant, double mutant, pedigree analysis
- Biochemistry: properties of amino acid side chains

Background skills:

- Ability to interpret diagrams of molecules
- Ability to interpret basic graphs

Pre-requisite Instructor Knowledge. Instructors should be familiar with the concepts and skills included in the *Pre-requisite Student Knowledge* section. We

recommend that instructors review the two *Science* papers covered in the activity (8, 9). For more information, Bender and Martinou's 2016 review of mitochondrial pyruvate carriers (MPC) provides a general overview and brief summary of the discovery of MPC (11).

Scientific Teaching Themes

Active Learning. Students engage in active learning by working collaboratively in small groups during the Lesson. Students must use their pre-existing knowledge to answer questions posed in the problem set. Students will collaborate to analyze data, form conclusions, and propose experimental designs to test hypotheses. At the end of the class, students lead a group discussion in which the instructor acts only as the facilitator. During discussion, students share their answers to the most difficult questions while displaying their problem sets on a document camera.

Assessment

Formative Assessment. Ongoing formative assessment occurs as the instructor circulates through the room during the Lesson, answering student questions. Formative assessment also occurs during the group discussion at the end of the Lesson. Student groups will share their answers in front of the class, including their experimental designs. The group discussion permits the instructor to provide clarification and feedback to the whole class.

Each group will turn in the one copy of the problem set for feedback. Students earn full credit if they make reasonable progress on the problem set and offer thoughtful answers. We give full credit for "good faith effort" because we want the Lesson to be a low-stakes assessment. Detailed written feedback is provided by either the instructor or the teaching assistant. We encourage students to take pictures of the written feedback when it is returned to the group, to ensure all students have a record of our comments. In the following class period, frequently-missed questions or common points of confusion

are addressed with the whole class. Finally, during the next class session, students are directed to review their graded problems sets and indicate whether they understand the feedback they received. Students are also asked to write down any remaining questions they have about the topic. This presents another avenue for instructors to formatively assess student learning.

Summative Assessment. Summative assessment occurs using matched-pair exam or isomorphic questions (12). On the corresponding exam, students are asked similar but not identical questions based on the problem set (Supporting File S3: Matched-Pair Exam Question). The goal of using matched-pair exam questions is to provide students the opportunity to transfer the knowledge they have gained from the Lesson to novel contexts.

Inclusive Teaching

Students work in groups of three and each student is assigned a rotating role: Manager, Recorder, and Presenter. See “The Lesson Plan” below for a full description of each role. These randomly-assigned roles promote inclusivity by allowing all students the chance to contribute in the classroom. Additionally, the collaborative nature of the problem set gives students the chance to share their own ideas about data analysis and experimental design.

Lesson Plan

Before the Lesson.

Student Preparation: Student pre-reading should be assigned no more than a week in advance of the Lesson. The pre-reading assignment should provide a review of cellular transport mechanisms (diffusion, facilitated diffusion, and active transport). These topics are covered in Alberts' Molecular Biology of the Cell (13) and Lodish's Molecular Cell Biology (14). The National Center for Biotechnology Information Bookshelf has free older versions of both textbooks

(https://www.ncbi.nlm.nih.gov/books/NBK21054/?term=alberts; https://www.ncbi.nlm.nih.gov/books/NBK21475/?term=molecular%20cell%20biology). Depending upon the background knowledge of the students, it may be beneficial to provide additional reading on topics students are already expected to know (see *Student Pre-Requisite Knowledge*). Pre-reading will allow students to complete the activity within the allotted class time.

Instructor Preparation: The instructor should prepare to teach the Lesson by reviewing the questions in the problem set carefully (Supporting File S2: MPC Problem Set-Instructor Version). Some students may not be familiar with some of the terminology in the questions. For example, the instructor should be prepared to clarify the difference between a gene that is necessary versus a gene that is sufficient. The instructor should be prepared to explain to students that the drug UK5099 inhibits mitochondrial pyruvate transporters. Instructors may also need to clarify question 4 by describing to students what it means to biochemically reconstitute a protein. For further discussion of each problem, see *Problem Set* below.

Classroom environment. This Lesson works well in a SCALE-UP (Student-Centered Active Learning Environment with Upside-down Pedagogies) classroom (15), but it has also been taught in traditional classrooms. Students will need to work collaboratively in groups of three. As such, it may be necessary to arrange the classroom seating to best facilitate small group discussion. We teach the Lesson in a SCALE UP classroom where each group of three students has access to one computer. Students can use a personal electronic device to look up terms if they do not have access to a computer. At the end of the Lesson, students share their experimental design with the class on whiteboards or large writing pads. As each group completes their problem set, we ask the Presenters to share their answer to question 4b on whiteboards around the room. It is helpful to use a document camera to best facilitate

the group discussion at the end of the Lesson. Students can place their problem sets under the document camera for the entire class to view as they present their answers.

