FUNCTIONAL ANALYSIS OF AN AUXIN-RESPONSIVE GENE AND ITS EFFECTS ON PLANT DEVELOPMENT AND GLOBAL TRANSCRIPTIONAL PROFILES

by

CHOON-MYUNG LEE

(Under the Direction of Dr. Joe L. Key)

ABSTRACT

Auxin mediates multiple aspects of plant growth and development. Auxin up-regulated genes have been studied to assess their role(s) in auxin-regulated plant growth and development. Aux/IAA genes are the most studied auxin-regulated genes. *Axr3-1* mutation results in an amino acid substitution in Domain II of the encoded IAA17 protein. To gain insight into how the Domain IV mutation of *axr3-1*, *axr3-1R4*, overcomes the severe pleiotropic phenotype caused by the Domain II mutation, effects on the expression of a large number of auxin-responsive genes were evaluated, and several protein-protein interactions of mutant proteins were compared to WT. The *axr3-1* mutant exhibited reduced message levels of auxin up-regulated genes, while the message levels in *axr3-1R4* were similar to those of WT. The revertant protein, axr3-1R4, showed no protein-protein interaction with other Aux/IAAs, with ARFs, or with itself. The axr3-1 protein exhibited the same protein-protein interactions as that of the WT protein.

To understand the function of IAA17, *IAA17* and *IAA19* knockouts and *axr3-1R4* were analyzed in more detail. The *IAA17* gene was expressed mainly in root tissue based on both Northern analysis and *IAA17* promoter-driven GUS expression in transgenic Arabidopsis plants. A double knockout mutant of IAA17/IAA19 exhibited WT-like phenotypes except that early stage roots had longer root hairs with shorter root cell size than those of the single knockouts, suggesting a synergistic effect of the two genes in early root development.

Affymetrix ATH1 GeneChips were used to produce global transcriptional profiles of WT, *axr3-1*, *axr3-1R4*, and *IAA17K* plants. A total of 524 genes were up- or down-regulated in *axr3-1* compared to WT. Expression of relatively fewer genes was changed in *axr3-1R4* and *IAA17K* compared to *axr3-1*, correlating with phenotypes of the revertant and the knockout. The global transcriptional patterns of WT and *axr3-1* were used to evaluate the relationship of auxin and light in their effects on plant development. A total of 169 genes were consistently up-regulated by auxin in etiolated WT seedlings. Auxin up-regulated genes were repressed by light, and auxin down-regulated genes were induced by light.

INDEX WORDS: Auxin, Aux/IAA, Axr3/IAA17, IAA19, Global Transcriptional Profiles

FUNCTIONAL ANALYSIS OF AN AUXIN-RESPONSIVE GENE AND ITS EFFECTS ON PLANT DEVELOPMENT AND GLOBAL TRANSCRIPTIONAL PROFILES

by

CHOON-MYUNG LEE

B.S., Chonnam National University, South Korea, 1989

M.S., Chonnam National University, South Korea, 1991

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2004

FUNCTIONAL ANALYSIS OF AN AUXIN-RESPONSIVE GENE AND ITS EFFECTS ON PLANT DEVELOPMENT AND GLOBAL TRANSCRIPTIONAL PROFILES

by

CHOON-MYUNG LEE

Major Professor:	Russell L. Malmberg
Committee:	Joe L. Key Ronald T. Nagao Claiborne Van C. Glover III Zheng-Hua Ye

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2004

DEDICATION

This dissertation is dedicated to my parents, my wife, Eun Yung Park, my two daughters, Eunice and Grace, and my brothers and sister. However, more importantly I would like to give this dedication to my Lord, Jesus Christ.

ACKNOWLEDGEMENTS

I would like to acknowledge the friendship and support of my family, friends, advisor and committee: Joe L. Key, Ronald T. Nagao, Russell L. Malmberg, Claiborne Van C. Glover III, and Zheng-Hua Ye. I also would like to thank Dr. Tom Guilfoyle at The University of Missouri for sending me DR5::GUS seed, Dr. Ottoline Leyser at York University for providing *axr3-1* and *axr3-1R4* seeds, Dr. Don Baldwin at The University of Pennsylvania for processing GeneChip hybridization and scanning, and Dr. Mark Farmer and Dr. John Shields for their help in SEM and confocal microscopy. I am grateful to the lab members of Dr. Key for their help and support: Dr. Kevin O'Grady, Dr. Joe Nairn, Mrs. Ginger Goekjian, Ms. Shelly Miller, Mrs. Joyce Kochert, and Dr. J. C. Hong.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Darwin concluded from studies on phototropism in 1880 that there is some influence moving down from coleoptile tips to elongation zone ("lower part"), causing the latter to bend (see Haagen-Smit, 1951 for review). Boysen-Jensen in 1913 showed that replacing the oat coleoptile tip restores phototropic sensitivity; in 1919, Pa?l showed that replacement of the coleoptile tip on the side of coleoptile stump produced curvatures away from the treated side without a unilateral light stimulus (see Haagen-Smit, 195 for review). In 1926, Went demonstrated that a substance(s) promoting growth of the coleoptile tip can diffuse into a gelatin block, and he improved the quantitative measurement of coleoptile bending by the substance(s) (auxin) (Went, 1974). In the mid-1930s, the chemical structure of an auxin from a urine sample was determined as indole-3-acetic acid (IAA) by the Kögel group, and Kögel and Haagen-Smit in 1931 suggested auxin to be the substance that stimulated elongation in coleoptiles (Haagen-Smit, 1951). Thimann in 1948 suggested that auxin is "an organic substance which promotes growth (i.e. irreversible increase in volume) along the longitudinal axis when applied in low concentrations to shoot of plants freed as far as practicable from their own inherent growth-promoting substances" (Thimann, 1948). Cleland (1996) recommended that auxin be defined as: "A compound that has a spectrum of biological activities similar to, but not necessarily identical with those of IAA. This includes the ability to: 1) induce cell elongation in isolated coleoptile or stem sections, 2) induce cell division in callus tissues in the presence of a cytokinin, 3) promote lateral root formation at the cut surfaces of stems, 4) induce parthenocarpic tomato fruit growth, and 5) induce ethylene formation."

Several other natural auxins such as 4-chloroindol-3-acetic acid and phenylacetic acid occur in at least some plants, but IAA is the most abundant and most active naturally occurring auxin (Bartel, 1997). The activity of several synthetic auxins including *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,4-dichlorophenoxy-a-propionic acid were compared by Thimann (1951). Indole-3-butyric acid, 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 4-chloro-2-methylphenoxyacetic acid, 2-(2,4-dichlorophenoxy) propionic acid, 2-(2-methyl-4-chlorophenoxy) propionic acid, and a-napthalene-1-acetic acid (NAA) have been shown to have auxin activity (Leopold, 1955; Sterling and Hall, 1997). These synthetic auxins have been used commercially in agriculture such as rooting of cuttings for plant propagation, prevention of fruit and leaf drop, herbicides, etc (Leopold, 1995; Taiz and Zeiger, 1998).

Exogenous auxin application promotes growth in stems and coleoptiles with a lag time of about 15 minutes in oat; the optimal concentration of the auxin-induced elongation in stems is typically 10^{-6} to 10^{-5} M of IAA (Leopold, 1950; Cleland, 1995), while the optimal concentration for auxin-induced root growth is about 10^{-10} to 10^{-9} M of IAA (Leopold, 1950; Audus, 1959; Cleland, 1995). Arabidopsis iaaM (having 35S-iaaM from *Agrobacterium tumefaciens* iaaM gene) transgenic plants have up to four-fold higher levels of IAA and display increased hypocotyl elongation in the light. A phenotypic effects of these transgenic plants are suppressed by the auxin-resistant *axr1-3* mutation (Romano et al., 1995), suggesting that the higher level of endogenous auxin can enhance cell elongation in a whole plant. *Arabidopsis* seedlings grown in the light at high temperature (29°C) exhibit dramatic hypocotyl elongation compared with seedlings grown at 20°C, and these plants have a higher level of IAA. This temperature-dependent growth response is dramatically reduced in auxin-related mutants such as *axr1-12* and

tir3-1 (mutation in ubiquitin-related protein-degradation pathway), and *tir1-1* (auxin transporter), while mutants with defects in gibberellin and abscisic acid biosynthesis or in ethylene responsiveness are not affected (Gray et al., 1998). These auxin-induced stem and hypocotyl elongation responses are associated with increased extensibility of cell walls (Cosgrove, 1993). Auxin regulates expressions of genes involved in cell wall modifications such as a xyloglucan endo-trans-glycosylase and an endo-1,4-ß-glucanase (Catala et al., 1997, 2000).

Tropic responses were critical in the discovery of the plant hormone IAA. Phototropism is mediated by the lateral redistribution of auxin in the presence of unilateral light; IAA starts to redistribute so that more IAA flows down to the shaded side and stimulates the cells to elongate faster than those cells on the lighted side (Briggs, 1963; Iino, 1991, 1995). This auxin redistribution can also be applied to gravitropism. Tomato hypocotyls exhibit asymmetrical ^{3}H-IAA redistribution from the lower side starting five to ten minutes after reorientation of the hypocotyl (Harrison and Pickard, 1989). Etiolated soybean hypocotyls respond to gravity so that higher levels of auxin-responsive SAUR gene expression occurs in the faster growing side (lower side) of hypocotyl when the hypocotyls are oriented longitudinally; a shift in SAUR gene expression to the lower side begins at 20 minutes, preceding the gravitropic response that begins at 45 minutes (Guilfoyle et al., 1990). In the root, IAA moves down from the shoot in the central vascular tissues (in the stele) to root cap, redistributed in the root cap, and transported in the epidermal and outer cortical cells. It has been suggested that more auxin flows into the lower side when roots are reoriented by 90º resulting in inhibition of cell elongation on the lower side because roots are more sensitive to auxin than shoots, and the root curves down (Hasenstein and Evans, 1988). Arabidopsis transgenic plants exhibit asymmetrical DR5::GFP expression in the lateral root cap and toward the elongation zone after gravistimulation (Ottenschläger et al.,

2003). In both phototropism and gravitropism, lateral redistribution of auxin plays an important role in differential cell elongation which causes stem and root curvature, and many mutants (*aux1* and *atpin1*) involved in auxin polar transport and in auxin response (*axr3-1* and *nph4*) have been shown to have defects in tropism responses (reviewed by Muday, 2001).

In 1933, Thimann and Skoog demonstrated that exogenous auxin inhibited lateral bud outgrowth in decapitated shoots of *Vicia faba* (bean), and subsequent results from many investigations have supported the results of Thimann and Skoog (Cline, 1996), demonstrating that the outgrowth of the axillary bud is inhibited by auxin transported from the apical bud. An exogenous synthetic auxin (NAA) application restores apical dominance in auxin- resistant Arabidopsis mutant *axr3-1* (Cline et al., 2001). Lateral roots are commonly found above the elongation zone, originating from small groups of cells in the pericycle, and auxin is known to enhance cell division in these areas (reviewed by Casimiro et al., 2003). It is known that auxin stimulates lateral root formation. Exogenous application of auxin stimulates lateral root formation (Evans et al., 1994). Roots deprived of endogenous auxin by the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) fail to initiate mitosis in pericycle cells and re-addition of auxin restores cell division in pericycle cells (reviewed by Casimiro et al., 2003). *Aux1* mutant, lacking the auxin-influx-carrier component AUX1, fails to accumulate IAA in root apices and showed 50% reduction in the number of lateral root primordia (Marchant et al., 2002). *Aberrant lateral root1* (*alf1*) mutant has a 17-fold higher endogenous auxin level than WT Arabidopsis and exhibits hyper-proliferation of lateral roots (Celenza et al., 1995). Arabidopsis *pin-formed* (*pin1-1*) mutant exhibits several floral abnormalities including wide petals, no stamens, and pistil-like structures with no ovules in the ovary (Okada et al., 1991) and encodes an auxin efflux carrier protein (Chen et al., 1998). The *ettin* mutant in Arabidopsis exhibits

various phenotypes related to flower development such as increases in perianth organ number, decreases in stamen number and anther formation, and apical-basal patterning defects in the gynoecium, and the *ettin* gene encodes an auxin response factor 3 (ARF3; Session et al., 1997). ARFs can bind an auxin responsive element (AuxRE, TGTCTC motif) found in auxin up-regulated genes, e.g., Aux/IAAs and GH3 (Ulmasov et al., 1997a, 1999a, 1999b). Above data suggest that auxin regulates floral organ development. Auxin also is known to be involved in induction of vascular differentiation. In *Zinnia elegans* cell cultures, single mesophyll cells "trans-differentiate" directly into tracheary elements (xylem vessel element) without further cell division in response to phytohormones (Fukuda and Komamine, 1980; reviewed by Fukuda, 1997). Auxin application can replace a vascular-inducing signal(s) from young leaf primordia, and local auxin application can induce vascular strands, suggesting that auxin plays a key role in the formation of vascular strands (reviewed by Sachs, 1991). Arabidopsis *MONOPTEROS* mutant exhibits a defect in axial-basal pattern formation as well as vascular differentiation (Hardtke and Berleth, 1998). Mattsson et al. (2003) finds that the activity of the Arabidopsis gene *MONOPTEROS*, which is required for proper vascular differentiation, is also essential in several auxin responses including the regulation of rapidly auxin-inducible AUX/IAA genes, and discovered the tissue-specific vascular expression profile of the class I homeodomain-leucine zipper gene, AtHB20. Interestingly, MONOPTEROS activity is a limiting factor in the expression of AtHB8 and AtHB20, encoding transcriptional regulators expressed early in procambial development.

Auxin affects reproduction such as flower initiation, development and growth, and fruit development (Leopold, 1955). Auxins are produced by the pollen tube as it grows through the style, and by the embryo and endosperm in the developing seeds. Fruit growth depends on these

sources of auxin. In some plants (tomato and cucumber) application of auxin to flowers before pollen is mature can promote parthenocarpy (the production of fruits without fertilization and seed formation).

IAA Biosynthesis and Metabolism

Plants produce active IAA by *de novo* synthesis and by releasing IAA from conjugates. From classic oat coleoptile experiments and more recent experiments (reviewed by Ljung et al., 2002), IAA biosynthesis has been known to occur in rapidly dividing tissue such as the shoot apical meristem and young leaves as primary auxin biosynthesis sites. Even though several biosynthetic pathways of IAA were proposed, IAA biosynthesis and its regulation and developmental signals remain poorly understood (reviewed by Ljung et al., 2002). In *de novo* synthesis, IAA can be synthesized from tryptophan-dependent and -independent pathways (reviewed by Bartel, 1997; see Fig. 1-1). Trp-dependent biosynthesis uses tryptophan as a precursor, and Trp-independent biosynthesis, discovered about ten years ago by using genetic tools, uses indole as a precursor and bypasses tryptophan for IAA biosynthesis (Cohen et al., 2003). Three major pathways, indole-3-pyruvate (IPA), indol-3-acetonitrile (IAN), and tryptamine (TAM) pathways, from L-tryptophan to IAA have been demonstrated (reviewed by Bartel, 1997). However, it is not clear whether all three proposed Trp-dependent pathways exist ubiquitously in all plant species (Bartel, 1997; Zhao et al., 2002). The IPA pathway involves a deamination reaction to form IPA, followed by decarboxylation to form indol-3-acetaldehyde (IAId), and then IAId is oxidized to IAA (right of Fig. 1-1). The TAM pathway is similar to the IPA pathway except the order of the deamination and decarboxylation reactions; in the IAN pathway, tryptophan is first converted to indol-3-acetaldoime (IAOx) and then to indole-3-

acetonitrile (IAN) (top left and center of Fig. 1-1, respectively). Activation tagging in Arabidopsis identified the *YUCCA* gene, which encodes a novel flavin monooxygenase that catalyzes N-hydroxylation of tryptamine (TAM) to N-hydroxyl tryptamine (NHT) (Zhao et al., 2002; top right of Fig. 1-1). Over-expression of *superroot2* (cytochrome P450 CYP83B1), or over-expression of a related cytochrome P450 (CYP83A1) results in increased indolic glucosinolate levels, but the morphological phenotype is consistent with underproduction of IAA. Mutations of *sur2* cause increased adventitious rooting and epinasty, consistent with IAA overproduction (Cohen et al., 2003).

The characterization of Trp auxotrophs (e.g. *orange pericarp*, *orp*; Wright et al., 1991) and stable isotope labeling of intact plants in maize and Arabidopsis led to propose Trp-independent IAA biosynthesis starting from indole or indole-3-glycerol phosphate (IGP) (top of Fig. 1-1; reviewed by Bartel, 1997; Glawischnig et al., 2000). The *orange pericarp* mutant has no tryptophan synthase and shows 50-fold higher levels of IAA than WT maize even with the block in tryptophan biosynthesis (Wright et al., 1991). After this, Trp-independent IAA biosynthesis has been found in several species including maize, Arabidopsis, pine, and bean (reviewed by Ljung et al., 2002). However, the intermediates from indole to IAA are not known, and the pathway remains to be elucidated in detail.

Plants can control IAA levels by several ways: IAA biosynthesis, the production of IAA-conjugates (inactive IAA form), IAA transport, compartmentation into chloroplasts (Sitbon et al., 1993), and IAA degradation either by oxidation or decarboxylation. Conjugates can be formed from IAA via hydrolase enzymes by modifying the side chain of IAA, and these have no auxin activity. It has been shown that IAA-conjugates make up as much as 90% of the total IAA in the plants during vegetative growth (Normanly, 1997), and IAA-conjugates play an important

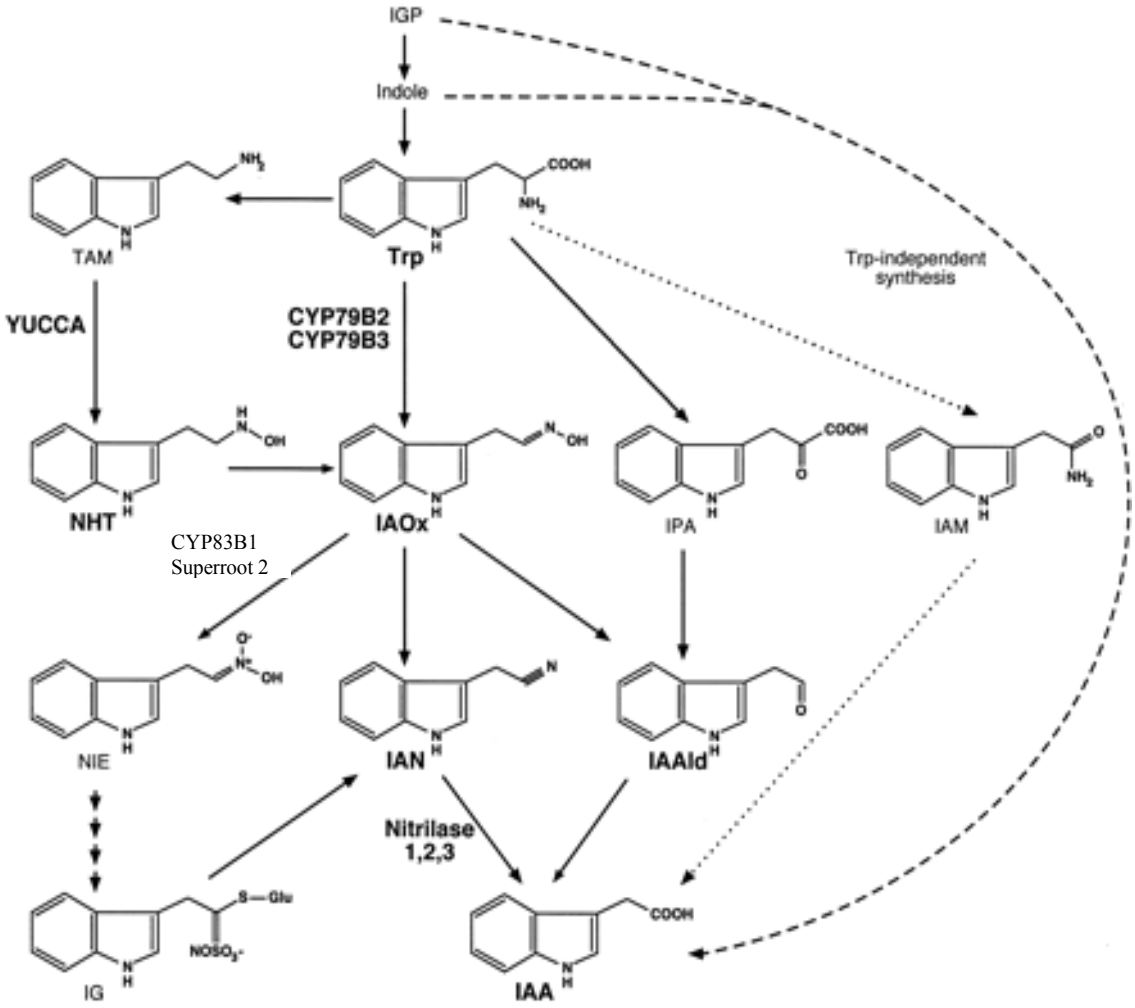


Figure 1-1. Proposed IAA Biosynthesis Pathways. Proposed Trp-dependent pathways are shown. Trp-independent IAA biosynthesis is shown by the dashed arrow, and *Agrobacterium* pathway for IAA biosynthesis is indicated by the dotted arrow. YUCCA, flavin monooxygenase; IAA, indole-3-acetic acid; IAAld, indole-3-acetaldehyde; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IAOx, indole-3-acetaldehyde; IG, indole glucosinolate; IGP, indole-3-glycerol phosphate; IPA, indole-3-pyruvic acid; NHT, N-hydroxyl tryptamine; NIE, 1-aci-nitro-2-indolyl-ethane; TAM, tryptamine; Trp, tryptophan. (Adopted from the Figure 1 of Zhao et al., 2002)

role in the level of free IAA (Östin et al., 1998). Two types of conjugates have been described in a variety of plant species: 1) ester-type conjugates where the carboxyl group of IAA is linked via the oxygen bridge to sugars (e.g. glucose), 2) amide-type conjugates where the carboxyl group forms peptide bonds with amino acids or polypeptides (reviewed by Normanly and Bartel, 1999;

Ljung et al., 2002; LeClere et al., 2002). Degradation of IAA is the final method of controlling auxin levels. This process also has two proposed mechanisms. The oxidation of IAA, as one mechanism of degradation, results in decarboxylation forming 3-methyleneoxindole as the major breakdown product by IAA oxidase (or possibly a peroxidase) and is considered a minor pathway (reviewed by Ljung et al., 2002). Non-decarboxylation of the indole ring is the major degradation pathway of IAA (Östin et al., 1998).

Polar Auxin Transport

Auxin is the only known plant hormone to transport from apex to base, i.e. polar auxin transport. It has been proposed that the shoot apex serves as the primary source of auxin for the entire plant (Ljung et al., 2001). It has been suggested that the auxin gradient resulting from polar auxin transport from shoot to root affects various developmental processes (Friml, 2003). Chemical polar transport inhibitors such as 1-N-naphthylpthalamic acid showed various auxin-related phenotypes suggesting the physiological importance of auxin transport (Rubery and Sheldrake, 1974; Scanlon, 2003). Plants require two types of auxin transporters, influx and efflux carriers, and recent investigations show that their subcellular locations are asymmetrical in that influx and efflux carriers localize at the top and bottom of cells (Friml et al., 2002a,b; Swarup et al., 2001; Blakeslee et al., 2004). Genetic and molecular biological approaches to identify putative auxin influx and efflux carriers resulted in the cloning of AUX and PIN gene families, respectively (Palme and Gälweiler, 1999). Although direct biochemical evidence to support their function as auxin carriers is still lacking, various evidences support that AUX and PIN proteins participate in the auxin transport process (Friml, 2002a, 2002b, 2003). Currently, many putative auxin carriers are identified by molecular genetic studies in Arabidopsis thaliana

such as AUX1 (Bennett et al. 1996) and AtPIN/AGR/EIR gene sequences (Chen et al. 1998; Luschnig et al. 1998; Müller et al. 1998; Utsuno et al. 1998) for influx and efflux carriers, respectively. AUX1 belongs to the auxin amino acid permease family of proton-driven transporters (Bennett et al. 1996), while AtPIN shows sequence homology with bacterial transporters (Palme and Gälweiler, 1999). Mutations in the Arabidopsis gene AtPIN1 disrupt polar auxin transport, embryo patterning, and vascular development, whereas aux1 mutants exhibit an agravitropic root phenotype (Bennett et al. 1996; Palme and Gälweiler, 1999).

Even though auxin was the first identified plant hormone, and its physiological effects on plant growth and development are well known, the molecular mechanism of auxin action is still not known in terms of signal (auxin) reception to transduction of its signal to gene transcription and translation. One putative auxin receptor is Auxin Binding Protein (ABP1) that was first identified based on its ability to bind with NAA in crude membrane preparations of etiolated coleoptiles (Hertel et al., 1972). After that, many auxin-binding proteins have been identified by biochemical studies, but few proteins remain a candidate receptor for auxin (Napier et al., 2002). In addition, genetic studies have been carried out screening for auxin-resistant mutants to identify putative auxin receptor(s), but so far, none of the gene products from the genetic studies show auxin binding activity (Napier et al., 2002). ABP1 has three domains of highly conserved motifs and an ER lumen retention signal (KDEL), and thus most of the protein was retained in ER lumen (Napier et al., 2002), indicating that ABP1 does not follow the criteria of classical receptor proteins. A knockout mutant for ABP1 has been identified from T-DNA screening, showing recessive trait, but the homozygous knockout mutant showed early embryogenic lethal phenotypes, suggesting a critical role in embryogenesis (Chen et al., 2001). However, ABP1 structure was analyzed recently with x-ray crystallography, showing structural similarity with

germin (an oxalate oxidase), and there are no structural differences in ABP1 with and without auxin binding (Woo et al., 2002). Auxin resistance, auxin-mediated cell division, or auxin-regulated gene expression related to ABP1 is still unproven. Other signaling intermediates such as MAP kinase cascade (Mockaitis and Howell, 2000), heterotrimeric G protein, phospholipase C, and inositol triphosphate (reviewed by Scherer, 2002) have been implicated in auxin action, but it is difficult to make a direct relationship with auxin signal transduction.

Auxin-Responsive Genes

Auxin affects plant growth and development in multiple ways. Since IAA has a simple chemical structure, auxin biologists suggest that complex downstream events may be required for the manifestation of such diverse effects on plant growth and development. Five major classes of auxin responsive up-regulated gene families have been identified (Abel and Theologis, 1996). Much evidence accumulated over the last forty years demonstrates that auxin-regulated gene expression is a significant component in effecting these growth and developmental responses (Key, 1969; Hagen, 1987; Key, 1989; Gee et al., 1991; Melissa et al., 1991; Guilfoyle, 1999). Some genes are also down-regulated by auxin based on both cDNA cloning (Baulcombe and Key, 1980) and *in vitro* translation of polyA RNA (mRNA) and 2-D gel analysis of the translation products (Baulcombe et al., 1980; Zurfluh and Guilfoyle, 1980, 1982). The five classes of genes are regulated specifically by auxin, including most notably the Aux/IAAs (Walker and Key, 1982; Hagen et al, 1984; Walker et al., 1985; Theologis et al., 1985; Conner et al., 1990), SAURs (Small Auxin Up-Regulated RNAs, McClure and Guilfoyle, 1987), and GH3s (Hagen et al., 1984) as major up-regulated classes with gluthation-S-transferase encoding genes (GH2/4, Hagen et al., 1988; pCNT103, Van der Zaal, 1987) and ACC synthase encoding genes

(CM-ACS2, Nakagawa et al., 1991; ACS4, Abel et al., 1995a) as minor auxin up-regulated classes. After that, many other less defined individual genes or groups of genes and a number of unrelated compounds have been isolated (reviewed by Abel and Theologis 1996; Guilfoyle, 1999). A detail list of genes that respond to auxin is listed in Table 1-1.

Table 1-1 Auxin-Responsive Genes

Gene	Plant Species	Response Time (min)	Other Inducers	Reference
Aux/lAA gene family				
Aux22	*Glycine*	15	n.d.	Ainley et al. (1988)
Aux28	*Glycine*	30	n.d.	Ainley et al. (1988)
AtAux2 11 (IAA4)	Arabidopsis	30	n.d.	Conner et al. (1990)
AtAux2-27 (IAAS)	Arabidopsis	90	n.d.	Conner et al. (1990)
ARG3	*Vignia*	20	CHX	Yamamoto et al. (1992)
ARG4	*Vignia*	20	CHX	Yamamoto (1994)
GH1	*Glycine*	15	-	Guilfoyle et al. (1993)
PS-IAA4/5	*Pisum*	5	CHX	Oeller et al. (1993)
PS-IAA6	*Pisum*	8	CHX	Oeller et al. (1993)
IAA1 - lAA6	Arabidopsis	5-25	CHX	Abel et al. (1995b)
IAA7, IAA8	Arabidopsis	60-120	-	Abel et al. (1995b)
IAA9 - lAA14	Arabidopsis	15-60	CHX	Abel et al. (1995b)
SAUR gene family				
SAURs	*Glycine*	3-5	CHX	McClure et al. (1989)
ARG7	*Vignia*	S	CHX	Yamamoto (1994)
SAUR-AC1	Arabidopsis	n.d.	CHX	Gil et al. (1994)
GH3 gene family				
GH3	*Glycine*	5	-	Hagen et al. (1991)
Genes encoding GST-like proteins[e]				
GH2/4 (Gmhsp26-A)	*Glycine*	15	CHX, Cd2+	Hagen et al. (1988)
parA	*Nicotiana*	20	CHX, Cd2+	Takahashi et al. (1995)
parB	*Nicotiana*	20	n.d.	Takahashi et al. (1995)
parC	*Nicotiana*	10	CHX	Takahashi et al. (1995)
pCNT103	*Nicotiana*	15	CHX, SA	Van der Zaal et al. (1987)
pCNT107 (parC)	*Nicotiana*	15	ABA, SA	Van der Zaal et al. (1987)
pCNT114 (parA)	*Nicotiana*	30	Cu2+ SA	Van der Zaal et al. (1987)
Genes encoding ACC synthase				
ACS4	Arabidopsis	25	CHX	Abel et al. (1995a)
CM-ACS2	*Cucurbita*	20	n.d.	Nakagawa et al. (1991)
OS-ACS1	*Oryza*	n.d.	CHX	Zarembinski and Theologis (1993)
VR-AC6	mungbean	30	n.d.	Yoon et al., (1997)
Miscellaneous genes				
ARG1	*Vignia*	20	n.d.	Yamamoto et al. (1992)
ARG2	*Vignia*	20	Heat shock	Yamamoto (1994)
ArcA	*Nicotiana*	60	n.d.	Ishida et al. (1993)
NT115/117	Tobacco	30-60	GA,ABA, CHX	Van der Zaal et al. (1987)
TCH4	Arabidopsis	10	Touch, dark, BR	Xu et al., (1995)
MHA2	Maize	40	n.d.	Frias et al., (1996)
Cdc2	Pea	10	Cytokinins	John et al., (1993)
MsPRP5	Alfafa	20	Heat, wonding	Gyorgyey et al., (1997)

n.d., Not determined. From Abel and Theologis (1996) and Guilfoyle (1999)

Members of the Aux/IAA gene family were isolated originally by differential screening of cDNA clones corresponding to mRNAs isolated from control and auxin-treated soybean hypocotyl tissue (Walker and Key, 1982). Ainley et al. (1988) later sequenced and characterized the two genomic clones corresponding to these cDNAs and designated them *GmAux22* and *GmAux28* (22 and 28 stand for deduced protein molecular weight in kDa). The *Ps-IAA4/5* and *Ps-IAA6* genes were isolated from auxin treated pea epicotyl tissue and defined as primary auxin response genes (Theologis et al., 1985). Arabidopsis homologs for *GmAux22* and *GmAux28* also were isolated and designated *AtAux 2-27* and *AtAux 2-11*, respectively (Conner et al., 1990). From the analysis of these four genes, Conner et al., (1990) identified four conserved domains in each putative protein with small regions of absolute identity; it was suggested that these conserved domains would have functional significance. At least 20 members of the Aux/IAA gene family in *Arabidopsis thaliana* have been isolated by PCR screening and yeast two-hybrid screening (Abel et al., 1995a; Kim et al., 1997). Later, with completion of Arabidopsis genomic sequencing, eight additional putative Aux/IAA genes were identified based on sequence homology (reviewed by Liscum and Reed, 2002). Currently, Arabidopsis contains a total of 30 Aux/IAA genes (reviewed by Liscum and Reed, 2002). However, six Aux/IAA genes (denoted as *IAA20*, *IAA30-IAA34*) have no Domains I and II, but the putative proteins have a similar molecular weight and in some cases (e.g. IAA20) have a partially conserved Domain I motif (Appendix A). Otherwise, the four conserved domains noted above are present in each of the identified Aux/IAA genes. The constitutive level of expression of Aux/IAA genes varies among the genes, as does the magnitude of the auxin-inducibility. The induction kinetics of these genes by auxin treatment varies from as little as five to ten minutes up to one to two hours (Walker and Key, 1982; Abel et al., 1995a; reviewed by Abel and Theologis, 1996). For example, *IAA3* and

IAA6 mRNAs are induced within 5 min of auxin treatment and peak after 10 min, whereas *IAA7* and *IAA8* respond more slowly (60 to 120 min) (reviewed by Abel and Theologis, 1996). Aux/IAA mRNAs are specifically induced by biologically active auxins and do not respond to other hormones or to a wide range of environmental and chemical stresses (Walker et al., 1985; Theologis et al., 1985; reviewed by Guilfoyle, 1999). In addition, the auxin-enhanced mRNA levels are shown by run-on (off) transcription studies to be the result, at least in part, from enhanced transcription of the genes (Hagen et al., 1984; Hagen and Guilfoyle, 1985). Aux/IAA genes encode proteins ranging from 20 to 35 kDa; these are found only in plants and are ubiquitous to plants (reviewed by Guilfoyle, 1999). In peas, Ps-IAA4 and Ps-IAA6 proteins were localized to the nucleus; these proteins have short half-lives, in the range of six to ten minutes (Abel et al., 1994). Of the conserved domains, Domains I and II contain typical bipartite NLS (nuclear localization signal) motifs, and the C-terminal Domain IV contains another NLS (Abel and Theologis, 1995b). It is proposed that conservation in amino acid sequence within these four domains in plants might play an important function(s) in auxin-regulated biological responses.

SAURs were identified by differential hybridization screening of cDNA clones from auxin-treated soybean hypocotyl (McClure and Guilfoyle, 1987). SAUR mRNAs are induced within two to five minutes of auxin application, and the induction of soybean SAURs is transcriptionally regulated (McClure et al., 1989). Auxin-inducible SAURs have been identified from mung bean, pea, Arabidopsis, and *Zea mays* (reviewed by Hagen and Guilfoyle, 2002). Some of SAURs are also induced by cycloheximide (Gil et al., 1994) and by the plant hormone cytokinin (Timpte et al., 1995). Deduced SAUR proteins have a molecular weight of nine to ten

kDa. (McClure et al., 1989). Arabidopsis contains at least 70 SAUR genes, and the function of SAUR proteins is not clear (reviewed by Hagen and Guilfoyle, 2002).

GH3 mRNA was also identified by differential screening of the cDNA clones from auxin-treated soybean etiolated seedlings (Hagen et al., 1984). Soybean GH3 mRNA starts to induce by auxin treatment within five minutes (Hagen and Guilfoyle, 1985) and is only induced by auxin treatment (Hagen et al., 1984). Soybean GH3 gene encodes a 70 kDa protein with unknown function (Hagen et al., 1991). GH3 genes have been identified from soybean, tobacco, Arabidopsis, and other dicots and monocots (reviewed by Hagen and Guilfoyle, 2002). Arabidopsis contains 20 members of the GH3 gene family encoding 65 to 70 kDa molecular weight proteins (reviewed by Hagen and Guilfoyle, 2002). *DFL1*, an Arabidopsis GH3-related gene, was isolated from activation tagged lines, and over-expression of this gene by the activation tagged mutation results in shorter hypocotyls under continuous red, blue and far-red light conditions (Nakazawa et al. 2001). *FIN219*, a member of the Arabidopsis GH3 gene family, exhibits a longer hypocotyl than wild type under continuous far-red light conditions (Hsieh et al. 2000). Over-expression of *DFL2* (At4g03400, also a member of Arabidopsis GH3 gene family) in transgenic Arabidopsis results in a short hypocotyl phenotype under red and blue light, and an antisense transgenic line of this gene displays a long hypocotyl under red light conditions (Takase et al., 2003). *Yadokari 1*-D (*ydk1*-D, an Arabidopsis GH3 gene), resulting from over-expression of *ydk1*-D by activation tagging, has a short hypocotyl in the light and the dark, a short primary root, a reduced lateral root number, and reduced apical dominance; *YDK*1 gene expression is induced by auxin treatment and regulated by ARF 7 (Takase et al., 2004). One Arabidopsis GH3 deduced protein shows a firefly luciferase superfamily-like structure by

the analysis of fold prediction, and some GH3 proteins can adenylate jasmonic acid or IAA *in virto* (Staswick et al., 2002).

Auxin Response Factor Identification and Characterization

Since enhanced transcription accounts at least in part for enhanced mRNA levels for the AuxIAAs, a number of studies primarily from the Guilfoyle lab were conducted in order to describe relevant promoter elements of these genes (AuxRE) and the transcription factor(s) that interacted with these elements (Ulmasov et al., 1997b). Transgenic plants and the carrot protoplast system were used to define the relevant sequences that make up the primary promoter element(s) of these genes (Ulmasov et al., 1995; Ulmasov et al., 1997a). The sequence, TGTCTC, was discovered to be the primary auxin response element (AuxRE) and is found typically to be associated with a second promoter element that varies from gene to gene (Ulmasov et al., 1995). A class of transcription factors (Auxin Response Factors, ARFs) that interact with the AuxRE was defined in these studies by using Yeast one-hybrid screening using a palindromic repeat of TGTCTC motif as a bait sequence (Ulmasov et al., 1997b). ARF1 (Auxin Response Factor 1) was first cloned by the above analysis (Ulmasov et al., 1997b). Independently, ARF genes were identified by Yeast two-hybrid screening by using IAA1 as bait protein, and originally named *IAA21* to *IAA25* (Kim et al., 1997). Yeast two-hybrid analyses (Kim et al., 1997; Ulmasov et al., 1999b; Ouellet et al., 2001) demonstrate that Domains III and IV serve as protein-protein interaction domains for Aux/IAAs and/or ARFs in homo- and (to a lesser extent) heterodimer formation.

ARFs are also a multigene family of transcriptional regulators, consisting of 23 members in Arabidopsis (reviewed by Liscum and Reed et al., 2002), and the encoded proteins range in

size from 70 to 130 kDa (reviewed by Hagen and Guilfoyle, 2002). ARFs have a DNA binding domain in the N-terminal region and the conserved Domains III and IV found in the Aux/IAA proteins in the C-terminal region; ARF3 (*Etten*, Sessions et al., 1997) and ARF17 represent exceptions in that they do not contain Domains III and IV (Liscum and Reed, 2002). In gel shift assays, ARFs show a preference for forming homodimers in binding to synthetic palindromic AuxREs (Ulmasov et al., 1999b). Some ARFs repress transcription while others activate transcription of reporter genes containing synthetic AuxREs based on the amino acid composition in the middle region (MR) in carrot protoplast transient assays (Ulmasov et al., 1999a). For example, some ARFs (ARF5, 6, 7, 8 and 19) have Q-rich MR and at least four ARFs from among the five ARFs are confirmed experimentally as transcriptional activators in carrot transient assay (Ulmasov et al., 1999a). Over expression of Aux/IAAs repressed transcription of reporter genes containing synthetic AuxRE promoter elements or the *GH3* promoter in carrot protoplast transient assays suggesting that over-expressed Aux/IAAs may interfere with ARF function in auxin-regulated gene expression (Ulmasov et al., 1997a). These observations led to the suggestion that Aux/IAAs function as repressors of ARF-mediated gene expression possibly by sequestering ARFs and/or by preventing the formation of homo-and/or heterodimers of ARFs resulting in repression of early auxin responsive genes (Guilfoyle et al., 1998). Since palindromic AuxREs do not exist in these plant genes, ARFs may bind as a monomer on AuxREs to turn on/off early auxin response genes including Aux/IAAs (Ulmasov et al., 1999a). How ARFs and Aux/IAAs regulate the transcription of early auxin-response genes is not yet fully understood.

Aux/IAA and ARF Mutants and Putative Functions

Genetic approaches to understand auxin action screened mutant populations for auxin-resistant or auxin-sensitive phenotypes. Such screens have yielded at least four classes of mutants with altered auxin-related phenotypes: 1) genes related to auxin signal transduction: putative receptor and kinase, *abp1*, *pinoid*, and *rcn1* (Chen et al., 2001; Christensen et al., 2000; Deruère et al., 1999); 2) a class of mutations which affect genes involved in auxin transport, e.g. *auxl*, *eirl*, *pinod*, *pin-formed, rcnl*, *pisl*, and *lop1* (Carland and McHale, 1996; Bennett et al., 1995; reviewed by Tian and Reed, 1999); 3) mutations affecting genes involved in activation of the ubiquitin-related protein RUB, such as *axr1* (auxin resistant) and/or its putative down-stream effector, *sar1*, *axr6*, and *tir1* (Cernac et al., l997; Hellmann et al., 2003; Gray et al., 2001); 4) mutations affecting Aux/IAA or Auxin Response Factor (ARF) transcriptional regulatory genes, such as *axr2*, *axr3*, *shy2, bdl, slr, msg2*, and *iaa28* (members of the Aux/IAA family) and *ettin*, *nph,* and *monopteros* (members of the ARF family) (Rouse et al., 1998; Tian and Reed, 1999; Hardtke and Berleth, 1998; Sessions et al., 1997; Hamann et al., 2002). The last class represents one example, where molecular and genetic approaches to understand the molecular action of auxin find common genes involved in auxin signaling. Here, Aux/IAA and ARF mutants are described in detail.

All Aux/IAA mutants characterized by screening for auxin-resistant phenotype(s) to date are gain-of-function mutants, and the mutations are located within Domain II of the Aux/IAA genes centered in the GWPPV motif. Each of these mutations results in multiple auxin-related pleiotropic phenotypes. Currently nine gain-of-function mutations in Aux/IAA genes have been characterized: *shy1-1* (*IAA6*, Kim et al., 1996), *shy2-2* (*IAA3*, Tian and Reed, 1999), *axr2-1* (*IAA7*, Nagpal et al., 2000), *bdl* (*IAA12*, Hamann et al., 1999), *slr* (*IAA14*, Fukaki et al., 2002),

axr3-1 (*IAA17*, Leyser et al., 1996; Rouse et al., 1998), *iaa18-1* (*IAA18*, reviewed by Reed, 2001), *msg2* (*IAA19*, Tatematsu et al., 1999), *iaa28-1* (*IAA28*, Rogg et al., 2001). All of these Aux/IAA Domain II gain-of-function mutants show auxin-related pleiotropic (semi-) dominant phenotypes, demonstrating the importance of Domain II and its critical role in auxin signaling by Aux/IAAs. Ramos et al. (2001) conjugated Domains I and II of IAA17/AXR3 with luciferase (Luc) and examined protein stability by measuring luciferase activity. They showed that mutated Domain II-conjugated protein is 20 times more stable than WT protein. Ouellet et al. (2001) carried out pulse chase analyses of IAA17 and axr3-1 proteins with peptide-raised IAA17 antibody from WT and the *axr3-1* mutant and showed that the axr3-1 protein is seven times more stable than WT IAA17. Finally Gray et al. (2001) showed axr3-1-GUS-conjugated protein is 20 times more stable than IAA17/AXR3-GUS conjugated protein. Domain II-mediated protein degradation is facilitated by auxin (Zenser et al., 2001; Tiwari et al., 2001 and 2003; Gray et al., 2001). Aux/IAA proteins interact with the ubiquitin ligase SCF^{TIR1}, and this interaction is facilitated by auxin, resulting in 26S proteosome-mediated degradation (Gray et al., 2001). The above data clearly show that Domain II of Aux/IAAs is involved in their stability and that mutations within Domain II correlate with enhanced protein stability and their gain-of-function mutant phenotypes.

Axr3-1 was the first gain-of-function mutant characterized (Rouse et al., 1998). *Axr3-1* is semi-dominant and encodes a modified Aux/IAA protein (IAA17). The *axr3-1* allele has a Pro to Leu change at position 88 within Domain II and shows the most severe phenotype changes such as agravitropic and short roots, strong apical dominance, short hypocotyls in dark, a small plant with upcurled leaves, etc. (Leyser et al., 1996). Intragenetic suppressors (or revertants) of *axr3-1* are cloned following EMS treatment of *axr3-1* seeds. Five revertants were isolated with primary

root length being used to measure allelic strength. One revertant, *axr3-1R4*, is the strongest of the five alleles and has a wild type-like phenotype (Rouse et al., 1998) and has an additional mutation within Domain IV. Over-expressed Aux/IAAs decrease expression of reporter genes containing either a synthetic AuxRE or the *GH3* promoter (Ulmasov et al., 1997a). Similarly, Domain II mutants of Aux/IAAs (*axr3-1*) repress the expression of the reporter gene containing a synthetic AuxRE (Tiwari et al., 2003).

Axr2-1 is also a Domain II dominant mutant, and the gene encodes IAA7 (Nagpal et al., 2000). *Axr2-1* exhibits various auxin-related phenotypes such as agravitropic roots with normal root growth rate, auxin-resistant root growth, more lateral and fewer adventitious roots than WT, fewer root hairs, wavy leaves, agravitropic and short hypocotyls and stems, short hypocotyl in dark, and leaf formation in dark (Wilson et al., 1990; Timpte et al., 1992; Nagpal et al., 2000). The intragenic revertants, *axr2-1r3* and *axr2-1r4*, have additional mutations resulting in change of Arg to Lys at position 138 and Leu to Phe at position 15, respectively, and display dominant traits to WT. Both revertants seem to be more auxin-sensitive than *axr2-5* (T-DNA knockout, null mutant), but their phenotypes are WT-like except slightly slower hypocotyl growth in light grown seedlings (Timpte et al., 1994; Nagpal et al., 2000), suggesting the intragenic revertant partially decreases the activity of axr2-1 protein (Nagpal et al., 2000).

Bodenlos is a Domain II dominant mutant, and its gene encodes IAA12 (Hamann et al., 1999,2002); the mutant displays defects in the primary root meristem, but normal post-embryonic roots develop; adult plants are fertile, are insensitive to the auxin 2,4-D, have upcurled leaves, reduced apical dominance, normal root hairs and lateral roots, and short inflorescence stem (Hamann et al., 1999, 2002).

An auxin-resistant Arabidopsis mutant, *iaa28-1*, is also a Domain II gain-of-function mutant and exhibits severe defects in lateral root formation, slight auxin-insensitive root growth, smaller adult size, and decreased apical dominance (bush-like); its gene encodes IAA28 (Roggs et al., 2001). IAA28 is preferentially expressed in roots and florescence stem, but its message level is not induced by the application of exogenous auxin; over-expression of iaa28-1 protein in a transgenic plant exhibits much more sever phenotypes than *iaa28-1* mutant, displaying much smaller and bushier plantlets than *iaa28-1* (Roggs et al., 2001).