Problem Set.

Introduction. Once all the students have entered the classroom assign students to groups. We prefer to assign groups rather than allow students to choose their own groups so that no student is left out. We randomly assign groups by asking students to count off 1-15 (for a class of 45 students). Each group will have a Manager, Recorder, and Presenter. We assign roles within each group randomly based on birthdays. The Manager is the student with the birthday closest to the date of the class, the Recorder is the student with the birthday next closest, and the Presenter is the student with birthday farthest away. The Manager will keep track of time and ensure everyone contributes to the problem set. The Recorder will write the group's answers on the official copy of the assignment that is submitted at the end of the period for a grade. The Presenter will share their group's answers with the class during the class discussion at the end of the Lesson. We give students copies of our published descriptions of these roles, see Supporting Materials from our previous *CourseSource* paper (10).

Once group roles have been assigned, introduce the problem set. The problem set contains five questions that ask students to use the results of previous scientific studies to draw conclusions and to design their own experiment to tests specific hypotheses. The questions are written so that each group should not need to use the internet to answer the question, although this depends on the background of the students. We ask students not to use their phones during class because phones distract members from interacting as a group. In our experience, even if a student is looking up something related to the problem set, phone usage promotes "parallel play" rather than collaboration because it is difficult for all group members to see the small screen. Give the class approximately 45-50 minutes to complete the activity. Write the time that

students need to be finished on the board. Remind students that the when their group is finished the Presenter should write their answer to Question 4b on a whiteboard.

Problem Set. Students will begin working on the questions after the introduction (Supporting File S1: MPC Problem Set-Student Version). Distribute copies of the problem set to each student. Although only one group copy will be submitted for grading at the end of the Lesson, all students should write answers on their own copy of the problem set during collaboration so they stay actively involved. Circulate throughout the room to answer any questions the student groups may have. We use questions to help guide students to the correct answer as opposed to telling them answers directly. It is also helpful to provide students with time updates to keep groups on-task and on-time for the group discussion.

- Question 1 introduces the context for the problem set by providing a summary of how mitochondrial pyruvate carrier (MPC) proteins were discovered. The terms integral membrane proteins, inner mitochondrial membrane, and the basics of methodologies such as purification and mass spectrometry should be familiar to upper-division cellular biology students. We encourage students who need a refresher to look up terms they do not remember on the group computer rather than on a personal electronic device. The question then asks students to recall what types of molecules require a transport protein to cross a membrane. Students should notice that pyruvate is relatively large and carries a charge, which makes it unlikely to pass through a membrane without a transport protein.
- Question 2 requires student to interpret data from MPC single and double mutant yeast strains and compare it to data from a wild-type yeast strain to determine which MPC proteins are necessary for pyruvate uptake. Background information about what it means for a protein to be necessary and sufficient in cell biology is provided in a textbox. To answer question 2a, students may need to make notes on the graph about which symbol represents which mutant. We want them to see that data published in top journals like *Science* may have graphs that are not easy to read, but that they can still extract information from them. In response to question 2b, some students will assume that conclusions about sufficiency can

be made from these data. We ask them what the textbox says about testing for sufficiency, and, if necessary, remind them that sufficiency cannot be tested in a system that already has the function of interest. An experiment to test sufficiency of MPC proteins is presented in Question 3.

- Question 3 asks students to interpret pyruvate import data from bacteria expressing mouse MPC genes. Students should recall key fundamental differences between eukaryotes and prokaryotes, especially that prokaryotes lack membrane-bound organelles. Question 3a requires students to explain why researchers expressed MPC genes in prokaryotic cells and not eukaryotic cells. Using bacteria allows the researchers to make conclusions about sufficiency because prokaryotic cells do not have mitochondria, and they do not have pyruvate mitochondrial transporters. In Question 3b, students analyze pyruvate import data to see that if expression of mouse MPC1 and/or mouse MPC2 is sufficient for high levels of pyruvate uptake into bacterial cells. Question 3c explains what the effect of the inhibitor UK5099 is on cells and asks students what conclusions can be made knowing the effect of the inhibitor in eukaryotes, and the observed effect in the bacterial cells expressing mouse MPC proteins. Students may need help realizing the positive control demonstrates that the pyruvate transport in bacteria is functioning similarly to what is seen in eukaryotic cells. Question 3d asks students to explain why pyruvate import increased in the prokaryotic cells when the extracellular pH dropped from 7.2 to 6.2. This question requires students to connect their pre-existing knowledge about transport mechanisms across membranes to experimental data. Students should recall that a lower extracellular pH translates to increased H^+ ions outside the cell. The increase in $[H^+]$ outside the cell could help power transport across the cell membrane through a variety of possible mechanisms such as symport of $[H^+]$ with pyruvate. Some students will hypothesize that pyruvate is protonated under these conditions, in which case we tell them that the pK_a of pyruvate is much lower than pH 6.2.
- Question 4 provides a basic experimental system to test different types of transport across membranes and asks students to design their own experiment. Question 4a asks students to compare and contrast diffusion, facilitated diffusion, and active transport. This is a review based on the pre-reading assignment. Question 4b requires students to design an experiment using the context