The dominant Domain II gain-of-function *solitary-root-1* (*slr-1*) mutant exhibits complete lack of lateral roots by blocking cell divisions of pericycle cells in lateral root initiation, and exogenous auxin application does not rescue this phenotype (Fukaki et al., 2002). The *slr-1* mutant also exhibits defects in root hair formation and in the gravitropic response of roots and hypocotyls; it also shows strong apical dominance, smaller leaves, and short and thin inflorescence stem, and the gene encodes IAA14 (Fukaki et al., 2002). *Slr-1r1*, containing an additional mutation within Domain I (Asp to Asn change), displays WT-like phenotype with fewer lateral roots, and green fluorescent protein (GFP)-tagged mutant IAA14 protein is localized in the nucleus (Fukaki et al., 2002).

Msg2-1 has a nucleotide change that resulted in a substitution of Pro to Ser at position 69 in Domain II of IAA19 (Tatematsu et al., 1999, 2004). Additional alleles of the IAA19 dominant mutant were isolated as follows: *msg2-3* with Pro to Leu change at position 69 and *msg2-2* with Gly to Arg change at position 67. Phenotypes of *msg2* mutants include no gravitropism, weaker phototropism, and weaker hook formation in hypocotyls as well as 2,4-D-resistant hypocotyl growth (Tatematsu et al., 1999, 2004).

Short hypocotyls 1 was originally screened for the suppressor of *hy2* mutant in Arabidopsis and exhibits partial photomorphogenic responses in the dark with apical hook opening, reduced hypocotyl elongation, and upcurled leaves (Kim et al., 1996); it also is a dominant Domain II mutant encoding IAA6 (Reed, 2001). *Iaa18-1* is also a Domain II gain-of-function mutant encoding IAA18 and exhibits long hypocotyls, fused cotyledons, short roots, and upcurled leaves (Reed, 2001)

Shy2 (*short hypocotyl*) is a dominant mutation in a gene that encodes the IAA3 protein. The *Shy2-2* mutation is located within conserved Domain II (Tian and Reed, 1999) with a Pro to Ser change in the core GWPPV motif. The phenotypes of *axr3-1* and *shy2-2* show many similarities such as a short root, increased adventitious root formation, upward curling of leaf edges, agravitropic roots, and formation of leaves in the dark. One of the intragenic revertants of *shy2-2*, *shy2-22*, has an additional mutation within Domain IV resulting in replacement of half of Domain IV with eight new amino acids, and this revertant also showed WT-like phenotypes. These two revertants and the failure of axr3-1R4 to undergo protein-protein interactions in yeast two-hybrid assays indicate the importance of Domains III and IV as interaction domains with Aux/IAAs and ARFs in homo- and heterodimer formation and their critical role in auxin signaling in plant growth and development.

The function of Domain I of Aux/IAAs is less clear. There is limited information, but no direct evidence, that Domain I may be involved in protein stability. *Axr3-1R3*, an intragenic revertant of *axr3-1,* has an additional mutation within Domain I (Leu to Phe change at position 18); this protein showed similar protein-protein interaction properties as those of WT IAA17 and axr3-1 proteins in yeast two hybrid analysis (Ouellet et al., 2001). Tiwari et al., (2001) showed that Domain II-mutated IAA17 (axr3-1 protein), IAA7 (axr2-1 protein), and IAA19 (msg2-1

protein) reduce the reporter gene activity containing the P3(4X) promoter (4X repeats of a palindromic synthetic AuxRE, **GAGACA**ACT**TGTCTC**) by three- to six-fold; however, these proteins with mutations within both Domains I and II (such as axr3-1R3 protein, axr2-1-r-3 protein, and artificial *in vitro* mutated Domain I protein from msg2-1) recover reporter gene activity to the level expressed in the presence of WT proteins in carrot transient assays. In addition, a mutation only in Domain I (such as iaa17R3 from Chapter II) results in decreased protein stability, while the Domain II mutation (axr3-1 protein) has much increased protein stability. However, axr3-1R3 protein (mutations in both Domains I and II) has an intermediate level of protein stability between WT IAA17 and axr3-1 protein (Tiwari et al., 2001). Taken together the data indicate that Domain I is somehow involved in protein stability. Other roles/functions of these conserved Domains have not been defined until recently. The data reported by Tiwari et al., (2004) indicate that Domain I (as core motif LxLxLx, L stands for Leu) serves as a general repressor domain. However, importance of Domain I as a general repressor is not clear in terms of plant development since Domain IV revertants (such as *axr3-1R4* and *shy2-22*) show phenotypic recovery.

Currently, three ARF mutants have been characterized and all are recessive. *Monopteros* (*mp*) mutant was the first identified ARF mutant, and originally identified for defects in embryo patterning (Berleth and Jürgens, 1993), and the gene encodes *ARF5* (Hardke and Berleth, 1998). *Mp* exhibits defects in post-embryo patterning and disruption of vascular tissues (Berleth and Jürgens, 1993; Hardke and Berleth, 1998). ARF5 is a transcriptional activator in carrot transient assays and can bind AuxRE DNA motif, and the binding strength is increased by dimmer formation (Ulmasov et al., 1999a and b).

Ettin (*ett*) mutant exhibits various floral organ defects such as increase in "perianth organ number, decrease in stamen number and anther formation, and apical-basal patterning defects in the gynoecium" (Session et al., 1997). *Ett* gene encodes ARF3 protein, which lacks Domains III and IV. ARF3 protein binds the palindromic AuxRE *in vitro* in band shift assays, but the interaction seems weaker and more unstable than other ARFs, which have Domains III and IV (Ulmasov et al., 1999b). ARF3 does not exhibit transcriptional activation or repression in carrot transient assay (Ulmasov et al., 1999a), suggesting this gene may not be involved in auxin-responsive gene expression. Thus, it is not clear whether ARF3 is involved in auxin-related gene expression and auxin signal transduction even though *ett* exhibits auxin-related phenotypes.

ARF7 mutant has been identified from three independent laboratories. *Non-phototrophic hypocotyls* (*nph*) originally identified by defects in hypocotyl phototropism in response to long-term rate unilateral blue light (Liscum and Briggs, 1995); the nph4 gene encodes ARF7 (Harper et al., 2000). *Transport inhibitor-resistant5* (*tir5-2*) originally was identified based on the resistance to NPA (Ruegger et al., 1997), and *massugu 1* (*msg1*) was identified based on its resistance to 2,4-D in hypocotyl growth and tropic responses (Watahiki and Yamamoto, 1997); both the mutants are allelic to ARF7. The *ARF7* mutant exhibits, in addition to that described above, defects in hypocotyl gravitropism, alteration of apical hook maintenance, and epinastic leaves, but the roots are as sensitive to 2,4-D as the wild type (Watahiki and Yamamoto, 1997; Harper et al., 2000). *Nph* null mutant exhibits repressed message levels of auxin up-regulated genes (*IAA6 and IAA30*) in control and (*IAA2*, *IAA5*, *IAA6*, *IAA12*, *IAA13*, *GH3*, and *SAUR-AC1*) in auxin-treated seedlings, respectively (Stowe-Evans et al., 1998).

Among *ARF* genes, seven ARFs are clustered on chromosome I (Hagen and Guilfoyle, 2002). Recently, several T-DNA knockouts of ARFs by reverse genetic approach have been

identified, but these mutants do not show distinct phenotypes (personal communication with Guilfoyle), suggesting redundant functionality among those ARFs. Analyzing more Aux/IAA and ARF mutants and double and/or triple mutants of Aux/IAA and/or ARFs should provide valuable insights into understanding auxin-related plant growth and development.

References

Abel S, Nguyen M, Theologis A (1995a). The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana.* J. Mol. Bio. **251**:533-549

Abel S, Oeller PW, Theologis A (1994). Early auxin-induced genes encode short-lived nuclear proteins. Proc. Natl. Acad. Sci. USA **91**:326-330

Abel S, Theologis A (1995b). A polymorphic bipartite motif signals nuclear targeting of early auxin-inducible proteins related to PS-IAA4 from pea (*Pisum sativum*). Plant J. **8**:87-96

Abel S, Theologis A (1996). Early genes and auxin action. Plant Physiol. **111**, 9-17

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997). Genes involved in organ separation in Arabidopsis: An analysis of the cup-shaped cotyledon mutant. Plant Cell **9:** 841–857

Ainley W, Walker J, Nagao R, Key J (1988). Sequence and characterization of two auxin-regulated genes from soybean. J. Biol. Chem. **263**:10658-10666

Audus LJ (1959). Plant growth substances, 2nd ed. London: Leonard Hill

Bartel B (1997). Auxin Biosynthesis. Annu. Rev. Plant Mol. Biol. **48**:51-66

Baulcombe D, Giorgini J, Key JL (1980). The effect of auxin on the polyadenylated RNA of soybean hypocotyls. *In* Nato Advanced Studies Institute Published in Genome Organization and Expression in Plants edited by CJ Leaver Plenum Press, pp 175-185

Baulcombe DC, Key JL (1980). Polyadenylated RNA sequences which are reduced in concentration following auxin treatment of soybean hypocotyls. J. Biol. Chem. 255:8907-8913

Behringer FJ, Davies PJ (1992). Indole-3-acetic acid levels after phytochrome-mediated changes in the stem elongation rate of dark- and light-grown *Pisum* seedlings. Planta **188:** 85-92

Bennett SRM, Alvarez J, Bossinger G, Smyth DR (1995). Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. Plant J **8**: 505–520

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schultz B, Feldmann KA (1996). Arabidopsis AUX1 gene: A permease-like regulator of root gravitropism. Science **273**: 948–950

Berleth T, Juergens G (1993). The role of the *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. *Development* **118**:575 -587

Blakeslee JJ, Bandyopadhyay A, Peer WA, Makam SN, Murphy AS (2004). Relocalization of the PIN1 auxin efflux facilitator plays a role in phototropic responses. Plant Physiol. **134**:28-31

Briggs WR (1963). Mediation of phototropic responses of corn coleoptiles by lateral transport of auxin. Plant Physiol **38**: 237–247

Carland FM, McHale NA (1996). *LOP1*: a gene involved in auxin transport and vascular patterning in *Arabidopsis*. Development **122**:1811–1819

Casimiro I, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G, Bennett MJ (2003). Dissecting Arabidopsis lateral root development. Trends Plant Sci. **8**:165-71

Catalá C, Rose JKC, Bennett AB (1997). Auxin regulation and spatial localization of an endo-1,4-ß-D-glucanase and a xyloglucan endotransglycosylase in expanding tomato hypocotyls. Plant J **12**: 417–426
Catalá C, Rose JKC, Bennett AB (2000). Auxin-Regulated Genes Encoding Cell Wall-Modifying Proteins Are Expressed during Early Tomato Fruit Growth. Plant Physiol. **122**: 527–534

Celenza JL Jr, Grisafi PL, Fink GR (1995). A pathway for lateral root formation in Arabidopsis thaliana. Genes Dev. **9**:2131-2142

Cernac A, Lincoln C, Lammer D, Estelle M (1997). The SAR1 gene of Arabidopsis acts downstream of the AXR1 gene in auxin response. Development **124**:1583–1591

Chen JG, Ullah H, Young JC, Sussman MR, Jones AM (2001). ABP1 is required for organized cell elongation and division in Arabidopsis embryogenesis. Genes Dev. **15**: 902-911

Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson P (1998). The Arabidopsis thaliana AGRAVITROPIC 1 gene encodes a component of the polar-auxin-transport efflux carrier. Proc. Natl. Acad. Sci. **95**: 15112–15117

Christensen S, Dagenais N, Chory J, Weigel D (2000). Regulation of auxin response by the protein kinase PINOID. Cell **100**: 469-478

Cleland RE (1995). Auxin and cell elongation. *In* Plant Hormones and Their Role in Plant Growth Development, 2nd ed., ed PJ Davies, Kluwer, Dordrecht, Netherlands, pp 214-227

Cleland RE (1996). Growth substance, *In* Units, Symbols, and Terminology for Plant Physiology ed by FB Salisbury, Oxford University Press, New York, pp. 126-128

Cline MG (1996). Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. Annals of Botany **78**:255-266

Cline MG, Chatfield SP, Leyser O (2001). NAA restores apical dominance in the axr3-1 mutant of Arabidopsis thaliana. Annals of Botany **87**:61-65

Cohen JD, Slovin JP, Hendrickson1 AM (2003). Two genetically discrete pathways convert tryptophan to auxin: more redundancy in auxin biosynthesis. Trends Plant Sci. **8**:197-199

Colon-Carmona A, Chen DL, Yeh KC, Abel S (2000). Aux/IAA proteins are phosphorylated by phytochrome in vitro. Plant Physiol. **124**:1728-38

Conner T, Goekjian V, LaFayette P, Key J (1990). Structure and expression of two auxin-inducible genes from *Arabidopsis*. Plant Mol. Biol. **15**:623-632

Cosgrove DJ (1993). Wall extensibility: its nature, measurement, and relationship to plant cell growth. New Phytol **124**: 1–23

Deruère J, Jackson K, Garbers C, Söll D, DeLong A (1999). The RCN1-encoded A subunit of protein phosphatase 2A increases phosphatase activity in vivo. Plant J. **20**:389-399

Evans ML, Ishikawa H, Estelle MA (1994). Responses of Arabidopsis roots to auxin studied with high temporal resolution: comparison of wild type and auxin response mutants. Planta **194**: 215–222

Firn RD (1994). Phototropism. *In* Kendrick RE, Kronrnberg GHM, eds, Photomorphogenesis in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 659–681

Frias I, Caldeira MT, Perez-Castineira JR, Navarro-Avino JP, Culianez-Macia FA, Kuppinger O, Stransky H, Pages M, Hager A, Serrano R (1996). A major isoform of the maize plasma membrane H(+)-ATPase: characterization and induction by auxin in coleoptiles. Plant Cell **8**:1533-44

Friml J (2003). Auxin transport - shaping the plant. Curr. Opin. Plant Biol. **6**:7-12

Friml J, Benkova E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002a). AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. Cell **108**: 661–673

Friml J, Winiewska J, Benková E, Mendgen K, Palme K (2002b). Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. Nature **415**:806-809

Fukaki H, Tameda S, Masuda H, Tasaka M (2002). Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsi*s. Plant J. **29**:153-168

Fukuda H (1997). Tracheary element differentiation. Plant Cell **9**: 1147–1156

Fukuda H, Komamine A (1980). Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans*. Plant Physiol **65**: 57–60

Gee MA, Hagen G, Guilfoyle TJ (1991). Tissue-specific and organ-specific expression of soybean auxin-responsive transcripts GH3 and SAURs. Plant Cell **3**:419-430

Gil P, Liu Y, Orbovic V, Verkamp E, Poff KL, Green PJ (1994). Characterization of the auxin-inducible SAUR-AC1 gene for use as a molecular genetic tool in Arabidopsis. Plant Physiol. **104**:777-784

Gil P, Green PJ (1997). Regulatory activity exerted by the *SAUR-AC1* promoter region in transgenic plants. Plant Mol. Biol. **34**: 803–808

Glawischnig E, Tomas A, Eisenreich W, Spiteller P, Bacher A, Gier A (2000). Auxin biosynthesis in maize kernels 1. Plant Physiol. **123**: 1109–1120

Gray WM, Östin A, Sandberg G, Romano CP, Estelle M (1998). High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. Proc Natl Acad Sci USA **95**: 7197–7202

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001). Auxin regulates SCF^{TIR1}-dependent degradation of the Aux/IAA proteins. Nature **414**:271–276

Guilfoyle T (1999). Auxin-regulated genes and promoters. *In* Biochemistry and Molecular Biology of Plant Hormones eds by Hooykaas P, Hall M, and Libbenga K, Elsevier Science B.V. pp423-459

Guilfoyle TJ, Hagen G, Li Y, Ulmasov T, Liu Z, Strabala T, Gee M (1993). Auxin-regulated transcription. Aust. J. Plant Physiol. **20**:489-502

Guilfoyle TJ, McClure BA, Hagen G, Brown D, Gee M, Franco A (1990). Regulation of plant gene expression by auxin *In* Gene Manupulation in Plant Improvement II, ed by Gustafson JP, Plenum Press, New York, pp 401-418

Guilfoyle TJ, Ulmasov T, Hagen G (1998). The ARF family of transcription factors and their role in plant hormone responsive transcription. Cell Mol. Life Sci. **54**: 619-627

Gyorgyey J, Nemeth K, Magyar Z, Kelemen Z, Alliotte T, Inze D, Dudits D (1997). Expression of a novel-type small proline-rich protein gene of alfalfa is induced by 2,4-dichlorophenoxyacetic acid in dedifferentiated callus cells. Plant Mol. Biol. **34**:593-602

Haagen-Smit AJ (1951). The History and Nature of Plant Growth Hormones, *In* Plant Growth Substances, ed by F Skoog, University of Wisconsin Press, pp 3-20

Hagen G (1987). The control of gene expression by auxin. *In* Plant Hormones and Their Role in Plant Growth and Development, ed by P.J. Davies, Dordrecht, The Netherlands: Martinus Nijhoff, pp 149-163

Hagen G, Guilfoyle TJ (1985). Rapid induction of selective transcription by auxins. Mol. Cell. Biol. **5 :**1197-1203

Hagen G, Guilfoyle TJ (2002). Auxin-responsive gene expression: Genes, promoters and regulatory factors. Plant Mol. Biol. **49:**373–385

Hagen G, Kleinschmidt A, Guilfoyle TJ (1984). Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections. Planta **162** :147-1 53

Hagen G, Martin G, Li Y, Guilfoyle TJ (1991). Auxin-induced expression of the soybean GH3 promoter in transgenic tobacco plants. Plant Mol. Biol. **17**:567-579

Hagen G, Uhrhammer N, Guilfoyle TJ (1988). Regulation of an auxin-induced soybean sequence by cadmium. J. Biol. Chem. **263**:6442–6446

Hamann T, Benkova E, Baurle I, Kientz M, Jürgens G (2002). The Arabidopsis *BODENLOS* gene encodes an auxin response protein inhibiting *MONOPTEROS*-mediated embryo patterning. Genes Dev. **16**: 1610**–**1615

Hamann T, Mayer U, Jürgens G (1999). The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the Arabidopsis embryo. Development **126**: 1387**–**1395

Hardtke CS, Berleth T (1998). The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J. **17**:1405–1411

Harper RM, Stowe-Evans EL, Luesse DR, Muto H, Tatematsu K, Watahiki MK, Yamamoto K, Liscum E (2000). The NPH4 locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial Arabidopsis tissue. Plant Cell **12:** 757–770

Harrison MA, Pickard BG (1989). Auxin asymmetry during gravitropism by tomato hypocotyls. Plant Physiol. **89**:652-657

Hasenstein K H, Evans ML (1988). The effect of cations on hormone transport in primary roots of *Zea mays*. Plant Physiol. **86**: 890-894

Hellmann H, Hobbie L, Chapman A, Dharmasiri S, Dharmasiri N, Del Pozo C, Reinhardt D, Estelle M (2003). Arabidopsis AXR6 encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis. EMBO J. **22**:3314-3325

Hertel R, Thompson KS, Russo VEA (1972). *In vitro* auxin binding to particulate cell fractions from corn coleoptiles. Planta **107**:325–340.

Hsieh HL, Okamoto H, Wang M, Ang LH, Matsui M, Goodman H, Deng XW (2000). *FIN219*, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development. Genes Dev. **14**: 1958–1970

Iino M (1991). Mediation of tropisms by lateral translocation of endogenous indole-3-acetic acid in Maize coleoptiles. Plant Cell Environ. **14**: 279–286

Iino M (1995). Gravitropism and phototropism of maize coleoptiles: evaluation of the Cholodny-Went theory through effects of auxin application and decapitation. Plant Cell Physiol **36**: 361–367

Jensen PJ, Hangarter RP, Estelle M (1998). Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown Arabidopsis. Plant Physiol. **116**: 455-4621

John PCL, Zhang K, Dong C (1993). A $p34^{cdc2}$-based cell cycle: its significance in monocotyledonous, dicotyledonous and unicelluar plants. *In* Molecular and Cell Biology of the Plant Cell Cycle, eds by Ormrod JC, Francis D, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 9–34

Jones AM, Cochran DS, Lamerson PM, Evans ML, Cohen JD (1991). Red light-regulated growth. I. Changes in the abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. Plant Physiol. **97:** 352-358

Kepinski S, Leyser O (2002). Ubiquitination and auxin signaling: a degrading story. Plant Cell **14:**S81 - S95

Key J (1969). Hormones and nucleic acid metabolism. Annu. Rev. Plant Physiol. **20**:449-473

Key J (1989). Modulation of gene expression by auxin. BioEssays **11**: 5248

Kim BC, Soh MS, Kang BJ, Furuya M, Nam HG (1996). Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2*. Plant J. **9:** 441–456

Kim J, Harter K, Theologis A (1997). Protein-protein interactions among the Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **94**:11786-11791

LeClere S, Tellez R, Rampey RA, Matsuda SP, Bartel B (2002). Characterization of a family of IAA-amino acid conjugate hydrolases from Arabidopsis. J Biol Chem. **277**:20446-20452

Leopold AC (1955). Auxins and Plant Growth. University of California Press

Leyser O, Pickett FB, Dharmasiri S, Estelle M (1996). Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. Plant J. **10**: 403-413

Liscum E, Briggs WR (1995). Mutations in the *NPH1* locus of Arabidopsis disrupt the perception of phototropic stimuli. Plant Cell **7**: 473–485

Liscum M, Reed J (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol. Biol. **49**:387-400

Ljung K, Bhalerao RP, Sandberg G (2001). Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. Plant J. **28**:465–474

Ljung K, Hull AK, Kowalczyk M, Marchant A, Celenza J, Cohen JD, Sandberg G (2002). Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana.* Plant Mol. Biol. **49:** 249-272

Luschnig C, Gaxiola RA, Grisafi P, Fink GR (1998). EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in Arabidopsis thaliana. Genes & Dev. **12**: 2175–2187

Marchant A, Bhalerao R, Casimiro I, Eklöf J, Casero PJ, Bennett M, Sandberg G (2002) AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. Plant Cell **14**:589-597

Mattsson J, Ckurshumova W, Berleth T (2003). Auxin signaling in Arabidopsis leaf vascular development. Plant Physiol. **131**: 1327- 1339

McClure BA, Hagen G, Brown CS, Gee MA, Guilfoyle TJ (1989). Transcription, organization, and sequence of an auxin-regulated gene cluster in soybean. Plant Cell **1**:229-239

McClure BA, Guilfoyle TJ (1987). Characterization of a class of small auxin-inducible soybean polyadenylated RNAs. Plant Mol. Biol. **6**:611-623

Melissa A. Hagen G, Guilfoyle TJ (1991). The tissue-specific and organ-specific expression of soybean auxin-responsive transcripts GH3 and SAURs. Plant Cell **3**:419-430

Mockaitis K, Howell SH (2000). Auxin induces mitogenic activated protein kinase (MAPK) activation in roots of Arabidopsis seedlings. Plant J. **24**:785-96

Muday GK (2001). Auxins and tropisms. J. Plant Growth Regul. 20:226-43

Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett MJ, Wisman E, Palme K (1998). AtPIN2 defines a locus of Arabidopsis for root gravitropism control. EMBO J. **17**: 6903–6911

Mussig C, Shin GH, Altmann T (2003). Brassinosteroids promote root growth in Arabidopsis. Plant Physiol. **133**: 1261-71

Nagpal P, Walker L, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW (2000). AXR2 encodes a member of the Aux/IAA protein family. Plant Physiol. **123**:563-573

Nakagawa N, Mori H, Yamazaki K, Imaseki H (1991). Cloning of a complementary DNA for auxin-induced ACC synthase and different expression of the gene by auxin and wounding. Plant Cell Physiol. **32**:1153-1163

Nakazawa M, Yabe N, Ichikawa T, Yamamoto YY, Yoshizumi T, Hasunuma K, Matsui M (2001). DFL1, an auxin-responsive GH3 gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. Plant J. **25**: 213–221

Napier RM, David KM, Perrot-Rechenmann CP (2002). A short history of auxin-binding proteins. Plant Mol. Biol. **49**:339–348

Normanly J (1997). Auxin metabolism. Physiol. Plant **100:** 431-442

Normanly J, Bartel B (1999). Redundancy as a way of life: IAA metabolism. Curr. Opin. Plant Biol. **2**:207-213

Oeller PW, Keller JA, Parks JE, Silbert JE, Theologis A (1993). Structural characterization of the early indoleacetic acid-inducible genes, PS-IAA415 and PS-IAA6, of pea (Pisum sativum L,). J. Mo1. Biol. **233**:789-798

Okada K, Ueda J, Komaki MK, Bell CJ, Simura Y (1991). Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell **3:** 677-684

Östin A, Kowalyczk M, Bhalerao RP, Sandberg G (1998). Metabolism of indole-3-acetic acid in Arabidopsis. Plant Physiol. **118**: 285-296

Ottenschlager I, Wolff P, Wolverton C, Bhalerao RP, Sandberg G, Ishikawa H, Evans M, Palme K (2003). Gravity-regulated differential auxin transport from columella to lateral root cap cells. Proc. Natl. Acad. Sci. USA **100**:2987-2991

Ouellet F, Overvoorde P, Theologis A (2001). IAA17/AXR3: biochemical insight into an auxin mutant phenotype. Plant Cell **13**: 829-842

Palme K, Gälweiler L (1999). PIN-pointing the molecular basis of auxin transport. Curr. Opin. Plant Biol. **2**: 375–381

Ramos JA, Zenser N, Leyser O, Callis J (2001). Rapid degradation of auxin/Indoleacetic Acid proteins requires conserved amino acids of Domain II and is proteasome dependent. Plant Cell **13:**2349 –2360

Reed JW (2001). Roles and activities of Aux/IAA proteins in Arabidopsis. Trends Plant Sci. **6 :**420–425

Rogg LE, Lasswell J, Bartel B (2001). A gain-of-function mutation in *IAA28* suppresses lateral root development. Plant Cell **13**:465-480

Romano CP, Robson PRH, Smith H, Estelle M, Klee HJ (1995). Transgene-mediated auxin overproduction in *Arabidopsis*: hypocotyl elongation phenotype and interactions with the *hy6–1* hypocotyl elongation and *axr1* auxin resistant mutants. Plant Mol Biol **27**: 1071**–**1083

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O (1998). Changes in auxin response from mutations in an *AUX/IAA* gene. Science **279**:1371-1373

Rubery PH, Sheldrake AR (1974). Carrier-mediated auxin transport. Planta **188**: 101**–**121

Ruegger M, Dewey E, Hobbie L, Brown D, Bernasconi P, Turner J, Muday G, Estelle M (1997). Reduced naphthylphthalamic acid binding in the tir3 mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. Plant Cell **9**: 745-757

Sachs T (1991). Cell polarity and tissue patterning in plants. Development Suppl. **91**: 83–93

Scanlon MJ (2003). The Polar Auxin Transport Inhibitor N-1-Naphthylphthalamic Acid Disrupts Leaf Initiation, KNOX Protein Regulation, and Formation of Leaf Margins in Maize Plant Physiol. **133**: 597-605

Scherer GFE (2002). Secondary messengers and phospholipase A_2 in auxin signal transduction. Plant Mol. Biol. **49**:357-372

Sessions A, Nemhauser, McColl J, Roe J, Feldmann K, Zambryski P (1997). ETTIN patterns the Arabidopsis floral meristem and reproductive organs. Development **124**:4481-4491

Sitbon F, Edlund A, Gardestrom P, Olsson O, Sandberg G (1993). Compartmentation of indole-3-acetic acid metabolism in protoplasts isolated from leaves of wild-type and IAA-overproducing transgenic tobacco plants. Planta **191**:274–279

Staswick PE, Tiryaki I, Rowe M (2002). The jasmonate response locus *JAR1* and several related Arabidopsis genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. Plant Cell **14**: 1405–1415

Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I (1999). Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development **126**:4235-4245

Sterling TM, Hall JC (1997). Mechanism of action of natural auxins and the auxinic herbicides. *In* Roe RM, Burton JD, Kuhr RJ, eds, Herbicide Activity: Toxicology, Biochemistry and Molecular Biology. IOS Press, Amsterdam, pp 111–141

Stowe-Evans EL, Harper RM, Motchoulski AV, Liscum E (1998). NPH4, a conditional modulator of auxin-dependent differential growth responses in Arabidopsis. Plant Physiol. **118**:1265-1275

Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M (2001). Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. Genes Dev. **15**: 2648-2653

Taiz L, Zeiger E (1998). Plant Physiology. Sinauer Associates Publishers, Sunderland, MA

Takahashi Y, Ishida S, Nagata T (1995). Auxin-regulated genes. Plant Cell Physiol. 36:383-390

Takase T, Nakazawa M, Ishikawa A, Kawashima M, Ichikawa T, Takahashi N, Shimada H, Manabe K, Matsui M (2004). ydk1-D, an auxin-responsive GH3 mutant that is involved in hypocotyl and root elongation. Plant J. **37**:471-483

Takase T, Nakazawa M, Ishikawa A, Manabe K, Matsui M (2003). *DFL2*, a new member of the Arabidopsis GH3 gene family, is involved in red light-specific hypocotyl elongation. Plant Cell Physiol. 44:1071-80

Tatematsu K, Watahiki K, Yamamoto K (1999). Evidences for a dominant mutation of IAA19 that disrupts hypocotyl growth curvature responses and alters auxin sensitivity. *In* 10th International Conference on Arabidopsis Research (Melbourne, Australia). Abstract No. 8-39

Tatematsu K, Kumagaia S, Mutob H, Satoa A, Watahikia MK, Harperc RM, Liscumc E, Yamamotoa KT (2004). *MASSUGU2* Encodes Aux/IAA19, an Auxin-Regulated Protein That Functions Together with the Transcriptional Activator NPH4/ARF7 to Regulate Differential Growth Responses of Hypocotyl and Formation of Lateral Roots in *Arabidopsis thaliana*. Plant Cell **16**:379-393

Theologis A, Huynh TV, Davis RW (1985). Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J. Mol. Biol. **183**:53-68

Thimann KV (1948). Plant Growth Hormones. *In* The Hormones, eds by Pincus G, Thimann K, vol. 1, New York, USA, Academic Press

Thimann KV (1977). Hormone Action *In* the Whole Life of Plants. Amherst, MA: University of Massachusetts Press

Tian Q, Reed JW (1999). Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. Development **126**:711-721

Tian Q, Uhlir JU, Reed JW (2002). Arabidopsis SHY2/IAA3 Inhibits Auxin-Regulated Gene Expression. Plant Cell **14**: 301-319

Timpte CS, Lincoln C, Pickett FB, Turner J, Estelle M (1995). The AXR1 and AUX1 genes of Arabidopsis function in separate auxin-response pathways. Plant J. **8**:561-569

Timpte CS, Wilson AK, Estelle M (1994). The *axr2-1* mutation of *Arabidopsis thaliana* is a gain-of-function mutation that disrupts an early step in auxin response. Genetics **138**: 1239-1249

Timpte CS, Wilson AK, Estelle M (1992). Effects of the *axr2* mutation of *Arabidopsis* on cell shape in hypocotyl and inflorescence. Planta **188**: 271-278

Tiwari SB, Wang WJ, Hagen G, Guilfoyle TJ (2001). AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell **13**:2809-2822

Tiwari SB, Hagen G, Guilfoyle TJ (2003). The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell **15**:533-543

Tiwari SB, Hagen G, Guilfoyle TJ (2004). Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell **16**:533-543.

Ulmasov T, Hagen G, Guilfoyle TJ (1997b). ARF1, a transcriptional factor that binds to auxin response elements. Science **276**:1865-1868

Ulmasov T, Hagen G, Guilfoyle TJ (1999a). Activation and repression of transcription by auxin-response factors. Proc. Natl. Acad. Sci. USA **96**:5844-5849

Ulmasov T, Hagen G, Guilfoyle TJ (1999b). Dimerization and DNA binding of auxin response factors. Plant J. **19**:309-319

Ulmasov T, Liu ZB, Hagen G, Guilfoyle TJ (1995). Composite structure of auxin response elements. Plant Cell **7:**1611–1623

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997a). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9**: 1963-1971

Utsuno K, Shikanai T, Yamada Y, Hashimoto T (1998). *AGR*, an *Agravitropic* locus of *Arabidopsis thaliana*, encodes a novel membrane-protein family member. Plant Cell Physiol. **39**:1111-1118

Van der Zaal EJ, Memelink J, Mennes AM, Quinn A, Libbenga KR (1987). Auxin-induced mRNA species in tobacco cell cultures. Plant Mol. Biol. **10**:145–157

von Arnim AG (2003). On again - off again: COP9 signalosome turns the key on protein degradation. Current Opinion in Plant Biology **6**:520-529

Walker J, Key J (1982). Isolation of cloned cDNAs to auxin-responsive poly(A)$^+$RNAs of elongating soybean hypocotyl. Proc. Natl. Acad. Sci. USA **79**:7185-7189

Walker J, Legocka J, Edelman L, Key J (1985). An analysis of growth regulator interactions and gene expression during auxin-induced cell elongation using cloned complementary DNAs to auxin-responsive messenger RNAs. Plant Physiol. **77**:847-850

Watahiki M-K, Yamamoto K-T (1997). The massugu1 mutation of Arabidopsis identified with failure of auxin-induced growth curvature of hypocotyl confers auxin insensitivity to hypocotyl and leaf. Plant Physiol. **115:** 419–426

Went FW (1926). On growth accelerating substances in the coleoptile of *Avena sativa.* Proc K Akad Wet Amsterdam **30**: 10–19

Went FW (1974). Reflections and speculations. Annu. Rev. Plant. Physiol. **25:** 1-26

Wilson AK, Pickett FB, Turner JC, Estelle M (1990). A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. Mol. Gen. Genet. **222**: 377-383

Woo E-J, Marshall J, Bauly J, Chen J-G, Venis M, Napier RM, Pickersgill RW (2002). Crystal structure of auxin-binding protein 1 in complex with auxin. EMBO J. **21**: 2877–2885

Wright AD, Sampson MB, Neuffer G, Michalczuk L, Slovin JP, Cohen JD (1991). Indole-3-acetic acid biosynthesis in the mutant maize *orange pericarp,* a tryptophan auxotroph. Science **254**: 998–1000

Xie Q, Guo HS, Dallman G, Fang S, Weissman AM, Chua NH (2002). SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. Nature **419:**167–170

Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J (1995). Arabidopsis *TCH4*, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. Plant Cell **7**: 1555–1567

Yamamoto KT (1994). Further characterization of auxin-regulated mRNAs in hypocotyl sections of mung bean [Vigna radiata (L.) Wilczek]: sequence homology to genes for fatty acid desaturases and

atypical late embryogenesis-abundant protein, and the mode of expression of mRNAs. Planta **192**:359-364

Yamamoto KT, Mori H, Imaseki H (1992). cDNA cloning of indole-3-acetic acid-regulated genes: Aux22 and SAUR from mung bean (Vigna radiata) hypocotyl tissue. Plant Cell Physiol. **33**:93-97

Yang T, Law DM, Davies PJ (1993). Magnitude and kinetics of stem elongation induced by exogenous indole-3-acetic acid in intact light-grown pea seedlings. Plant Physiol. **102**: 717–724

Yoon IS, Mori H, Kim JH, Kang BG, Imaseki H (1997). VR-ACS6 is an auxin-inducible 1-aminocyclopropane -1-carboxylate synthase gene in mungbean (Vigna radiata). Plant Cell Physiol. **38**:217-224

Zarembinski TI, Theologis A (1993). Anaerobiosis and plant growth hormones induce two genes encoding 1-aminocyclopropane-1-carboxylate synthase in rice (Oryza sativa L.). Mol. Biol. Cell. **4**:363-373

Zenser N, Dreher KA, Edwards SR, Callis J (2003). Acceleration of Aux/IAA proteolysis is specific for auxin and independent of *AXR1*. Plant J. **35**: 285-294

Zenser N, Ellsmore A, Leasure C, Callis J (2001). Auxin modulates the degradation rate of Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **98 :** 11795–11800

Zhang Y, Brown G, Whetten R, Loopstra CA, Neale D, Kieliszewski MJ, Sederoff RR (2003). An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. Plant Mol Biol. **52**:91-102

Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, Ecker JR, Normanly J, Chory J, Celenza JL (2002). Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev. **16**:3100-3112

Zurfluh LL, Guilfoyle TJ (1980). Auxin-induced changes in the patterns of protein synthesis in soybean hypocotyls. Proc. Natl. Acad. Sci. USA **77**:357-361

Zurfluh LL, Guilfoyle TJ (1982). Auxin-induced changes in the population of translatable messenger RNA in elongationg sections of soybean hypocotyl. Plant Physiol. **69**:332

CHAPTER II

MOLECULAR CHARACTERIZATION OF IAA17/AXR3 MUTATION[1]

[1] Lee CM, O'Grady K, Nagao RT, Key J To be submitted to Plant Physiology

Introduction

The plant hormone auxin affects plant growth and development in multiple ways including mediation of primary root growth, promotion of root hair formation as well as adventitious and lateral root formation, hypocotyl and stem elongation, mediation of root and stem tropisms, vascular tissue differentiation, apical dominance, and phyllotaxy (Leopold, 1955; Guilfoyle, 1999).

The mechanism(s) by which auxin mediates these multiple developmental and physiological processes is not fully understood. However, a large body of evidence accumulated over the last forty years demonstrates that auxin-regulated gene expression is a significant component in effecting these growth and developmental responses (Key, 1969; Hagen, 1987; Key, 1989; Melissa et al., 1991; Guilfoyle, 1999). The expression of a number of families of genes are regulated specifically by auxin, including most notably the Aux/IAAs (Walker and Key, 1982; Hagen et al, 1984; Walker et al.,1985; Theologis et al., 1985; Conner et al., 1990), SAURs (Small Auxin Up-Regulated RNAs, McClure and Guilfoyle, 1987), GH3s (Hagen et al., 1984), and other less defined individual genes or groups of genes which may respond to auxin and a number of unrelated compounds (reviewed by Guilfoyle, 1999).

Members of the Aux/IAA gene family were isolated originally by differential screening of cDNA clones corresponding to mRNAs isolated from control and auxin-treated soybean hypocotyl tissue (Walker and Key, 1982). Ainley et al. (1988) later sequenced and characterized the two genomic clones corresponding to these cDNAs and designated them *GmAux22* and *GmAux28* (22 and 28 stand for deduced protein molecular weight in kDa). The *Ps-IAA4/5* and *Ps-IAA6* genes were isolated from auxin-treated pea epicotyl tissue and defined as primary auxin response genes (Theologis et al., 1985). Arabidopsis homologs for *GmAux22* and *GmAux28* also

were isolated and designated *AtAux 2-27* and *AtAux 2-11*, respectively (Conner et al., 1990). From the analysis of these four genes, Conner et al. (1990) identified four conserved domains in each putative protein with small regions of absolute identity, and suggested that these conserved domains would have functional significance. At least 20 members of the Aux/IAA gene family in *Arabidopsis thaliana* have been isolated by PCR screening and yeast two-hybrid screening (Abel et al., 1995a; Kim et al., 1997). The four conserved domains noted above were present in each of the identified Aux/IAA genes. The constitutive level of expression of Aux/IAA genes varies among the genes as does the magnitude of the auxin-inducibility. The induction kinetics of these genes by auxin treatment varies from as little as five to ten minutes up to one to two hours (Walker and Key, 1982; Abel et al., 1995a). For example, *IAA3* and *IAA6* mRNAs are induced within 5 min of auxin treatment and peak after 10 min, whereas *IAA7* and *IAA8* respond more slowly (60 to 120 minutes) (reviewed by Abel and Theologis, 1996). Aux/IAA mRNAs are specifically induced by biologically active auxins and do not respond to other hormones or to a wide range of environmental and chemical stresses (Walker et al., 1985; Theologis et al., 1985; reviewed by Guilfoyle, 1999). Also the auxin-enhanced mRNA levels were shown by run-on (off) transcription studies to be the result, at least in part, from enhanced transcription of the genes (Hagen et al., 1984; Hagen and Guilfoyle, 1985).

Since the current study is based on *IAA17*, this family of genes will be described in some detail. Aux/IAA genes encode proteins ranging from 20 to 35 KDa; these are found only in plants, and are ubiquitous to plants. In peas, Ps-IAA4 and Ps-IAA6 proteins were localized to the nucleus; these proteins have short half-lives, in the range of 6 to 10 minutes (Abel et al., 1994). Of the conserved domains, Domains I and II contain typical bipartite NLS (nuclear localization signal) motifs, and the C-terminal Domain IV contains another NLS (Abel and Theologis,

1995b). It was proposed that conservation in amino acid sequence within these four domains in plants might play an important function(s) in auxin-regulated biological responses.

One approach to understanding the function of the Aux/IAAs in auxin action has been the screening of mutant populations for auxin-resistant mutants. All Aux/IAA mutants characterized to date are gain-of-function mutants, and the mutations are located within Domain II of the Aux/IAA genes. Each of these mutations results in multiple auxin-related pleiotropic phenotypes. Currently nine gain-of-function mutations in Aux/IAA genes have been characterized: *shy1-1* (*IAA6*, Kim et al., 1996), *shy2-2* (*IAA3*, Tian and Reed, 1999), *axr2-1* (*IAA7*, Nagpal et al., 2000), *bdl* (*IAA12*, Hamann et al., 1999), *slr* (*IAA14*, Fukaki et al., 2002), *axr3-1*(*IAA17*, Leyser et al., 1996; Rouse et al., 1998), *iaa18-1* (*IAA18*, reviewed by Reed, 2001), *msg2* (*IAA19*, Tatematsu et al., 1999), and *iaa28-1* (*IAA28*, Rogg et al., 2001). *Axr3-1* was the first gain-of-function mutant characterized (Rouse et al., 1998). *Axr3-1* is semi-dominant and encodes a modified Aux/IAA protein (IAA17). The *axr3-1* allele has a Pro to Leu change at position 88 within Domain II and shows the most severe phenotype changes such as agravitropic and short roots, strong apical dominance, short hypocotyls in dark, a small plant with upcurled leaves, etc. (Leyser et al., 1996). Intragenetic suppressors (or revertants) of *axr3-1* were cloned following EMS treatment of *axr3-1* seeds. Five revertants were isolated with primary root length being used to measure allelic strength. One revertant, *axr3-1R4*, was the strongest of the five alleles and has near wildtype phenotype (Rouse et al., 1998).

Since enhanced transcription accounts at least in part for enhanced mRNA levels for the AuxIAAs, a number of studies primarily from the Guilfoyle laboratory were conducted in order to describe relevant promoter elements of these genes (AuxRE) and the transcription factor(s) that interacted with these elements (Ulmasov et al., 1997b). Transgenic plants and the carrot

protoplast system were used to define the relevant sequences that made up the primary promoter element(s) of these genes (Ulmasov et al., 1995;Ulmasov et al., 1997a). The sequence, TGTCTC, was discovered to be the primary auxin response element (AuxRE) and was found typically to be associated with a second promoter element which varied from gene to gene (Ulmasov et al., 1995). A class of transcription factors (Auxin Response Factors, ARFs) that interacted with the AuxRE was defined in these studies (Ulmasov et al., 1997b).

ARF1 (Auxin Response Factor 1) was first cloned using a yeast one-hybrid screen with a highly active synthetic auxin-responsive element (AuxRE) containing a palindromic repeat of the TGTCTC element as bait (Ulmasov et al., 1997b). ARFs are also a multigene family of transcriptional regulators, consisting of 23 members in Arabidopsis (reviewed by Liscum and Reed et al., 2002), ranging in size from 70 to 130 kDa in protein size (reviewed by Hagen and Guilfoyle, 2002). ARFs have a DNA-binding domain in the N-terminal region and the conserved Domains III and IV found in the Aux/IAA proteins in the C-terminal region. ARF3 (*Etten*, Sessions et al., 1997) and ARF17 represent exceptions in that they do not contain Domains III and IV. Domains III and IV serve as protein-protein interaction domains (Ulmasov et al., 1997b, 1999a; reviewed by Liscum and Reed, 2002, and Hagen and Guilfoyle, 2002). ARFs interact with Aux/IAAs through Domains III and IV (Kim et al., 1997; Ulmasov et al., 1997b). ARFs bind AuxREs found in the promoter region of early (primary) auxin response genes including Aux/IAAs. In gel shift assays, ARFs show a preference for forming homodimers in binding to synthetic palindromic AuxREs (Ulmasov et al., 1999b). Some ARFs repress transcription while others activate transcription of reporter genes containing synthetic AuxREs in carrot protoplast transient assays (Ulmasov et al., 1999a). Over-expression of Aux/IAAs in carrot protoplast transient assays repressed transcription of reporter genes

containing synthetic AuxRE promoter elements or the *GH3* promoter, an early auxin up-regulated gene from soybean, suggesting that over-expressed Aux/IAAs may interfere with ARF function in auxin-regulated gene expression (Ulmasov et al., 1997a). These observations led to the suggestion that Aux/IAAs function as repressors of ARF-mediated gene expression possibly by sequestering ARFs and/or by preventing the formation of homo- and/or heterodimers of ARFs, resulting in repression of early auxin-responsive genes (Guilfoyle et al., 1998a and 1998b). Since palindromic AuxREs do not exist in these plant genes, ARFs may bind as a monomer on AuxREs to turn on/off early auxin response genes including Aux/IAAs (Ulmasov et al., 1999a). How ARFs and Aux/IAAs regulate the transcription of early auxin-response genes is not yet fully understood.

An analysis of the expression level of auxin-responsive genes from WT, a gain-of-function mutant (*axr3-1*), and a revertant background (*axr3-1R4*) is proposed for a number of reasons. First, all known gain-of-function mutants of Aux/IAA genes have mutations within Domain II and have decreased levels of mRNAs of their own genes. They also show similar auxin-related pleiotropic phenotypes and auxin resistance in plant roots and/or hypocotyls. Second, in carrot transient assays, over-expressed Aux/IAAs decreased expression of reporter genes containing either a synthetic AuxRE or the *GH3* promoter (Ulmasov et al., 1997a). Similarly, Domain II mutants of Aux/IAAs (*axr3-1*) repressed the expression of the reporter gene containing a synthetic AuxRE (Tiwari et al., 2003). Third, Aux/IAA proteins have a short half-life, and their transcriptional levels are rapidly induced by auxin, and in some cases by cycloheximide treatment as well (reviewed by Abel and Theologis, 1996). Since cycloheximide inhibits *de novo* protein synthesis, the induction by cycloheximide indicates that some Aux/IAA genes are primary response genes and that their transcription is repressed by a negative regulator

which is also a short half-life protein. Finally, *axr3-1* showed severe auxin-related phenotypes, but its intragenic revertant, *axr3-1R4*, showed a WT-like phenotype.

The interaction between ARFs and Aux/IAAs in the yeast two-hybrid system suggests that such interactions may play a role in auxin-related gene expression as well as in auxin-responsive plant growth and development. The phenotypic change(s) might result from the alteration of protein-protein interactions by the mutation within Domain II (axr3-1 allele) and/or by the additional mutation within Domain IV (axr3-1R4 allele). The additional mutation within Domain IV resulted in the substitution of about half of Domain IV with a sequence of 37 amino acids not found in the WT IAA17 (Rouse et al., 1998). Measuring the interaction of IAA17, axr3-1, and axr3-1R4 through Domains III and IV may be relevant to understanding the phenotypic reversions.