presented in the question to test whether diffusion, facilitated diffusion, or active transport is occurring in the described system. Sometimes students have are unsure what it means to “biochemically reconstitute” proteins. In this question, it means that the MPC proteins, which are integral membrane proteins, are incorporated into the bilayer of the lipid vesicles (or liposomes). We remind students to design an experiment that makes sense given what they know about biology. The Presenter from each group should use a white board to share the group answer to Question 4b.

- Question 5 transitions from model systems to mitochondrial disease in humans. Students will learn about a disease resulting from defects in mitochondrial pyruvate oxidation linked to mutations in the human MPC1 gene. Pedigrees from three different families affected by this disease are presented, along with descriptions of the mutations in the MPC1 gene. Question 5a asks students to determine which human MPC1 mutation is most deleterious. A key is provided to help students interpret the pedigree symbols. Question 5b asks students to explain why one type of mutation can be more detrimental to normal protein function than another type of mutation. Question 5c asks students to conclude whether the data presented in the pedigrees supports the hypothesis, “*MPC1 encodes a gene involved in pyruvate import in mitochondria*.” To answer this question, students may need to recall that pyruvate oxidation occurs inside the mitochondria. Finally, Question 5d asks students to briefly design an experiment to test the hypothesis that human MPC1 encodes a protein involved in pyruvate transport. Some students may be unsure about how to design an experiment to test this hypothesis. In those cases, we suggest that students review the experiments they have already read about in the problem set for ideas. (The experimental design used in Question 3 would work well).

Group Discussion. A few minutes before the time deadline, ask students who have not posted their answers on the whiteboard to question 4b do so. Once the deadline has been reached, you can ask for Presenters to volunteer to share their group’s answer for the more challenging questions. Depending on the amount of time, we ask for one or two Presenters to report on question 2, question 3, and question 4b. Not all groups will finish question 5, in which case,

we ask them to do this as homework. We use a random number generator to select a group, then that group's Presenter shares the group's answers to a specific question. Presenters may use a document camera to project their group's answers if available. After students share their group answers, the instructor asks other groups to add or edit the first student's response until consensus is reached. The instructor should take opportunities to mention key components of the scientific process for each question. Once the questions have been discussed as a class, you can revisit the learning objectives of the Lesson to provide a conclusion to the Lesson if time allows. Collect the Recorders' copies of the completed problem set for assessment.

Next Class Period. During the next session of the class, students will answer two debriefing questions after receiving feedback on their problem set answers (Supporting File S1: MPC Problem Set-Student Version). These questions encourage students to read the feedback and identify any areas of confusion from the Lesson. This is another opportunity to collect formative assessment regarding the activity.

Teaching Discussion

Effectiveness in achieving learning goals and objectives. The activity effectively meets the stated learning goals and objectives. Students perform well on summative assessment related to this Lesson. When we have given matched-pair questions on the corresponding unit exam, nearly all students are able to correctly interpret graphs with data from transport mutants. Most students are able to correctly determine whether proteins are necessary or sufficient for transport. The majority of students can also apply their understanding of pyruvate transport to a more challenging question that asks them to explain the effect of an ionophore on pyruvate transport. Student have also performed well when we have given matched-pair questions on a

cumulative final exam or an in-class assessment three months after completing the Lesson. Yet some student confusion is revealed about what type of experiment allows a scientist to make conclusions about sufficiency of a protein or proteins for transport. This suggests that time should be spent revisiting the concept of how to test for sufficiency in order to promote long-term understanding.

Student and instructor reactions to the Lesson. Students respond positively to the Lesson based on classroom observation by multiple instructors and student participation in during class discussion. Instructors who have taught the Lesson note that students struggle at first to understand the difference between necessity and sufficiency, but they become more comfortable after answering the questions and hearing the class discussion. Students seem to be pleased when these concepts finally “click”. Students also appear excited to analyze the pedigree data and some students have explained that this is because they appreciate the connection between pyruvate transport and human health. Students seem to enjoy applying their prior knowledge of pedigree analysis, which they learned in the pre-requisite Genetics course, during the Lesson. When we’ve taught the Lesson in our Cell Biology course, our students readily volunteer to share their answers with the class.