The transcriptional patterns of the Aux/IAAs and other auxin-responsive genes and protein-protein interaction assays should provide some insight into understanding the rather severe phenotypic changes of the *axr3-1* plants and the phenotypic recovery in the *axr3-1R4* plants. Some insight into other Domain II gain-of-function Aux/IAA mutants might also be gained.

Materials and Methods

Screening of *axr3-1R4*

Original *axr3-1R4* plants (Rouse et al., 1998) were backcrossed to WT Arabidopsis (Columbia) and then screened for the *axr3-1R4* allele by the PCR-RFLP (Restriction Fragment Length Polymorphism) method. DNA was extracted with the NaOH-boiling method from a single leaf (Klimyuk et al., 1993). PCR amplification was done with *IAA17* primer sets, and then

PCR products were ethanol-precipitated. The product was incubated overnight with restriction endonuclease Age I (New England Biolab, Beverly, MA). Digested DNA was separated by agarose gel electrophoresis and photographed. The DNA fragment size from the restriction enzyme digestion was measured for identification of the *axr3-1* allele.

Probe Isolations and Labeling

Gene specific probes for Aux/IAA genes were isolated from either 3' UTR (untranslated region) or unique coding regions by RT-PCR with appropriate primers (See Table 2-1 for Primer sets) since Aux/IAA genes show high sequence similarity. Total RNA was isolated by the Pine Tree method (http://afgc.stanford.edu/afgc_html/site2Rna.htm#pinetree) after 2-hr auxin treatment. Probes for other auxin-responsive genes and ARFs also were synthesized by RT-PCR. After purifying mRNA with oligo-dT cellulose (Ambion, Austin, TX), first strand cDNA was synthesized by using Enhanced Avian HS RT-PCR kit (Sigma, St. Louis, MO) with gene specific 3' UTR primers or oligo-dT-V primer (23 oligo dT and A, C, or G at 3' end of 24^{th} position), and then 3' UTR regions were amplified by touchdown PCR. Only a single band was amplified from reverse transcription and touchdown PCR. After isolating a single band from agarose gel following electrophoresis, DNA was reamplified with the same primer set and sequenced for the confirmation of probe identity. Probes were random-labeled with ^{32}P-dATP (3,000 μCi/mole, NEG012H, Perkin Elmer, Boston, MA) using the StripEZ^{TM} DNA kit (Ambion, Austin, TX), and unincorporated nucleotide was removed by using a Bio-Rad P-30 spin column (Bio-Rad, Hercules, CA).

IAA Treatment

WT (Columbia), *axr3-1*, and *axr3-1R4* plants were grown in the dark (etiolated) for 5 days, and 20 μM IAA in 0.5X MS (Murashige and Skoog Basal media) salt was sprayed under dark conditions. After 2 hours of IAA treatment, plants were harvested and total RNA was isolated by the PineTree method. Three independent IAA treatments and RNA extractions were done for Northern analysis.

Northern Analysis

Northern blot analysis was done with ^{32}P-radiolabeled probes (Sambrook et al., 1992). Total RNA was separated by 1% formaldehyde agarose gel electrophoresis and transferred to Biodyne B membrane (Pall, Ann Arbor, MI) by downward capillary transfer with 3X SSC (diluted from 20X SSC stock solution containing 3M NaCl and 0.3M sodium citrate, pH 7.0). After U.V. fixation of RNA to the membrane, the blot was prehybridized and hybridized with radiolabeled probes with about 10^6 CPM/ml of Sigma perfect Hyb plus (Sigma, St. Louis, MO) overnight, and then the blot was washed with low stringency washing buffer (2X SSC/0.1% SDS) and 3 times with high stringency washing buffer (0.5X SSC/0.1% SDS) for 15 min each. After developing the X-ray film, the images were scanned with imaging software (Microtek scanner ScanWizard 5 and Photoshop 5.0).

Site-Directed Mutagenesis

Site-directed mutagenesis was carried out to generate *iaa17R3* and *axr3-1R3* constructs for yeast two-hybrid analysis from *IAA17* and *axr3-1* clones, respectively, by using QuickChange® Site-Directed Mutagenesis kit (Stratagene, LaJolla, CA) with two primer sets for

axr3-1R3 allele (5'-GGAGACTGAGCTGTG TTTTGGTCTTCCCGGTG-3' and 5'-CACCGGGAAGACCAAAAC ACAGCTCAGTCTCC-3')

Yeast Two-Hybrid Analysis

Yeast two-hybrid analysis was conducted following procedures from Clontech (Palo Alto, CA). Open reading frames of *Aux28*, *Aux22*, *ARF1*, *IAA17*, *axr3-1*, *axr3-lR4A*, *axr3-lR4B*, *axr3-1R3* and *iaa17R3* were cloned into yeast two-hybrid vectors containing Gal4 binding or activation domains. PCR was carried out with primers containing EcoRI and Sma I sites 3' and 5', respectively. The PCR products were digested with EcoR I and Sma I (NEB, Beverly, MA) and purified with Qiaquick® PCR Purification Kit (Valencia, CA). Two vectors, pGBT9 (containing the Gal 4 binding domain, BD, and TRP1 selection marker) and pGAD424 (containing the activation domain, AD, and LEU2 selection marker) from the original Clontech Matchmaker yeast two-hybrid system were used to clone the eight genes noted above inframe into the EcoR I and Sma I sites. In addition, pGBKT7 and pGADT7 vectors (containing the c-myc and HA epitope tag, respectively) for the Matchmaker Two-Hybrid System III were used to clone the Aux/IAA genes. All vector constructs of activation and binding domain combinations were verified by DNA sequencing. Nomenclature for the construct for the original Matchmaker system vectors are *AD-IAA17, BD-IAA17, AD-axr3-1*, and so on, and for the Matchmaker System III are *AT-IAA17, BT-IAA17*, and so on. The combinations for the two-hybrid analyses are shown in Tables 2-3 and 2-4.

For the original Matchmaker system, a yeast strain HF7c was used. HF7c contains two reporter genes, *HIS3* and *lacZ*, fused to a promoter controlled by Gal4 DNA-binding domain. Yeast strain AH109 containing three reporter genes (*ADE2*, *HIS3*, and *lacZ*) was used for the

new Matchmaker System III. Two appropriate plasmids from the above constructs were cotransformed into the yeast strains and plated on SD/-Leu/-Trp to select for cotransformants. The surviving positive clones were streaked on plate SD/-His/-Leu/-Trp to select for colonies expressing the HIS3 reporter for the original system or on SD/-His/-Ade/-Leu/-Trp plate for the Matchmaker System III. For confirmation, an additional *b-galactosidase* assay was done for the His-positive clones.

The *b*-galactosidase filter assays were done as described in the Matchmaker manual (Clone Tech, Palo Alto, CA). Three separate cotransformants were grown in liquid YPD medium, and aliquots of culture were blotted onto Whatman #1 filter paper using a vacuum dot blot apparatus. The filter was frozen in liquid nitrogen, thawed at room temperature, placed on another piece of Whatman #1 filter paper saturated with Z buffer (39 mM β-mercaptoetanol, 334 μg/ml X-gal), and incubated at 30°C for color development for the original Matchmaker system. Positive interaction of yeast cotransformants in the Matchmaker System III was screened on SD/X-α-gal/-Trp/-Leu/-His/-Ade medium.

Results

Screening of *Axr3-1R4*

Axr3-1 was the first characterized Domain II gain-of-function mutant (Leyser et al., 1996; Rouse et al., 1998). Among the intragenic revertants of *axr3-1*, the *axr3-1R4* allele showed the most WT-like phenotypic reversion. *Axr3-1* and *axr3-1R4* were used to study the function of *IAA17/AXR3* relative to their phenotypes and the possible molecular mechanism(s) involved in these changes. The seeds for *axr3-1* and *axr3-1R4* were obtained from Dr. O. Leyser (Leyser et al., 1996; Rouse et al., 1998) and propagated for further experiments.

Most of the *axr3-1R4* plants showed WT-like phenotypes. However, some *axr3-1R4* plants showed phenotypes including no trichomes, pale green leaves, and late flowering after propagation of plants (by selfing) through two or three generations in addition to those phenotypic changes reported by Rouse et al. (1998) suggesting heterozygosity. In order to clean the genetic background of the revertant, *axr3-1R4*, plants were backcrossed twice with WT and screened for the *axr3-1* allele by the PCR-RFLP method. F1(+/*axr3-1R4*) plants did not show any of the phenotypes mentioned above (data not shown). Since the *axr3-1R4* did not show distinct penotypes, and could not be visually distinguish with WT, PCR-RFLP analyses were done (an example of results is shown in Figure 2-1). If a plant is WT, the PCR products would be digested by Age I to produce a 600 bp fragment (lower band). However, the mutant and the revertant produce about a 1.2 kb fragment because the Age I site is not present in the mutated *IAA17* gene (*axr3-1* allele). Seeds from homozygous *axr3-1R4* plants were selected and propagated.

Northern Analysis

Arabidopsis contains at least 23 (or 29 listed in the review of Liscum and Reed (2002); of the 29 Aux/IAA genes, 6 genes do not contain distinct Domains I and II). Some of these (6 genes) do contain a LxLxLx motif in Domain I which is believed to function as a general repressor (Tiwari et al., 2004). Such genes were separated as a subgroup of Aux/IAA genes (see details in Appendix). Significant homologies are shared among Aux/IAAs. Accordingly, it is difficult to identify unique coding sequences for each gene for use in Northern analysis; therefore, PCR amplifications of the 3' untranslated regions were used to prepare unique Aux/IAA probes. Only a single band after PCR amplification was utilized, and uniqueness for

each was confirmed by DNA sequencing. All Northern blot results were repeated in three independent experiments and presented in Figure 2-2.

It was of interest to evaluate mRNA steady state levels of auxin-responsive genes from *axr3-1* and the revertant, *axr3-1R4*, since most dominant mutants of Aux/IAA genes show inhibition of their own transcription (Tian and Reed, 1999; Nagpal et al., 2000; Fukaki et al., 2002; Rogg et al., 2001). The *axr3-1R4* plants are intragenic revertants of *axr3-1* and show WT-like phenotypes. The mRNA levels were determined for several families of auxin-responsive genes to see if a correlation exists between the expression of auxin-responsive genes and the phenotypes of the two mutants (*axr3-1* and its revertant, *axr3-1R4*) relative to expression in WT plants. Several auxin-responsive gene families have been isolated following treatment with auxin and the screening of relevant cDNA libraries. Those gene families can be classified as follows: 1) Aux/IAA family, 2) SAUR family, 3) GH3 family, 4) GST-like proteins (GH2/GH4 family, and 5) ACC synthase (ACS) family (reviewed by Abel and Theologis, 1996). Four of these gene families including 20 members of Aux/IAA genes were included in these studies to evaluate the steady state levels of mRNAs and the levels of auxin-responsiveness in WT, mutant, and revertant backgrounds.

Results of Northern analyses of the above noted classes of auxin-responsive genes are shown in Figure 2-2 and summarized in Table 2-2. Auxin-responsive genes were grouped based on the mRNA levels of the three background genotypes of interest in these studies (WT, *axr3-1*, and the revertant, *axr3-1R4*) as follows: 1) highly auxin-responsive genes (*IAA1, 2, 6, 10, 11, 12, 13,* and *19,* AtGH3 (*dfl1*), and a GST (*At103-1a*)), 2) highly auxin-responsive but not expressed in the *axr3-1* mutant (*IAA5/At2-27* and *SAUR-AC1*), 3) less auxin-responsive genes (*IAA4/At2-11 and IAA9*), 4) high constitutive expression but weakly auxin-responsive in the *axr3-1*

background (*IAA3/SHY2*, *IAA7/AXR2*, *IAA8*, and *IAA17/AXR3*), 5) non-auxin-responsive genes (*IAA14, IAA16, IAA18, IAA27/PAP2*, and *IAA28*) expressed in mutant and revertant, and 6) highly auxin-responsive in WT and revertant, but not auxin-responsive in *axr3-1* (*IAA20*).

The highly auxin-responsive genes (Groups 1 and 2) showed low levels of message in control or non-auxin-treated seedlings. The less auxin-responsive genes (Groups 3, 4, 5) showed higher constitutive message levels without auxin treatment. In Group 1, the highly auxin-responsive genes showed high auxin-responsiveness in WT and revertant as well as in the *axr3-1* mutant. In addition, the message level of Group 1 genes without auxin treatment was detectable but much lower in *axr3-1* compared to WT and revertant both in control and auxin-treated plants. Group 2 genes showed high auxin-responsiveness in both WT and revertant, but the level of expression was somewhat lower in the revertant background; these mRNAs were not detectable in the mutant background even with auxin treatment (no transcript was detected even after over-exposure of the x-ray film, data not shown). Group 3 genes (*IAA4* and *IAA9*) were less auxin-responsive in both WT and revertant than Groups 1 and 2 but showed similar auxin responsiveness in *axr3-1*. The mRNA level of Group 3 genes was also reduced in *axr3-1* plants. Group 4 genes showed high constitutive patterns of expression without auxin treatment and showed a moderate response to auxin. However, the transcript levels were dramatically lower in *axr3-1* plants but showed similar auxin-responsiveness as those of WT and revertant plants. Group 5 genes showed no auxin-responsiveness in any of the three backgrounds; the transcript levels in *axr3*-1 were as high as in WT and revertant. Interestingly, most auxin-responsive genes (Groups 1, 2, 3, and 4) were down-regulated (much less transcript) in *axr3-1*, but the level and auxin responsiveness were similar to WT levels in the revertant. In addition, non-auxin-responsive Group 5 genes showed similar levels of transcript in all three genetic backgrounds.

Expression of *IAA20* was unique in that it was highly auxin-responsive in WT and revertant, was expressed at a level equal to that of auxin-treated WT plants in *axr3-1*, but was not affected by auxin treatment. The level of expression was even slightly higher with or without auxin treatment in *axr3-1* compared to that of WT plants. *Axr3-1* plants also expressed lower levels of the transcript of other auxin up-regulated genes such as a GH3 (*dfl1*), a SAUR gene, and a glutathione-S-transferase (GST*, At 103-1a*).

In conclusion, *axr3-1* plants showed reduced transcript levels of all auxin-responsive genes. However, Group 5 genes which did not respond to auxin did not have a reduced message level in *axr3-1*. The phenotype and the transcription level of auxin-responsive genes correlated well between *axr3-1* and *axr3-1R4* in that the revertant had WT mRNA levels of most auxin-responsive genes compared to much lower levels in *axr3-1*. The fact that expression of the non-auxin-responsive Group 5 genes was not altered by the *axr3-1* mutation indicates that *axr3-1* rather specifically and significantly alters expression of auxin-responsive genes.

Axr3-1R4 Plants Produce Two Forms of mRNAs from the *IAA17* Gene

The *IAA17/AXR3* and its mutated genes were cloned by RT-PCR from WT, *axr3-1*, and *axr3-1R4* plants. *Axr3-1* and WT generate only one band of the *IAA17* gene, but *axr3-1R4* generated two bands of mRNA from the *IAA17* gene (Fig. 2-3A). These gene products were isolated and reamplified for DNA sequencing and cloning. The structures of these two forms are shown in Figures 2-3B and -3C. The *IAA17/AXR3* gene has five exons. The revertant *axr3-1R4* allele has a "G" to "A" nucleotide change resulting in the activation of a cryptic 5' splice site 4 nt downstream from the base substitution at the end of exon 3. This four-base insertion in *axr3-1R4A* causes a reading frame shift resulting in replacement of half of Domain IV by 37 new

amino acids (Band A, Fig 2-3A, 3B, and 3C). The two alternatively spliced forms were designated *axr3-1R4A* and *axr3-1R4B*. In *axr3-1R4B*, the intron from exon 3-intron 3-exon 4 junction was present. Intron 3 contains a stop codon; thus, the new amino acids were translated from intron 3 resulting in 36 new amino acids in half of Domain IV (Band B, Fig 2-3A and C). The new amino acid sequences from the alternative spliced transcripts were searched via the BLAST program (NCBI), and no sequence homologies against the two new sequences were found. The two alternative spliced transcripts were included in the yeast two-hybrid system studies (see below) as one approach to understanding the phenotypic reversion of *axr3-1R4* to WT.

Yeast Two-Hybrid Assay

The question was addressed as to how the mutation within Domain II caused the severe phenotype while the additional mutation within Domain IV of *axr3-1* caused phenotypic reversion. One testable hypothesis to address this question relates to potential changes in protein-protein interactions. The interactions between ARFs and Aux/IAA proteins in the yeast two-hybrid system suggest a potentially important role in auxin-regulated gene expression (Guilfoyle et al., 1998a). It was proposed that a regulatory mechanism of Aux/IAA proteins in auxin-responsive plant growth and development is to form homo- or heterodimers among Aux/IAA and ARF family proteins (Guilfoyle et al., 1998b). The cause of the phenotypic change might be the result of altered protein-protein interactions by the mutation within Domain II (axr3-1 allele) and/or the additional mutation in Domain IV (axr3-1R4 allele).

Yeast two-hybrid analysis was used to evaluate the protein-protein interactions. The original Matchmaker System was used with the pGBT9 and pGAD242 vectors containing Gal4

DNA-binding domain (BD) and activation domain (AD), respectively. These vectors have an AdH1 yeast constitutive promoter that expresses a lower level of protein than the Matchmaker System III vectors. To reduce false positive clones, an additional *ß*-galactosidase filter assay was done with the positive clones.

The schematic diagrams of various yeast two-hybrid constructs are shown in Figure 2-4B. The controls for the yeast two-hybrid assay worked well as expected except for the BD-IAA17 and AD combinations (Table 2-3). The BD-IAA17 and AD combination showed positive colony growth, but the *ß*-galactosidase filter assay showed very low color development. The results from yeast two-hybrid analyses using the original Matchmaker System are summarized in Table 2-3. IAA17 and axr3-1 proteins interacted with the other Aux/IAA proteins analyzed and with each other, whereas axr3-1R4 proteins did not show interactions. The AUX22 protein transcribed from a soybean auxin-responsive gene showed the strongest interaction with other Aux/IAAs. BD-Aux22 and BD-Aux28 showed the strongest color development when cotransformed with their own gene in the activation domain (AD-Aux22 and AD-Aux28, respectively) showing that homodimer formation was preferred to heterodimer formation. The BD-ARF1 and AD-IAA17 combination showed colony growth and weak color development, but the BD-ARF1 and AD-axr3-1 combination did not show color development (Table 2-3).

To analyze these potential interactions more thoroughly, the Matchmaker System III was employed. Matchmaker System III was designed to significantly reduce false positives because it uses the AH109 yeast strain containing three reporter systems, namely *HIS3*, *ADE2*, and *ß*-galactosidase. The controls worked especially well in this system. The BT-IAA17 and AD combination which showed some apparent leaky survivals with the Matchmaker System I, were negative in this system (Tables 2-3 and 2-4). Site-directed mutated *axr3-1R3* and *iaa17R3* from

axr3-1 and *IAA17/axr3* genes were used to further assess protein-protein interactions (for schematic diagram; see Fig. 2-4B, Table 2-4). The *axr3-1R3* allele was generated from EMS-treated *axr3-1* seeds. The intragenic revertant contains an additional mutation (Leu to Phe) within Domain I of the *axr3-1* gene at position 18 (Rouse et al., 1998; Fig. 2-4A). The *axr3-1R3* allele was not as strong as *axr3-1R4* in terms of phenotypic reversion, but the appearance of the plants was similar to WT.

Axr3-1R3 and iaa17R3 proteins also showed similar protein-protein interactions as was observed with IAA17/AXR3 and axr3-1 proteins (Table 2-4). When ARF1 was conjugated to the activation domain (AT-ARF1), there were distinct differences in colony growth and color development (Table 2-4). AT-ARF1 interacted with BT-IAA17, BT-ARF1, and BT-iaa17R3 at similar interaction strength, but showed weak interactions with BT-axr3-1 and BT-axr3-1R3 in terms of colony growth and color development on the plates. In the Matchmaker System III, the results showed basically the same pattern as in Matchmaker System I where axr3-1 and IAA17/AXR3 showed the same protein-protein interaction properties, whereas the axr3-1R4 protein showed no interactions with IAA17, axr3-1, and ARF1 proteins.

Based on these results from the yeast two-hybrid analyses, mutation within Domain II did not affect protein-protein interaction through Domains III and IV. However, an additional mutation within Domain IV of axr3-1R4 resulted in loss of interaction (or caused interference) with IAA17, other Aux/IAAs, and ARFs as a result of replacement of half of Domain IV. An additional mutation within Domain I of axr3-1 (axr3-1R3) also did not affect protein-protein interactions, interacting similarly as did in IAA17 and axr3-1.

Discussion

To gain insight into how Domain IV of *axr3-1R4* overcomes the severe phenotypes caused by Domain II mutations, two approaches were taken: Northern analysis profiling a large number of auxin-responsive genes representing several different classes, and yeast two-hybrid analyses of protein-protein interactions. It was interesting to test transcript levels of a large number of auxin-responsive genes since most dominant gain-of-function mutants of Aux/IAAs show reduced levels of their own transcripts (Tian and Reed, 1999; Nagpal et al., 2000; Fukaki et al., 2002; Rogg et al., 2001). An intragenic revertant from *axr3-1*, *axr3-1R4*, was added in these studies to compare the phenotypes and transcriptional patterns of auxin-responsive genes from both the mutant and revertant relative to expression in WT. Five day-old etiolated seedlings were used to compare the transcriptional profiling of early auxin-responsive genes. This was also the system used for work with the *axr2* mutant (Abel et al., 1995a). This provides a basis for direct comparisons of results from the studies done here with the *axr3-1* and *axr3-1R4* and with *axr2-1* mutant, though the *axr2-1* studies were more limited in scope.

Four classes of early auxin-responsive genes were tested, with emphasis on Aux/IAA genes in the three genetic backgrounds, WT, *axr3-1*, and *axr3-1R4*. Comparisons to the data of Abel et al. (1995a), who extracted RNA from five day-old etiolated seedlings and examined mRNA levels of some Aux/IAA genes in an auxin-resistant mutant, *axr2-1* (*IAA7*), showed some differences and some similarities in the data sets on Aux/IAA genes. The data presented here and data of Abel et al. (1995a) show that Aux/IAAs are expressed at different constitutive levels and have varied tissue- and organ-specific patterns of expression (see also Chapter III). The expression of all auxin-responsive Aux/IAAs is reduced in both *axr2-1* and *axr3-1* mutants compared to WT; those that are detectably expressed remain auxin responsiveness (Fig. 2-2 and

Table 2-2). There are some notable differences in that *IAA6* is expressed at a reduced, but substantial level in *axr3-1*. However, *IAA6* was not detectably expressed in *axr2-1*. *IAA5* transcripts are not detectable in either mutant, and *IAA3* was not expressed in *axr3-1* but was in *axr2-1*.

All auxin-responsive genes including SAUR, GST, and GH3 that were tested showed reduced message levels in the *axr3-1* background (Fig. 2-2). However, Group 5 genes (*IAA14, IAA16, IAA18, IAA28*, and *PAP2*) which are not auxin-responsive in WT or revertant did not show reduced message levels in *axr3-1*. Thus auxin-responsiveness correlates with reduced message levels in the *axr3-1*. All auxin-responsive genes tested (four families) showed markedly decreased levels in the mutant while expression of the non-auxin-responsive Aux/IAAs was not affected in the mutant background. Group 4 genes (*IAA3, IAA7, IAA8*, and *IAA17*) showed relatively high levels of constitutive expression but lower levels of auxin-responsiveness compared to Groups 1, 2, and 3. Groups 3 and 4 genes, except *IAA4* and *IAA9*, showed dramatically reduced message levels in *axr3-1*. The transcriptional patterns of *IAA3*, *IAA7*, and *IAA8* are similar to the data on expression in the *axr2-1* mutant (Abel et al.,1995a), where the transcript levels are reduced. *IAA20* is unique among the Aux/IAA genes because it showed an enhanced level of mRNA in *axr3-1* (Fig. 2-2); the gene is highly auxin-responsive in WT and revertant backgrounds, but not in *axr3-1*. The constitutive transcript level of *IAA20* is slightly higher in the revertant. These data show that *IAA20* has a unique pattern of expression compared to the other Aux/IAA genes. Thus it would be useful to know if it has unique spatial and temporal expression patterns among Aux/IAAs.