Colleagues who’ve taught this Lesson report their satisfaction with it as well. They mention their appreciation of the way the questions build from simple to complex concepts, which helps students work in groups without much facilitation and with less frustration. They note that the scaffolding is especially important when the Lesson is being taught by only one instructor without the help of a TA. Colleagues also appreciated the way questions give students multiple opportunities to practice analyzing real data and considering experimental design. They note that their students can design experiments to test a specific hypothesis related to transport once they understand the

way scientists have tested similar hypotheses. Nearly all the colleagues who have taught the Lesson once continue to use it in their subsequent Cell Biology courses.

Suggestions for possible adaptations. The Lesson can be adapted for other classes and groups of students. The Lesson could be used in a genetics or biochemistry course if cellular transport is covered. Different levels of students may also find this Lesson effective. Students without a background in genetics and biochemistry may need help with some key concepts used in this activity. For example, students need to be familiar with wild type versus mutant genes and recessive versus dominant traits. Students must also be familiar with the potential impacts amino acid mutations have upon protein function. Finally, students need to be aware of key differences between prokaryotic and eukaryotic cells. If using this Lesson in a class without this background knowledge, it may be helpful to assign additional pre-reading, increase the time students have to complete the activity, and encourage them to use outside resources to look up terms, like the internet or textbooks.

The Lesson can be adapted for larger or smaller groups of students. POGIL is designed for use in large lecture formats and this particular activity has been used in a class as large as 135. It is more difficult for the instructor to interact with individual groups in larger classes, but the questions are written so that each question builds on the knowledge from the question before it. It would be appropriate in such a setting to ensure that students were still able to share their answers during a group discussion. Instead of using white boards, large poster boards could be used. It is also possible to complete this Lesson in 50 minutes instead of 75 minutes. This can be done by assigning Questions 1 and 4a as homework to complete before arriving to the class session.

Supporting Materials[2]

S1. MPC Problem Set-Student Version. A copy of the student version of the problem set is given to all students to work on during the Lesson.

S2. MPC Problem Set-Instructor Version. The instructor version of the problem set contains a key with possible answers to all questions.

S3. Matched-Pair Exam Questions. These questions are similar to but different than the problem set questions and can be used for summative assessment.

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[2] The supporting materials are available from *CourseSource.*

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Table A1. Lesson Plan and Timeline for "Necessary and Sufficient? Solving the Mystery of the Mitochondrial Pyruvate Transporter"

Activity	Description	Time (min)
Preparation for Class		
Student Prep	Complete assigned background reading on cellular transport mechanisms before coming to class.	30-60
Instructor Prep	Read through problem set and review two MPC papers covered in the problem set. (Supporting File: S2. MPC Problem Set-Instructor Version).	60-90
Class Session (75-minute class period)		
Introduction	Briefly provide an overview of activity to students. Help students form groups of three and designate roles (Manager, Recorder, and Presenter).	5
Question 1	Learn background on mitochondria pyruvate carrier (MPC) proteins MPC 1, 2, and 3 Predict whether pyruvate can cross a membrane by diffusion by considering its structure (Supporting File: S1. MPC Problem Set-Student Version, question 1).	3-5
Question 2	Learn about what it means for a protein to be necessary and/or sufficient. Analyze pyruvate import data from mitochondria pyruvate carrier (MPC) single and double mutants in yeast. (Supporting File: S1. MPC Problem Set-Student Version, question 2).	5
Question 3	Analyze pyruvate import data from prokaryotic cells expressing mouse MPC genes. Consider the use of inhibitors to characterize transporters. Evaluate the possible role of pH (i.e., $[H^+]$) in pyruvate transport. (Supporting File: S1. MPC Problem Set-Student Version, question 3).	10-12
Question 4	Distinguish between diffusion, facilitated diffusion, and active transport.	8-10

	Design an experiment to test whether transport of a molecule across a membrane is occurring by diffusion, facilitated diffusion, or active transport occurs, using a given experimental system. (Supporting File: S1. MPC Problem Set-Student Version, question 4).	
Question 5	Analyze pedigree data from families with mutations in MPC genes. Explain why some amino acid mutations are more deleterious than others. Design an experiment to test the hypothesis that human MPC1 encodes a gene involved in pyruvate transport, using experiments in question 2 and 3 as models. (Supporting File: S1. MPC Problem Set-Student Version, question 5).	8-10
Group Discussion	Presenters lead a group discussion of their answers.	15-20
Conclusion	Highlight key concepts from the problem set. Collect the Recorder's copy of each group's problem set for grading. (Supporting File: S2. MPC Problem Set-Instructor Version).	3
Next Class Session		
"Questions for Next Session"	Return graded problem sets to groups. Ask students to answer the two questions to review written feedback and to complete the Lesson. (Supporting File: S1. MPC Problem Set-Student Version, "Questions for Next Session").	5