Aux/IAA interacts with other Aux/IAAs and ARFs through Domains III and IV in the yeast two-hybrid system (Kim et al., 1997; Ulmasov et al., 1997a and 1997b; unpublished data,

K. O'Grady, personal communication). While not studied in detail, there appears to be substantial preferential selectivity in interactions leading to homo- and heterodimer formation and the relative strength of interactions between different pairs. The gain-of-function mutants of Aux/IAAs show auxin-related pleiotropic phenotypes such as loss of tropic responses, small-sized roots and shoots, and strong apical dominance (Leyser et al., 1996; Tian and Reed, 1999; Nagpal et al., 2000; Fukaki et al., 2002; Rogg et al., 2001). The revertant protein, axr3-1R4, showed no protein-protein interaction with other Aux/IAAs or with ARFs (Tables 2-3 and 2-4), and *axr3-1R4* had a WT-like phenotype. However, the axr3-1 protein showed the same protein-protein interactions as that of the WT protein (Tables 2-3 and 2-4). Ouellet et al. (2001) showed that IAA17, axr3-1, and axr3-1R3 proteins exhibited similar interaction patterns with ARF1 and ARF5. *Axr3-1R2* is an intragenic revertant of *axr3-1* with an additional point mutation (Asp to Asn change at position 118) in conserved Domain III (Rouse et al., 1998). *Axr3-1R2* responded gravitropically and had a longer primary root than *axr3-1*, but the allele was not as strong as the *axr3-1R3* and *axr3-1R4* alleles in producing the primary root phenotype (Rouse et al., 1998). Ouellet et al. (2001) showed that the axr3-1R2 protein did not interact with IAA17, ARF1, or ARF5. They suggested that phenotypic suppression of *axr3-1* is mediated by altered protein-protein interactions (Ouellet et al., 2001). However, axr3-1R2 protein showed slight protein-protein interactions with IAA17 and ARFs, whereas axr3-1R4 protein showed complete loss of protein-protein interactions with ARFs and other Aux/IAAs (Tables 3 and 4). The phenotypic recoveries (or allele strength) between *axr3-1R2* and *axr3-1R4* from *axr3-1* to WT show a positive correlation with protein-protein interaction with Aux/IAAs and ARFs (Rouse et al., 1998; Ouelllet et al. 2001; Table 2-3 and 2-4).

The data presented here, which combine Northern analyses of mRNA levels of multiple auxin-responsive genes (Fig. 2-2) and protein-protein interaction analyses (Tables 2-3 and 2-4), suggest that recovered transcript levels of auxin up-regulated genes in *axr3-1R4* to WT levels seems to result from the loss of protein-protein interactions with other Aux/IAAs and ARFs. This probably results in *axr3-1R4* plants having a WT-like phenotype. However, it may be possible that the revertant, *axr3-1R4*, may mislocalize axr3-1R4 protein since the additional mutation of Domain IV removed the C-terminal region which may contain a nuclear localization signal (NLS) as described for Ps-IAA4/5 (Abel et al., 1995b); this might also contribute to reversion of *axr3-1R4* to WT. However, the revertant protein did localize in the nucleus as did WT IAA17 and axr3-1 proteins (unpublished data, J. Nairn, personal communication), demonstrating that altered localization did not contribute to the revertant phenotypes. This adds credence to the importance of protein-protein interactions as the cause of phenotypic reversion. The *Shy2-2* mutation is located within conserved Domain II with a Pro to Ser change (Tian and Reed, 1999). The phenotypes of *axr3-1* and *shy2-2* show many similarities such as a short root, increased adventitious root formation, upward curling of leaf edges, agravitropic root, and formation of leaves in the dark. One intragenic revertant of *shy2-2*, *shy2-22*, also has an additional mutation within Domain IV resulting in eight amino acid changes in half of Domain IV. This revertant also has a WT-like phenotype. The phenotypes of these revertants (*axr3-1R4, axr3-1R2,* and *shy2-22*) and lack of protein-protein interactions in yeast two-hybrid assays indicate the importance of Domains III and IV as interaction domains with Aux/IAAs and/or ARFs and their critical role in auxin signaling in plant growth and development.

The short half-life of Aux/IAAs in plants (Abel et al., 1994; Colón-Carmona et al., 2000; Worley et al., 2000) and the semi-dominant phenotype of *axr3-1* and other Domain II gain-of-

function mutants suggest that Domain II is involved in protein stability of Aux/IAAs. Ramos et al. (2001) conjugated Domains I and II of *IAA17* with the luciferase (Luc) reporter gene and examined protein stability by measuring Luc activity. They showed that the mutated Domain II conjugated protein was 20 times more stable than WT protein. Ouellet et al. (2001) did pulse-chase analysis of IAA17 and axr3-1 proteins from WT and *axr3-1* using IAA17 peptide-raised antibody and showed that axr3-1 protein is seven times more stable than WT IAA17/AXR3. Finally, Gary et al. (2001) showed that axr3-1-GUS conjugated protein was 20 times more stable than IAA17-GUS conjugated protein. These data demonstrate a definitive role(s) for Domain II of Aux/IAAs in protein stability, and those mutations within Domain II correlate with their gain-of-function mutant phenotypes.

Axr3-1R3, an intragenic revertant of *axr3-1* has an additional mutation within Domain I (Leu to Phe change at position 18) (Rouse et al., 1998), and showed similar protein-protein interaction properties as WT IAA17 and axr3-1 proteins (Ouellet et al., 2001). Site-directed mutation of *axr3-1R3* and *iaa17R3* were used to study protein-protein interaction of these modified proteins (Table 2-4). The *axr3-1R3* allele is not the strongest allele in terms of phenotypic reversion, but the appearance of the plant is WT-like (Rouse et al., 1988). Since axr3-1R3 and iaa17R3 proteins showed similar protein-protein interaction as the IAA17 and axr3-1 proteins, Domain I might also have some role in protein stability. Tiwari et al. (2001) showed that an Aux/IAA protein with a mutation only in Domain I (such as iaa17R3 from Table 2-4) had less protein stability than its WT protein, while the Domain II mutation (axr3-1 protein) had much increased protein stability. However, axr3-1R3 protein (mutations in both Domains I and II) had an intermediate level of protein stability between WT IAA17 and axr3-1 protein (Tiwari et al., 2001). These data indicate that Domain I also may be involved in protein stability.

The single amino acid change within Domain II of *axr3-1* (Pro to Leu at position 88) may result in a rather dramatic change of structural conformation. The additional mutation within Domain I in axr3-1R3 may result in some conformational change, altering or compensating in part for the structural conformation of axr3-1. Other Domain I revertants (*axr3-1R3* of *IAA17/axr3-1*, *axr2-1-r-3* and *axr2-1-r-4* of *IAA7/axr2-1*, and *slr-1R1* of *IAA14/slr-1*, respectively) also did not revert the phenotype completely to WT and/or to their null phenotypes (Chapter II; Rouse et al., 1998; Nagpal et al., 2000; Fukaki et al., 2002), perhaps due to an intermediate level of protein stability between WT and their Domain II mutants as mentioned above from Tiwari et al. (2001), or some unidentified Domain I function.

The middle region (MR) of ARFs is nonconserved and has been proposed to be a transcriptional repression or activation domain depending upon the specific amino acid sequence (Ulmasov et al., 1999a). Tiwari et al. (2003) showed that ARF DNA-binding domains (DBDs) alone are sufficient to recruit ARFs to their DNA target sites and auxin does not affect this recruitment. In addition, reporter gene activity driven from a synthetic AuxRE P3(4X) was not affected by the cotransfection with both MR5 (containing the middle region of ARF5, a transcriptional activator, lacking Domains III and IV of ARF5) and *IAA17*, and with both MR5 and *axr3-1* upon auxin treatment. The reporter activity was reduced when MR5-CTD (carboxy terminal domain, Domains III and IV) and *IAA17* were cotransfected into carrot protoplast in + auxin media. The level of reduction was even greater with cotransfection of MR5-Domains III and IV plus *axr3-1*. From these data, the authors concluded that the auxin response is mediated by the recruitment of Aux/IAA proteins to AuxRE promoters with a DNA-binding protein containing a Q-rich MR-CTD (an attached Domains III and IV) such as ARF5. Data on protein stability, protein-protein interactions, and phenotypes of gain- and loss-of-function mutants

(revertants) of Aux/IAAs taken together indicate that one area of auxin signaling is mediated by protein-protein interactions throughout Domains III and IV with Aux/IAAs and/or ARFs in a concentration-dependent manner and specificity for heterodimer partners.

Tian et al. (2002) showed that both auxin-responsive genes and non-auxin-responsive genes were affected by the mutation of *shy2-1*. Auxin up-regulated genes were generally down-regulated in *shy2-2*, whereas expression of these genes recovered to WT levels in *shy2-24 (*a putative null mutant which is an intragenic revertant with introduction of a stop codon just upstream of *shy2-2* mutation) (Tian and Reed, 1999). Hayashi et al. (2003) showed that Yokonolide B blocked the degradation of Aux/IAA proteins; GUS activity of IAA17-GUS fusion protein remained constant for at least 100 min after Yokonolide B treatment, whereas only about 20 percent of GUS activity remained after mock treatment. Further, Yokonolide B treatment reduced the reporter gene activity driven by *DR5*, *pIAA3*, and *pIAA7* promoters in transgenic plants, suggesting that stable Aux/IAA proteins reduce the expression of auxin up-regulated genes.

Here, a model is suggested that more stable Aux/IAAs resulting from the Domain II mutations interfere with the normal protein-protein interaction cycles with other Aux/IAAs, ARFs, and/or other proteins, resulting in down-regulation of most auxin up-regulated genes. This may lead to changes in the transcription of many genes including transcription factors, enzymes involved in metabolism, and other auxin-related genes (Chapter III). These changes may result in abnormal responses to auxin (e.g., auxin insensitivity, Chapter II), so that plants have auxin-related abnormal phenotypes (i.e., show severe pleiotropic phenotypes). However, the intragenic revertants (*axr3-1R4*, *axr3-1R2,* and *shy2-22*) negate the effect of protein stability by not interacting with other Aux/IAAs, ARFs, and/or other proteins. This hypothesis may be

apply to other Domain II gain-of-function mutants, which show various auxin-related pleiotropic phenotypes (Table 2-2 of Chapter III).

Table 2-1. Primer Sets of Aux/IAA, ARFs, and Other Auxin Up-Regulated Gene for RT-PCR to Generate Northern Hybridization Probes.

IAA16>
F: AATT TTGAGGCCAC GGAGCT
R: AG GTA CGG TGC ACC GTCCA

IAA17>
F: GTCTCATGAAAGGATCGGAT
R: CTACATACCAAATCCAGATCA

IAA18>
F: CTTCTGAGATTTCTTCAGCA
R: GCCTAAAAGGGTTTGTAAATT

IAA19>
F: GGGTTTGGGCTGCAGCCTA
R:TCTTTCTGAAGATAATTATGCA

IAA20>
F:CTTCAATAGAGAGTAGCAGCA
A
R:AAT CAA GGG TTC TGA TCA
AGT

IAA2>
F: GGAGATGATGACTATGATCGA
R: TGATCTTATAGGACATAACTA
CT

IAA3>
F: CGGGCAAGATCTATGTTCAT
R: CTTTGATCAATGAGAACGCAA

IAA7>
F: AGTACTGCAAGAACAGATCTT
R: AATACTGCCCTATATACCCAT

IAA8>
F: ACATGCCAGAAACTGAAGA
R: GTCAAAGTAGAAACACACA
CA

IAA9>
F: GGTGTGATGCTATTGGGTT
R: CCTTACATTCGTAGTCTTACT

IAA10>
F: TAGGAGATGTTCCTTGGCA
R: GGTTAAGCTGTTGTTTGATAT

IAA11>
F: TACTGGTAAAGCTCAGATGA
R: GTGCAAGAAAGGGTTTTCTTA

IAA12>
F: GAGGTTTGCAATTTACTCGA
R: GGAATCATAAACATAACTCT
TATCA

IAA13>
F:TGATGAACCTAAAGATGTGAC
AA
R: ATC TAA AAG CCT CAA CGG
TT

IAA14>
F: TCAAGAACAGATCATGAACA
R: CGAACTCTATAGATTTACTAT
CA

IAA1>
F: CCTTATGATCCATTGTCTCAA
R: TGTTAGTATCAAATATCTTGA
GCA

IAA4>
F: AACCTAATTGAGAGATAAA
GATCA
R: ATGGAGACAGAGTTACAGC
TA

IAA5>
F: CCGGGTTTGGAAGAACCAA
R: AAATCTGTGGCGGTTCTCA

ARF3>
F: CAGCTGTTCAAAGCAATCAT
R: CCAAGTCTACAAGTCTCTCA

ARF6>
F: GTAGATGGAAACCTCCCTT
R: GGAAAGTGACATATATAGA
GTTCA

ARF8>
F: TCCCGCAAAACCCGACCCA
R: TAGTTACCCTGAGACAGCTA

ARF7>
F: CTGGAAACTTGTTTATGTCGA
R: CTCTTCTGCCATCACCGGT
ARF4>
F: AGCCAGCCTGATTCTTCT
R: CTTAAAATCCAATGGCATGCT

ARF1>
F: TCATCTATGGCGGGATCAA
R: GGTAGAACAAGACGTGAAA
CT

ARF5/MP>
F: CAACTGAGGTCCAGCAGAT
R: CATTCATTCATCACTCTACT
ACA

ARF9>
F: TGGCCTGAGTTCTGCAACA
R: GCAACAAAACACAGACACAA

ARF2>
F: TGCATCAAATCCTTCATTGT
R: GGCTTATAAAAGAGCTTTTC
ATA

SAUR-AC1>
F: AGG AGAGAATGATCAGAA
GAAGA
R: TCC TCT CAT TGA AAC AAT
TTA CA

GH3-DEF-1>
F: AGT ACACTAGCTA TGCG
GACA
R: TTG TGA CCA GGG GAC CAT

At103-1a (GST)>
F: TCT TTG CTA AAC TCG TCG
AT
R: GAT CTC ACT CTC TCT GCC
AT

Table 2-2. Summary of Relative Transcriptional Patterns of Auxin-Responsive Genes of WT, *Axr3-1*, and *Axr3-1R4* by Northern Analyses

Groups		**WT (CO)**		***Axr3-1***		***Axr3-1R4***	
	Auxin Treatment	**-**	**+**	**-**	**+**	**-**	**+**
I. Highly auxin-responsive genes	***IAA1***	+	++++++	-	+	+	++++++
	IAA2	++	++++	+	++	++	++++
	IAA6	++	++++	+	++	++	++++
	IAA10	++	++++	+	++	++	++++
	IAA11	+	+++	+	++	+	+++
	IAA12	+	+++	+/-	+	+	+++
	IAA13	++	+++++	+	++	+	++++
	IAA19/bodenols	++	++++++	-	+	++	++++++
	GH3/*dfl1*	+	++++++	-/+	++	+	++++++
	GST(*At103-1a*)	++	++++	-/+	+	++	++++
II. Plus no expression on mutant	***IAA5(AT2-27)***	-	+++++	-	-	-	+++++
	SAUR-AC1	+/-	+++++	-	-	+/-	++++
III. Less auxin Responsesive	***IAA4 (AT2-11)***	+++	++++	+	++	+++	++++
	IAA9	++	+++	+	++	++	+++
IV. High constitutive activity	***IAA3/sh2***	+++	++++	-	-/+	+++	++++
	IAA7/axr2	+++	++++	+	++/-	+++	++++
	IAA8	++	+++	+	+	++	+++
	IAA17/axr3	+++	++++	-	+/-	+++	++++
V. No response to auxin	***IAA14***	++	++	++	++	++	++
	IAA16	+++	+++	+++	+++	+++	+++
	IAA18	+	+	+	+	+	+
	IAA28/iaa28-1	++++	++++	++++	++++	++++	++++
	IAA17/PAP2	+++	+++	+++	+++	+++	+++
Unique	***IAA20***	+	++++	++++	++++	++	++++

Table 2-3. Protein-Protein Interaction Analysis by Matchmaker Yeast Two-Hybrid System I

Binding Domain	Activation Domain	Growth on -HIS	Lac Z filter assay
BD	-	-	-
-	AD	-	-
BD-IAA17	AD	+++++	+/-
BD	AD-IAA17	-	-
BD	AD-Axr3-1	-	-
BD-axr3-1R4A	AD	-	-
BD-axr3-1R4B	AD	-	-
BD	AD-axr3-1R4A	-	-
BD	AD-axr3-1R4B	-	-
BD-IAA17	AD-IAA17	+++++	+++
BD-axr3-1	AD-IAA17	+++++	++
BD-axr3-1R4A	AD-IAA17	-	-
BD-axr3-1R4B	AD-IAA17	-	-
BD-aux22	AD-IAA17	+++++	++++++
BD-aux28	AD-IAA17	+++++	+
BD-ARF1	AD-IAA17	+++++	+
BD-aux22	AD-axr3-1	+++++	++++++
BD-aux28	AD-axr3-1	+++++	+
BD-ARF1	AD-axr3-1	+++++	-
BD-aux22	AD-axr3-1R4A	-	-
BD-aux28	AD-axr3-1R4A	-	-
BD-ARF1	AD-axr3-1R4A	-/+	-
BD-ARF1	AD	-	
BD-aux22	AD	-	-
BD-aux28	AD	-/+	-
BD-axr3-1	AD-axr3-1	+++++	++
BD-axr3-1	AD-axr3-1R4A	+/-	-
BD-aux22	AD-aux22	+++++	+++++++
BD-aux28	BD-aux28	+++++	+++++++
BD-IAA17	AD-axr3-1	+++++	++++

AD: pGAD424 containing Gal4-activation domain and LEU2 selection marker.
AD-IAA17: pGAD424-IAA17 (C-terminal fused to Gal4-AD).
BD: pGBT9 containing Gal4 binding domain and Trp selection marker.
BD-IAA17: pGBT9-IAA17 (C-terminal fused to Gal4-BD).
Aux22 and Aux28 are auxin-responsive genes from soybean .

Above combinations were cotransformed into yeast HFC7 strain and plated on SD/-Lue/-Trp media. After 3 days, positive clones were replica plated (streaked) on SD/-Leu/-Trp/-His media.

All results shown above were from 3 repeated experiments from 3 independent transformations.

Table 2-4. Protein-Protein Interaction Analyses by Matchmaker Yeast Two-Hybrid System III

	AT	AT-IAA17	AT-axr3-1	AT-axr3-1R3	AT-axr3-1R4	AT-ARF1	AT-IAA17R3
BT	-	-	-	-	-	-	-
BT-IAA17	-	++++++	++++	+++++	-	+++++	+++++
BT-axr3-1	-	+++++	++++	+++++	-	+++	+++++
BT-Axr3-1R3	-	+++++	++++	++++	-	++	+++++
BT-axr3-1R4	-	-	-	-	-	-	-
BT-ARF1	-	+++++	++++	+++++	-	++++++	+++++
BT-IAA17R3	-	++++	++++	++++	-	+++++	+++++

AT: pGADT7 containing Gal4-activation domain, HA epitope tag, and Leu2 selection marker
AT-IAA17: pGAD424-IAA17 (C-terminal fusion to Gal4-AD)
BT: pGBKT7 containing Gal4 binding domain, c-Myc epitope tag, and Trp selection marker
BT-IAA17: pGBT9-IAA17 (C-terminal fused to Gal4-BD)
AT- or BT-IAA17R3 contain only the R3 allele in Domain I and the other part is the same as WT IAA17

Above combinations were cotransformed to yeast AH109 strain and plated on SD/-Lue/-Trp media. After 3 days, positive clones were replica plated (streaked) on SD/-Leu/-Trp/-His/-Ade/-His media containing X-a-Gal.

Figure 2-1. PCR-RFLP of *Axr3-1* Allele as a Tool for Screening *Axr3-1R4* Plant

After *axr3-1R4* plants were backcrossed to WT, F1 plants were selfed and then F2 plants were screened for *axr3-1R4* allele. DNA was extracted from plants and then PCR amplified with designed primers, producing a 1200 bp fragment from *IAA17* gene. The fragment was purified and then Age I-digested overnight, and separated by electrophoresis. If a plant has the WT *IAA17* allele, the 1200 bp frgment cannot be digested by Age I. 600 bp product will be produced with Age I digestion if a plant has the *axr3-1* allele. M, marker; Lanes 1 to Lane 11, individual plants; Lanes 1 and 10 are heterozygotes of WT and *axr3-1* allele; Lane 11, homozygote of *axr3-1* allele. Since *axr3-1* plants shows very severe phenotypes and the revertant, *axr3-1R4*, primarily screened by Rouse et al. (1998), was similar to WT, a plant which shows WT-like phenotype and has *axr3-1* allele is considered as *axr3-1R4* plant.

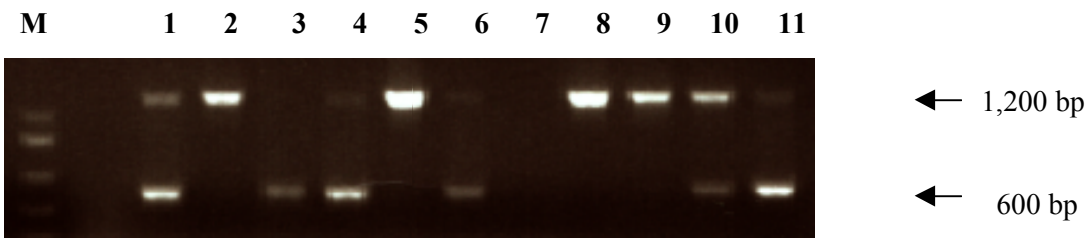

M
1
2
3
4
5
6
7
8
9
10
11
1,200 bp
600 bp

Figure 2-2. Northern Analysis of Auxin-Responsive Genes from WT, *Axr3-1*, and *Axr3-1R4*.

Plants were grown in the dark (etiolated) for 5 days, and 20 μM IAA/0.5X MS salt was sprayed under dark conditions. After 2 hours of IAA treatment, plants were harvested and total RNA was isolated by the PineTree Method. Twenty μg of total RNA was loaded on the gel. Above results are from three independent auxin treatments and RNA extractions. The three independent RNA samples were run on the same gel and hybridized with indicated probes.

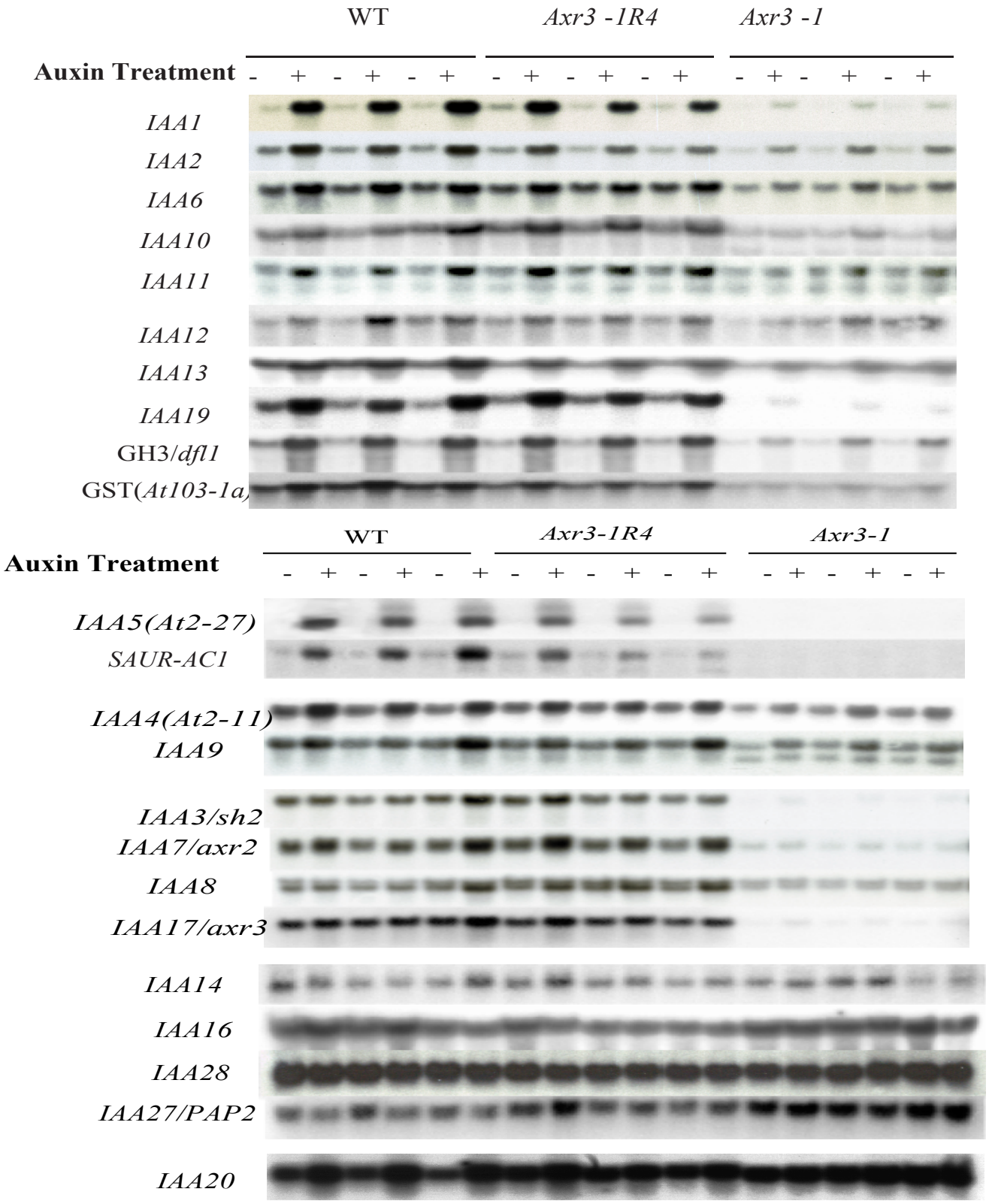

WT
Axr3 -1R4
Axr3 -1
Auxin Treatment
- + - + - + - + - + - + - + - + - + - +
IAA1
IAA2
IAA6
IAA10
IAA11
IAA12
IAA13
IAA19
GH3/dfl1
GST(At103-1a)
WT
Axr3-1R4
Axr3-1
Auxin Treatment
- + - + - + - + - + - + - + - + - + - +
IAA5(At2-27)
SAUR-AC1
IAA4(At2-11)
IAA9
IAA3/sh2
IAA7/axr2
IAA8
IAA17/axr3
IAA14
IAA16
IAA28
IAA27/PAP2
IAA20

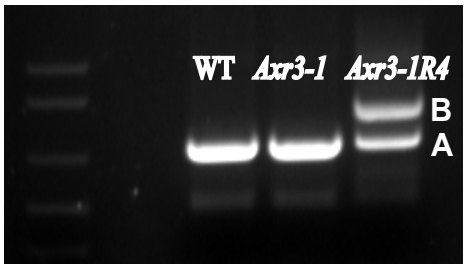
Figure 2-3. The Alterate Splicing Pattern of *Axr3-1R4*.

A. PCR products generated with reverse transcription and touchdown PCR from WT, *axr3-1*, and *axr3-1R4*. A and B represent products of two alternate splicing forms, *axr3-1R4A* and *axr3-1R4B*, respectively.

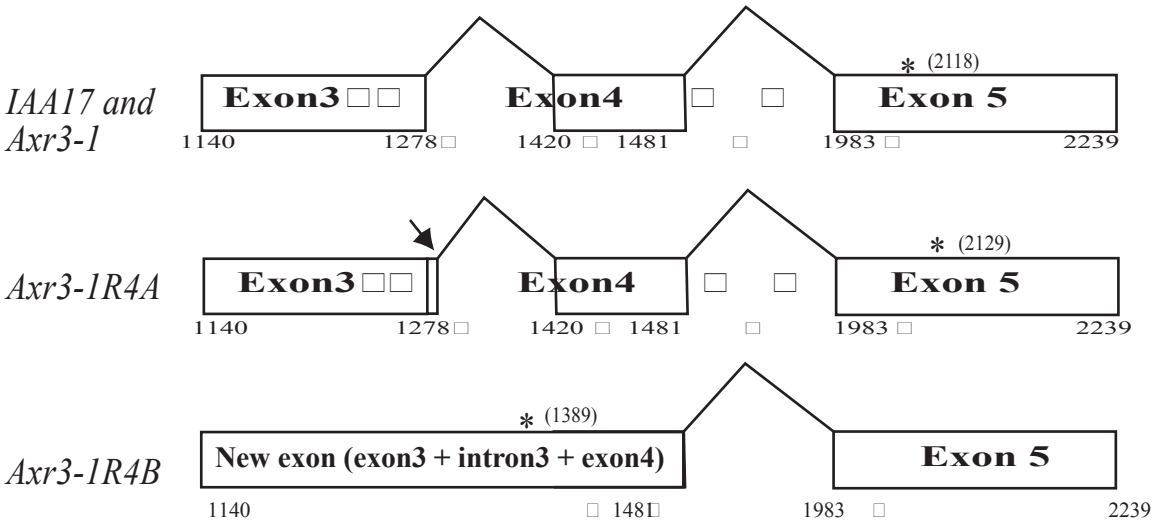
B. Two alternate splicing structures of *axr3-1R4A* and *axr3-1R4B*. Arrow indicates the new splicing site of *axr3-1R4A*. The new splicing site (position 1282) is located four nucleotides down from position 1278. Stars (*) indicate the locations of stop codons, and parentheses indicate the actual position of stop codon.

C. The results of alternate splicing. A and B splicing forms change half of conserved Domain IV with 37 and 36 new amino acids, respectively.

A. Gel Electrophoresis of RT-PCR Products



B. Splicing Structure of WT and Revertants



C. Modification of Domain IV by *Axr3-1R4* Allele

Conserved Domain IV

IAA17/Axr3 **WDYVPSYEDKDGDWMLVGDVPWP**MFVDTCKRLRLMKGSDAIGLAPRAMEKCKSRA

Axr3-1R4A TNVRRYMQAFTSHEAIGCHWSRSEGDGEVQEQSLKSN

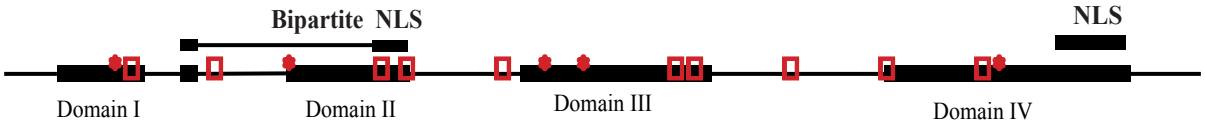
Axr3-1R4B TVFFFSLINYHLIRALLFRLKFFLYFCLCCHLFVSN

Figure 2-4. Structure and Mutations of *IAA17/AXR3*

A. Structures of axr3-1 and its four revertants: Red dots represent for the relative locations of mutations, and red characters represent the mutated amino acids.

B. IAA17 and various mutant constructs used for yeast two-hybrid analyses; * represents the mutated locations

A.



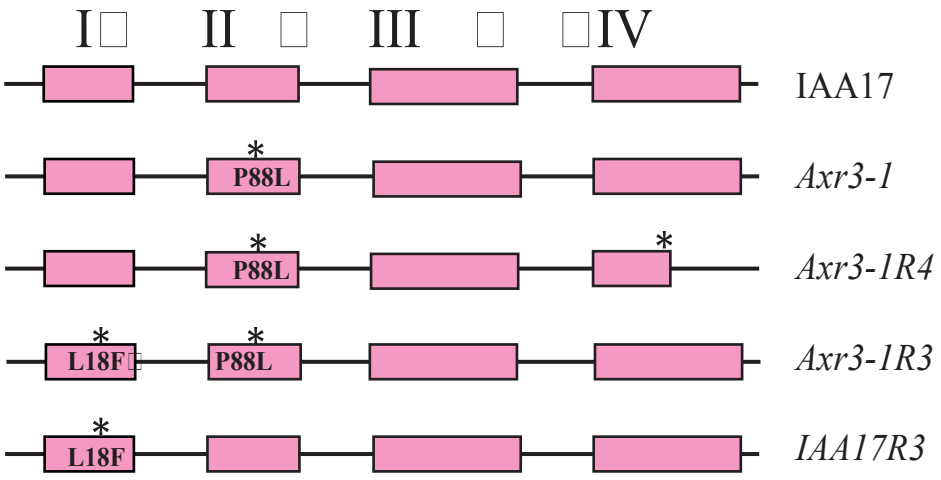
Consensus tELrLgLpg KR PP . k . q v VGWPP v r s yR..kn y vKV smDG a p yl RK.Dl se.vptYeDKdgDWMLvGDVPW.mF..sckrLrimkgsea...gl

AXR3/IAA17 TELRLGLPG KR PPAKAQVVGWPPVRSYR..KN FVKVSMDGAPYLRKIDL WDYVPTYEDKDGDWMLVGDVPWPMFVDTCKRLRIMKGSDA..IGL

axr3-1 PPAKAQVVGWP **L**VRSYR..KN

axr3-1R3 TELRLG **F**PG KR PPAKAQVVGWP**L**VRSYR..KN
axr3-1R2 PPAKAQVVGWP **L**VRSYR..KN FVKVSM**N**GAPYLRKIDL
axr3-1R1 PPAKAQVVGWP **L**VRSYR..KN FVKVSMDGA**S**YLRKIDL
axr3-1R4 PPAKAQVVGWP **L**VRSYR..KN WDYVPTYEDKDGDWMLVGDVPWP*--Replaced by 37 amino acids

B.



References

Abel S, Oeller PW, Theologis A (1994). Early auxin-induced genes encode short-lived nuclear proteins. Proc. Natl. Acad. Sci. USA **91**:326-330

Abel S, Nguyen M, Theologis A (1995). The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana.* J. Mol. Bio. **251**:533-549

Abel S, Theologis A (1995). A polymorphic bipartite motif signals nuclear targeting of early auxin-inducible proteins related to PS-IAA4 from pea (*Pisum sativum*). Plant J. **8**:87-96

Abel S, Theologis A (1996). Early Genes and Auxin Action. Plant Physiol. **111**:9-17

Ainley W, Walker J, Nagao R, Key J (1988). Sequence and characterization of two auxin-regulated genes from soybean. J. Biol. Chem. **263**:10658-10666

Conner T, Goekjian V, LaFayette P, Key J (1990). Structure and expression of two auxin-inducible genes from *Arabidopsis*. Plant Mol. Bio. **15**:623-632

Colón-Carmona A, Chen DL, Yeh K, Abel S (2000). Aux/IAA proteins are phosphorylated by phytochrome in vitro. Plant Physiol. **124**:1728-1738

Fukaki H, Tameda S, Masuda H, Tasaka M (2002). Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsi*s. Plant J. **29**:153-168

Gray W, Estelle M (2000). Function of the ubiquitin-proteasome pathway in auxin response. Trends in Biochem. Sci. **25**:133-138

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M. (2001). Auxin regulates SCF TIR1-dependent degradation of Aux/IAA proteins. Nature **414:** 271–276

Guilfoyle T, Hagen G, Ulmasov T, Murfett J. (1998a). How does auxin turn on genes? Plant Physiol. **118**: 341–347

Guilfoyle T, Ulmasov T, Hagen G (1998b). The ARF family of transcriptional activators and their role in plant hormone-responsive transcription. Cell. Mol. Life Sci. **54**:619-627

Guilfoyle T (1999). Auxin-regulated genes and promoters. *In* Biochemistry and Molecular Biology of Plant Hormones edited by Hooykaas P, Hall M, and Libbenga K. Elsevier Science B.V. pp. 423-459

Hagen G (1987). The control of gene expression by auxin. *In* Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed (Dordrecht, The Netherlands: Martinus Nijhoff). pp. 149-163

Hagen G, Guilfoyle T (1985). Rapid induction of selective transcription by auxins. Mol. Cell. Biol. **5 :**1197-1203

Hagen G, Guilfoyle T (2002). Auxin-responsive gene expression: Genes, promoters and regulatory factors. Plant Mol. Biol. **49:**373–385

Hagen G, Kleinschmidt A, Guilfoyle T (1984). Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections Planta **162** :147-153

Hamann T, Mayer U, Jurgens G (1999). The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in *Arabidopsis* embryo. Development **126**:1387-1395

Hayashi K, Jones A, Ogino K, Yamazoe A, Oono Y, Inoguchi M, Kondo H, Nozaki H (2003). Yokonolide B, a novel inhibitor of auxin action, blocks degradation of AUX/IAA factors. J. Biol. Chem., **278**:23797-23806

Key J (1969). Hormones and nucleic acid metabolism. Annu. Rev. Plant Physiol. **20**:449-473

Key J (1989) Modulation of gene expression by auxin. BioEssays **11**: 5248

Kim BC, Soh MS, Kang BJ, Furuya M, Nam HG (1996). Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2*. Plant J. **9:** 441–456

Kim J, Harter K, Theologis A (1997). Protein-protein interactiuons among the Aux/IAA proteins. Pro. Natl. Acad. Sci. USA **94**:11786-11791

Klimyuk VI, Carroll BJ, Thomas CM, Jones JD (1993). Alkali treatment for rapid preparation of plant material for reliable PCR analysis. Plant J. **3**:493-494

Leopold AC (1955). Auxins and Plant Growth, University of California press

Leyser O, Pickett F, Dharmasiri S, Estelle M (1996). Mutations in the AXR3 gene of Arabidopsis result in altered auxin response including ectopic expression from the SAUR-AC1 promoter. Plant J. **10**:403-413

Liscum M, Reed J (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol. Biol. **49**:387-400

McClure B, Guilfoyle T. (1987). Characterization of a class of small auxin-inducible soybean polyadenylated RNAs. Plant Mol. Biol. **6**:611-623

Melissa A. Hagen G, Guilfoyle T (1991). The tissue-specific and organ-specific expression of soybean auxin-responsive transcripts GH3 and SAURs. Plant Cell **3**:419-430

Nagpal P, Walker L, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW (2000). AXR2 encodes a member of the Aux/IAA protein family. Plant Physiol. **123**:563-573

Ouellet F, Overvoorde P, Theologis A (2001). IAA17/AXR3: biochemical insight into an auxin mutant Phenotype. Plant Cell **13**: 829-842

Ramos JA, Zenser N, Leyser O, Callis J (2001). Rapid degradation of Auxin/Indoleacetic Acid proteins requires conserved amino acids of domain II and is proteasome dependent. Plant Cell **13:** 2349–2360

Reed JW (2001). Roles and activities of Aux/IAA proteins in Arabidopsis. Trends Plant Sci. **6 :**420–425

Rogg l, Lasswell J, Bartel B (2001). A gain-of-function mutation in IAA28 suppresses lateral root development. Plant Cell **13**: 465-480

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O (1998). Changes in auxin response from mutations in an AUX/IAA gene. Science **279**:1471-1373

Sambrook J, Fritsch EF, Maniatis T. (1992). Molecular cloning: a laboratory manual, second edition. Cold Spring Harbor Laboratory. Cold Spring Harbor. New York

Sessions A, Nemhauser, McColl J, Roe J, Feldmann K, Zambryski P (1997). ETTIN patterns the Arabidopsis floral meristem and reproductive organs. Development **124**:4481-4491

Tatematsu K, Watahiki K, Yamamoto K (1999). Evidences for a dominant mutation of IAA19 that disrupts hypocotyl growth curvature responses and alters auxin sensitivity. *In* 10th International Conference on Arabidopsis Research (Melbourne, Australia). Abstract No. 8-39

Theologis A, Huynh TV, Davis RW (1985). Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J. Mol. Biol. **183**:53-68

Tian Q, Reed J (1999). Control of auxin-regulated root development by the arabidopsis thaliana SHY2/IAA3 gene. Development **126:** 711–721

Tian Q, Uhlir NJ, Reed J (2002). Arabidopsis SHY2/IAA3 inhibits auxin-regulated gene expression. Plant Cell **14**:301-319

Tiwari S, Wang WJ, Hagen G, Guilfoyle T (2001). AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell **13**:2809-2822

Tiwari S, Hagen G, Guilfoyle T (2003). The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell **15**:533-543

Ulmasov T, Liu ZB, Hagen G, Guilfoyle T (1995). Composite structure of auxin response elements. Plant Cell **7:**1611–1623

Ulmasov T, Murfett J, Hagen G, Guilfoyle T (1997a). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9**: 1963-1971.

Ulmasov T, Hagen G, Guilfoyle T (1997b). ARF1, a transcriptional factor that binds to auxin response elements. Science **276**:1865-1868

Ulmasov T, Hagen G, Guilfoyle T (1999a). Activation and repression of transcription by auxin-response factors. Proc. Natl. Acad. Sci. USA **96**:5844-5849

Ulmasov T, Hagen G, Guilfoyle T (1999b). Dimerization and DNA binding of auxin response factors. Plant J. **19**:309-319

Walker J, Key J (1982). Isolation of cloned cDNAs to auxin-responsive poly(A)$^+$RNAs of elongating soybean hypocotyl. Proc. Natl. Acad. Sci. USA **79**:7185-7189

Walker J, Legocka J, Edelman L, Key J (1985). An analysis of growth regulator interactions and gene expression during auxin-induced cell elongation using cloned complementary DNAs to auxin-responsive messenger RNAs. Plant Physiol. **77**:847-850

Worley CK, Zenser N, Ramos J, Rouse D, Leyser O, Theologis A, Callis J (2000). Degradation of Aux/IAA proteins is essential for normal auxin signaling. Plant J. **21**:553-562

CHAPTER III

FUNCTIONAL CHARACTERIZATION OF *IAA17/AXR3* AND ITS EFFECT ON PLANT DEVELOPMENT [2]

[2] Lee CM, Nagao RT, Key J To be submitted to Plant Cell

Introduction

Auxin, indole-3-acetic acid (IAA), was the first identified plant hormone. It is known to affect many aspects of plant growth and development including cell elongation, differentiation, and organogenesis (Guilfoyle, 1999). Since IAA has a simple chemical structure, auxin biologists suggested that complex downstream events may be required for the manifestation of such diverse effects on plant growth and development. Two general approaches have been taken to try to understand auxin function: molecular and genetic. From the molecular approach, five major classes of auxin-responsive up-regulated gene families were identified (Chapter II). Some genes are also down regulated by auxin based on both cDNA cloning (Baulcombe and Key, 1980) and *in vitro* translation of poly-A RNA (mRNA) and 2-D gel analysis of the translation products (Baulcombe et al., 1980; Zurfluh and Guilfoyle, 1980, 1982).

Genetic approaches to understand auxin action involved screening mutant populations for auxin-resistant or auxin-sensitive phenotypes. Such screens have yielded at least four classes of mutants with altered auxin-related phenotypes: 1) genes related to auxin signal transduction: putative receptor and kinase, *abp1*, *pinoid*, and *rcn1* (Chen et al., 2001; Christensen et al., 2000; DeLong et al., 2002); 2) a class of mutations that affect genes involved in auxin transport, e.g., *auxl*, *eirl*, *pinod*, *pin-formed, rcnl*, *pisl*, and *lop1* (Carland and McHale, 1996; Bennett et al., 1996; reviewed by Tian and Reed, 1999); 3) mutations affecting genes involved in activation of the ubiquitin-related protein RUB, such as *axr1* (auxin-resistant) and/or its putative down-stream effector, *sar1*, *axr6*, and *tir1* (Cernac et al., l997; Hellmann et al., 2003; Gray and Estelle , 2000; Gary et al., 2001); 4) mutations in Aux/IAA or Auxin Response Factor (ARF) transcriptional regulator genes, such as *axr2*, *axr3*, *shy2, bdl, slr, msg2*, and *iaa28* (members of the Aux/IAA family), and *ettin*, *nph,* and *monopteros* (members of the ARF family) (Rouse et al., 1998; Tian

and Reed, 1999; Hardtke and Berleth, 1998; Sessions et al., 1997; Hamann et al., 2002). The last class represents one example in which molecular and genetic approaches to understand the molecular action of auxin find common genes involved in auxin signaling.

Currently nine gain-of-function mutants have been characterized from the Aux/IAA family of genes: *shy1-1* (*IAA6*, Kim et al., 1996), *shy2-2*(*IAA3*, Tian and Reed, 1999), *axr2-1*(*IAA7*, Nagpal et al., 2000), *bdl* (*IAA12*, Hamann et al., 2002), *slr* (*IAA14*, Fukaki et al., 2002), *axr3-1*(*IAA17*, Leyser et al., 1996; Rouse et al., 1998), *iaa18-1* (*IAA18*, Reed, 2001), *msg2-1* (*IAA19*, Tatematsu et al., 1999), and *iaa28-1* (*IAA28*, Rogg et al., 2001). All of the above have mutations within Domain II, with a single amino acid change centered within a core GWPPV motif (reviewed by Kepinski and Leyser, 2002). These mutants show auxin-related pleiotropic (semi-) dominant phenotypes, demonstrating the importance of Domain II and its critical role in auxin signaling by Aux/IAAs. Ramos et al. (2001) conjugated Domains I and II of IAA17/AXR3 with luciferase (Luc) and examined protein stability by measuring Luciferase activity. They showed that mutated Domain II-conjugated protein is 20 times more stable than WT protein. Ouellet et al. (2001) did pulse chase analyses of IAA17 and axr3-1 proteins with peptide-raised IAA17 antibody from WT and the *axr3-1* mutant and showed that the axr3-1 protein is 7 times more stable than WT IAA17. Finally, Gray et al. (2001) showed that axr3-1-GUS-conjugated protein was 20 times more stable than IAA17/AXR3-GUS conjugated protein. Domain II-mediated protein degradation is facilitated by auxin (Zenser et al., 2001; Tiwari et al., 2001 and 2003; Gray et al., 2001). Aux/IAA proteins interact with the ubiquitin ligase SCF^{TIR1}, and this interaction is facilitated by auxin, resulting in 26S proteosome-mediated degradation (Gray et al., 2001). The above data clearly show that Domain II of Aux/IAAs is involved in their

stability and that those mutations within Domain II correlate with enhanced protein stability and their gain-of-function mutant phenotypes.

Yeast two-hybrid analyses (Chapter II; Kim et al., 1997; Ulmasov et al., 1999b; Ouellet et al., 2001) demonstrate that Domains III and IV serve as protein-protein interaction domains for Aux/IAAs and/or ARFs in homo- and (to a lesser extent) heterodimer formation. *Axr3-1R4*, which has an additional mutation within Domain IV, showed WT-like phenotypes, and the revertant protein did not undergo protein-protein interactions as did the WT and axr3-1 proteins. *Shy2* (*short hypocotyl*) is a dominant mutation in a gene that encodes the IAA3 protein. The *Shy2-2* mutation is located within conserved Domain II (Tian and Reed, 1999) with a Pro to Ser change in the core GWPPV motif. The phenotypes of *axr3-1* and *shy2-2* show many similarities such as a short root, increased adventitious root formation, upward curling of leaf edges, agravitropic roots, and formation of leaves in the dark. One of the intragenic revertants of *shy2-2*, *shy2-22*, has an additional mutation within Domain IV resulting in replacement of half of Domain IV with 8 new amino acids, and this revertant also showed WT-like phenotypes. These two revertants and the failure of axr3-1R4 to undergo protein-protein interactions in yeast two-hybrid assays indicate the importance of Domains III and IV as interaction domains with Aux/IAAs and ARFs in homo- and heterodimers formation and their critical role in auxin signaling in plant growth and development.

The function of Domain I of Aux/IAAs is less clear. There is limited information, but no direct evidence, that Domain I is involved in protein stability. *Axr3-1R3*, an intragenic revertant of *axr3-1,* has an additional mutation within Domain I (Leu to Phe change at position 18); this protein showed similar protein-protein interaction properties as those of WT IAA17 and axr3-1 proteins in yeast two-hybrid analysis (Chapter II; Ouellet et al., 2001). A mutation only within

Domain I of IAA17 (Leu to Phe change at position 18, iaa17R3) showed the same protein-protein interaction behavior as IAA17, axr3-1, axr3-1R3 (Chapter II). Tiwari et al. (2001) showed that Domain II-mutated IAA17 (axr3-1 protein), IAA7 (axr2-1 protein), and IAA19 (msg2-1 protein) reduced the reporter gene activity containing the P3(4X) promoter (4X repeats of a palindromic synthetic AuxRE, **GAGACA**ACT**TGTCTC**) by 3- to 6-fold; however, these proteins with mutations within both Domains I and II (such as axr3-1R3 protein, axr2-1-r-3 protein, and artificial *in vitro* mutated Domain I protein from msg2-1) recovered reporter gene activity to the level expressed in the presence of WT proteins in carrot transient assays. In addition, they showed that a mutation only in Domain I (such as iaa17R3 from Chapter II) resulted in decreased protein stability, while the Domain II mutation (axr3-1 protein) had much increased protein stability. However, axr3-1r3 protein (mutations in both Domains I and II) had an intermediate level of protein stability between WT IAA17 and axr3-1 protein (Tiwari et al., 2001). Taken together the data indicate that Domain I is somehow involved in protein stability. Other roles/functions of these conserved domains have not been defined until recently. The data reported by Tiwari et al. (2004) indicate that Domain I (as core motif LxLxLx, L stands for Leu) serves as a general repressor domain.

Domain I revertants of gain-of-function mutants of Aux/IAAs (*axr3-1R3*, *axr2-1-r-3*, *axr2-1-r-4*, and *slr-1R1*) did not recover completely to WT phenotypes and/or to their null mutant phenotypes (Rouse et al., 1998; Nagpal et al., 2000; Fukaki et al., 2002), perhaps because of the intermediate level of protein stability between WT and the Domain II mutant proteins mentioned by Tiwari et al. (2001). ARFs have a conserved N-terminal DNA-binding domain (DBD), and in most cases the conserved C-terminal Domains III and IV (CTD) found in Aux/IAAs. The middle region (MR) of ARFs is nonconserved and has been proposed to

function as a transcriptional repressor or activator depending upon the presence of a P-rich or Q-rich motif, respectively (Ulmasov et al., 1999a). Tiwari et al. (2003) showed that ARF DBDs alone are sufficient to recruit ARFs to their DNA target sites and that auxin does not affect this recruitment. In addition, the reporter gene activity driven by the P3(4X) promoter was not affected by cotransfection with a combination of MR5 (containing the MR of *ARF5*, a transcriptional activator lacking the conserved CTD, Domains III and IV) and *IAA17*, or the combination of MR5 and *axr3-1* in the absence or presence of auxin; but the activity was reduced when MR5-CTD and *IAA17* were cotransfected into carrot protoplasts in auxin-containing media. The level of reduction was even greater with cotransfection of MR5-CTD and *axr3-1*. From these data, Tiwari et al. (2003) concluded that the auxin response is mediated by the recruitment of Aux/IAA proteins to promoters that contain a DNA-binding protein (ARF) with a Q-rich MR and an attached CTD such as ARF5. This implies that one area of auxin signaling is mediated by protein-protein interactions through Domains III and IV between Aux/IAAs and ARFs in an Aux/IAA protein dose-dependent manner as measured in the carrot protoplast system.

The *Axr3-1* mutant was the first gain-of-function mutant characterized among the Aux/IAAs. *Axr3-1* is semi-dominant and encodes a modified Aux/IAA protein (IAA17). The *axr3-1* allele (where a Pro to Leu change occurs within Domain II at position 88) shows the most severe phenotype having agravitropic and short roots, very few root hairs, strong apical dominance, short hypocotyls in the dark, small-sized upcurled-leaves, small plants, etc. (Leyser et al., 1996). Intragenic suppressors (or revertants) of *axr3-1* were cloned following EMS treatment of *axr3-1* seeds. Five revertants were isolated using primary root length as a measurement of allelic strength. One revertant, *axr3-1R4*, was the strongest allele with a WT-

like phenotype (Rouse et al., 1998). The axr3-1 protein showed similar protein-protein interaction properties as did the WT IAA17 in that it interacted with IAA17, other Aux/IAAs, and ARF1 in yeast two-hybrid assays (Chapter II). Axr3-1R4, however, did not interact with IAA17, some other Aux/IAAs, or ARF1 in yeast two-hybrid assays (Chapter II). Thus, *axr3-1R4* was concluded to be a loss-of-function mutant that did not show distinct phenotypes. Since *axr3-1* is a gain-of-function mutant that down-regulated most auxin-responsive genes such as Aux/IAAs, GH3, SAURs, and GST (Chapter II), it was difficult to study the function of WT IAA17 from *axr3-1* in auxin-related plant growth and development responses. *Axr3-1R4* and *IAA17K* (IAA17 knockout) were used in an attempt to further analyze the function(s) of IAA17.

Msg2-1 has a nucleotide change that resulted in a substitution of Pro to Ser at position 69 in Domain II of IAA19 (Tatematsu et al., 1999). Additional alleles of the IAA19 dominant mutant were isolated as follows: *msg2-3* with Pro to Leu change at position 69, and *msg2-2* with Gly to Arg change at position 67. Phenotypes of *msg2* mutants include no gravitropism, weaker phototropism, weaker hook formation in hypocotyls, as well as 2,4-D-resistant hypocotyl growth. Since the Domain II-mutated IAA19 showed increased protein stability (Tiwari et al., 2001), these mutants may be similar to *axr3-1* such that dominant mutant phenotypes would not provide functional information of WT IAA19. An IAA19 knockout (*IAA19K*) was also isolated and used in the analysis of Aux/IAA gene function(s). Double knockout of IAA17 and IAA19 was generated to extend the study of the possible function(s) and interactions of Aux/IAA genes.

Materials and Method

IAA17 Expression in Transgenic Arabidopsis Plants

Genomic DNA was prepared from WT (Columbia) Arabidopsis seedlings using Qiagen DNeasy® Plant Mini (or Maxi) Kit (Qiagen Inc, Valencia, CA). The 2.3 kb of the IAA17 promoter from 5' translation start site and 0.7 kb of the terminator region of IAA17 were amplified from genomic DNA with primers (3' UTR_F: 5'-AAA CGA GCT CAA AAG GAT AAG TGG TAT CGA-3' TT-3' and 3' UTR_R: 5'-AAA GGC GCG CCT TCC CTA TGG GTC CTA TTT CTC TA-3'; Pro_F: 5'-AAA GGC GCG CCT TCC CTA TGG GTC CTA TTT CTC TA-3' , and Pro_R: 5'-AAA AGG ATC CAC CTT TCT TCT TCT TTG GTG TT-3') by PCR with PFU (Stratagene, LaJolla, CA) and cloned into *pUPC5-GUS* vector to replace the ubiquitin-3 promoter and nos terminator. The new construct was subcloned into *pUNPT-1* vector containing *nptII* as a selection marker and inserted into *pPZP-201BK* transformation vector containing the kanamycin resistance gene for plant selection. The new transformation plasmid was named *pIAA17*::*GUS*.

Transformation was done by the standard floral dip method (Clough and Bent, 1998). The *pIAA17*::*GUS* construct was transformed into *Agrobacterium tumefacinecs* AGLO 101 strain and harvested at mid-log phase. Arabidopsis plants were dipped in Agrobacterium media, air-dried, returned to the growth chamber to finish the life cycle of Arabidopsis, and then seeds were harvested. Transgenic plants were screened on plates containing 50 μg/ml kanamycin media. At least 10 independent transgenic lines from each experiment were selected to confirm similarity of expression patterns among the transgenic lines.

GUS expression in transgenic plants was observed by developing the X-gluc color (Martin et al., 1992). Seedlings or tissue sections were incubated for 30 min on ice in 2%

paraformaldehyde, 100 mM Na-phosphate, pH 7.0, 1 mM EDTA, and then washed in 100 mM Na-phosphate, pH 7.0. After submerging samples in 2 mM X-gluc in buffered solution (50 mM Na-phosphate, pH 7, 0.5% Triton X-100), samples were vacuum infiltrated for 10 sec, and then incubated 10 min to 3 hr while checking staining strength from time to time to avoid overstaining. GUS reaction was stopped by washing samples in water and then bleached with several changes of 70% ethanol.

Knockout Mutant Screenings for IAA17 and IAA19

An internet search was conducted to find T-DNA insertional lines for Aux/IAA genes. Garlic_1233_C09 line was found in the Syngenta collection (http://www.nadii.com/pages/collaborations/garlic_files/GarlicDescription.html, San Diego, CA) for an *IAA17* knockout (*IAA17K*), and Salk_000337 line was found from the Arabidopsis Biological Resource Center (Columbus, OH) for an *IAA19* knockout (*IAA19K*). Stocks obtained for the two lines putatively have T-DNA insertions in the two respective mutant alleles.

To confirm T-DNA insertions, an individual plant leaf was cut from the putative knockout plants, and DNA was extracted by the NaOH boiling method (Klimyuk et al., 1993). Primers were designed from the T-DNA left border (LB) region (for Syngenta knockout: 5'-CAGAAATGG ATAAATAGCCTTGCT-3', and for Salk line knockouts: 5'-GGTGTAAACAA ATTGACGCTT AGACAA-3'), and gene-specific primers for *IAA17* (forward : 5'-CAT AGT CCC AGC TAT TCA CCA A-3' and reverse: 5'-CAAATCCAGA TCA AAACACAGACAA-3') and *IAA19* (forward: 5'-ATGGAGAAGGAAGGACTCGGG CTT-3' and reverse: 5'-TCATCA CTCGTCTACTCCTCTA-3'). Touchdown PCR was carried out with the appropriate

primer sets. The PCR products were separated by agarose gel electrophoresis, and the size of the band was determined for confirmation of T-DNA insertions in the allele.

Southern Analysis

Genomic DNA was extracted with Qiagen DNeasy® Plant Maxi Kit (Qiagen Inc, Valencia, CA) and further purified by phenol:chloroform (1:1) extraction and ethanol precipitation. Southern blot analysis was done with ^{32}P-radiolabeled probe (Sambrook et al., 1992). Purified genomic DNA was digested with restriction enzymes (NEB, Beverly, MA), separated by 1% agarose gel electrophoresis, and transferred to Biodyne B membrane (Pall, Ann Arbor, MI) by downward capillary transfer with 3X SSC (3 M Sodium Chloride and 0.3 M Sodium Citrate, pH 7). After UV fixation of DNA to the membrane, the blot was prehybridized and then hybridized with radiolabeled probes (about 10^6 cpm/ml of Perfecthyb^{TM} plus Hybridization buffer, Sigma, St. Louis, MO) overnight. The blot was washed with low stringency washing buffer (2X SSC/0.1% SDS) and 3 times with high stringency washing buffer (0.5X SSC/0.1% SDS) for 15 min each. After developing the X-ray film, the images were scanned with imaging software (Microtek scanner ScanWizard 5 and Photoshop 5.0).

Northern Analysis of *IAA17* and *IAA19* Expression

Northern blot analysis was done with ^{32}P-radiolabeled probe (Sambrook et al., 1992). Total RNA was separated by 1% formaldehyde agarose gel electrophoresis and transferred to Biodyne B membrane by downward capillary transfer with 3X SSC. After UV fixation of RNA to the membrane, the blot was prehybridized and hybridized with radiolabeled probes (about 10^6 cpm/ml of Perfecthyb^{TM} plus Hybridization buffer) overnight, and the blot was then washed with

low stringency washing buffer (2X SSC/0.1% SDS) and 3 times with high stringency washing buffer (0.5X SSC/0.1% SDS) for 15 min each. After developing the X-ray film, the images were scanned with imaging software (Microtek scanner ScanWizard 5 and Photoshop 5.0).

Plant Root Growth Measurements in Various Auxin Concentrations

Plants (WT, *axr3-1*, *axr3-1R4*, and *IAA17K*) were germinated in 0.5X MS media for 2 days and then transferred onto 0.5X MS/0.2% Sucrose media containing various NAA (a-naphthalene acetic acid) concentrations (10^{-9} M to 10^{-3} M). After 4 days of vertical growth (16 hr light: 8 hr dark), plants were photographed with a digital camera (Nikon Coolpix 995, Nikon, Tokyo, Japan), and then root length was measured using Scion Image software (Scion Inc, MD). Each data point was generated from at least 30 measurements from three independent experiments.

Observation of Root Cells by Scanning Electron Microscopy and Confocal Microscopy

Plants were grown vertically on 0.5X MS media plates for 4 days with 16 hr light:dark 8 hr and then prepared for scanning electron microscopy (SEM) by cryoprep methodology using Gatan Alto 2500 Cryostage and cryoprep chamber (Gatan UK, Oxford, UK). The samples were rapidly frozen in liquid nitrogen slush and transferred to the cryoprep chamber. Samples were kept frozen and coated with platinum in the chamber, then moved to the cryostage within the SEM (LEO 982 field emission scanning electron microscope, LEO Electron Microscopy, Inc. Thornwood, NY). Samples were kept frozen while viewed.

For confocal microscopy, plants grown as above were submerged in 1 mg/ml propidium iodide solution (Sigma) and washed twice with water. Samples were slide mounted and

observed with a Bio-Rad MRC 600 Laser Scanning Confocal Microscope (Hercules, CA). Root cell length was measured by using Scion Image software.

Double Mutant Analysis

Crossing of knockout mutants to make a double knockout mutant was done by anthers of *IAA17K* (pollen) to fertilize carpels of *IAA19K* plants. A dissecting microscope was used to facilitate the pollination. F1 plants were selected for herbicide resistancy after spraying 30-fold diluted Finale® (AgrEvo Environmental Health, Montvale, NJ). F1 plants were selfed, and F2 plants were screened for both knockout alleles for *IAA17K* and *IAA19K* with touchdown PCR. DNA extraction and PCR with primers were carried out as described in Knockout Mutant Screening section. The double knockout was designated as *IAA17K/IAA19K*.

Results

Screening for *IAA17* and *IAA19* Knockouts

Phenotypes from gain-of-function and loss-of-function mutants and molecular aspects of the gene/protein will contribute some insight into the function(s) of a gene. However, a null allele of a gene is often very important in order to define its role(s). An internet search identified two T-DNA insertional lines for Aux/IAA genes. Garlic_1233_C09 line was obtained from Syngenta for an *IAA17* knockout, and Salk_000337 line was obtained from the Arabidopsis Biological Resource Center for an *IAA19* knockout. The two lines were screened for the T-DNA insertions in their respective alleles by PCR. Primers were designed from the T-DNA left border (LB) region, and gene-specific primers were designed for *IAA17* and *IAA19*. Touchdown PCR was conducted on genomic DNA from these lines. However, homozygotes were not found from

the original seeds from the two sources. The hemizygotes of the two T-DNA insertion lines were selfed, and then F2 plants were screened to find homozygous insertional lines. Figure 3-1A shows examples of the PCRs. Upper bands (1 kb) were PCR products from two gene-specific primers of *IAA17*, and lower bands (0.5 kb) represent the PCR product from *IAA17* and T-DNA LB primers. Figure 3-1B shows a schematic diagram of the location of the T-DNA insertion in the *IAA17* and *IAA19* alleles. After confirming T-DNA insertions by analyzing the band size, Southern blot analysis was conducted to identify any additional T-DNA insertions in the genome. *IAA17K* seemed to have at least one additional insertion in the genome (Fig. 3-2), so a homozygous *IAA17K* was backcrossed to WT (Co.) to remove the additional insertions, and then F2 plants were re-screened to find homozygous knockouts for the *IAA17* allele. F2 plants showed 3:1 segregation ratio for Finale® resistance from F1 selfing (data not shown) suggesting a single T-DNA insertion in the genome. Figure 3-2 shows homozygous *IAA19K* with only a single insertion in the genome. Since the T-DNA insertion in the *IAA17* and *IAA19* alleles were located near the C-terminal end (Fig. 3-1B), Northern analysis was conducted to ascertain if the knockouts produced transcripts for *IAA17* and *IAA19*. Probes for *IAA17* and *IAA19* were PCR-synthesized from the 5' region of Open Reading Frames (ORF) of the genes. No transcripts for *IAA17* and *IAA19* were detected by Northern analysis in the knockouts (Fig. 3-3). It was concluded that the two selected knockouts were null alleles for *IAA17* and *IAA19* based on the following: 1) sequence information showed the T-DNA insertions in the two alleles; 2) the PCR results showed the expected band sizes for T-DNA insertions; 3) Southern analysis revealed that each line had only one insertion in the genome, and 4) finally, the two knockout lines did not produce detectable transcripts for their respective genes.

Auxin Sensitivity Test of IAA17/AXR3 and Its Mutants

A previous study (Leyser et al., 1996) showed that *axr3-1* was 500-fold more insensitive (or resistant) to IAA (indol-3-acetic acid) in root growth inhibition than the WT. It was of interest to examine the auxin sensitivity of the revertant and *IAA17K* since the phenotypes of these plants were WT-like. In addition, the Northern data showed that the gain-of-function mutant, *axr3-1*, had greatly reduced message levels of most auxin up-regulated genes. Further, the intragenic revertant, *axr3-1R4*, had near WT message levels of auxin up-regulated genes (Chapter II). Seeds were germinated for two days on vertical growing plates, and uniform seedlings were selected and transferred onto agar plates containing various concentrations of NAA. Plants were grown on vertically oriented agar plates for four days followed by root length measurement to determine auxin sensitivity. NAA was selected as the auxin source instead of IAA because of its stability and permeability into cells.

Figure 3-4 shows auxin sensitivity of root growth of the four different genetic backgrounds. Root growth of *axr3-1* plants was 100-fold less sensitive to auxin than WT. Inhibition of root growth did not occur until the NAA concentration reached 10^{-5} M versus 10^{-7} M for the other three genetic backgrounds. *Axr3-1R* and *IAA17K* showed slightly more auxin sensitivity than WT, but the difference was not statistically significant. *Axr3-1* did not show auxin inhibition of root growth up to 10^{-6} M NAA, whereas WT was significantly inhibited by 10^{-7}M NAA. Basically, WT, *IAA17K*, and *axr3-1R4* showed the same auxin sensitivity throughout the range of NAA concentrations.

The root length of *axr3-1R4* and *IAA17K* plants was shorter than that of WT between 0 and 10^{-8} M NAA (Fig. 3-5). *Axr3-1* showed strong root growth up to 10^{-6} M NAA and showed root growth even at 10^{-5} M NAA although significantly reduced. WT, *axr3-1*, and *axr3-1R4*

showed many bulged lateral roots which did not elongate at 10^{-5} M NAA, so the roots were short and fat (radially enlarged). However, WT, *IAA17K*, and *axr3-1R4* plants had very many elongated lateral roots with a short primary root at 10^{-6} M NAA, whereas axr3-1 showed root growth without lateral roots out to 10^{-5} M NAA (Fig. 3-5B). *IAA17K* and *axr3-1R4* did not show phenotypic differences and were similar to the WT in terms of auxin sensitivity and plant morphology at various NAA concentrations.

GUS Staining Patterns from *pIAA17*::*GUS* as a Measure of IAA17 Spatial and Temporal Expression

Detailed studies of the phenotypic analysis of loss-of-function mutants *axr3-1R4* and *IAA17K* were done in order to gain some insight into the function of the gene. Spatial and temporal expression patterns of a gene may provide valuable insights into gene-related phenotypes and the gene's possible role(s) in plant development. The *IAA17* promoter (2.3 kb, 5' of the translation start site) and terminator (0.7 kb 3' of the stop codon) were cloned onto a *GUS* ORF and transformed into Arabidopsis. Transformants were screened on kanamycin selection media after harvesting seeds from putative T1 plants. At least 10 independent transgenic lines (T2 generation) from 10 T1 lines were examined to confirm that the GUS expression patterns resulted from the *IAA17* promoter/ terminator construct activity. All plants tested showed similar GUS expression patterns (data not shown).

Based on the GUS staining pattern, IAA17 was expressed primarily in the root tissue, but not in the root hairs (Fig. 3-6). Very strong GUS staining was observed in the bending area of roots (Fig. 3-6A). GUS staining was observed from the elongation zone up to the root-hypocotyl junction, but was not observed in the root apical meristem or root cap (Fig. 3-6A). However,

there was a window of expression of GUS in the root because staining became weaker in 4-day old root tissue. In older plants, strong staining occurred in the elongation and mature zone of root (new growth and subsequently developed tissue) (data not shown). Staining was also observed in lateral roots, but again not in the root apical meristem (Fig. 3-6F and G). The pattern was similar to that of primary roots in that GUS staining started in the elongation zone and was present throughout the entire mature region. *DR5*::*GUS* transgenic plants containing seven tandem repeats of a synthetic auxin response element (CCTTTTGTCTC, AuxRE) coupled to GUS showed very high auxin-responsiveness of GUS expression (Ulmasov et al., 1997b). It was interesting to compare *pIAA17*::*GUS* staining with *DR5*::*GUS* since *DR5*::*GUS* was strongly expressed in regions of the transgenic plants such as meristems where auxin concentrations are expected to be high. *DR5*::*GUS* showed very strong staining in the root tip, and the stain extended throughout the elongation zone (Fig. 3-6H and I). Also, *DR5*::*GUS* plants showed staining in lateral root tips, elongation zone, and emerging and pre-emerging lateral roots, all of which are known to have high auxin concentrations. In contrast, *pIAA17*::*GUS* expressed from the elongation zone throughout the mature region, showing unique differences relative to *DR5*::*GUS* and *AtAux2-11*::*GUS* (promoter of *AtAux2-11/IAA4* plus GUS) compared to IAA17 promoter-driven expression in the transgenic plants.

In the shoot, GUS expression appeared on the edge of the cotyledon and in the mid vein of rosette leaves (Fig. 3-6B and C). Also, the area below the shoot apical meristem where active cell elongation takes place showed expression (Fig. 3-6E and Fig. 3-7H). Cauline leaves, stem, and various floral organs as well as old siliques showed GUS staining (Fig. 3-7). Part of the stem did not stain, possibly because cutin inhibited penetration of GUS substrate solution into stem, but stain was seen at the end of stems where the solution can penetrate a short distance

(Fig. 3-7I). The staining patterns in the floral organ were very interesting since the parts are actively growing and differentiating. GUS staining was confined to the base of siliques when the siliques were young, but at some discrete point of silique maturation GUS expression extended through the entire silique. GUS staining also showed a similar developmental pattern in flowers and pollen grains in that 1) the base of flower showed GUS stain and 2) pollen grains showed stain only after a certain stage of maturation (Fig. 3-7).

Northern Analysis of Tissue-Specific Expression of *IAA17* and *IAA19*

Steady state message levels of *IAA17* and *IAA19* from various tissues and organs were examined by Northern analysis (Fig. 3-8). Auxin-responsiveness in whole seedlings was measured. *IAA17* showed high message levels primarily in root tissue and seven-day old young seedlings. Much lower message levels of *IAA17* were present in other tissues, such as leaves, primary inflorescence stem, base (stem and hypocotyl junction where rosette leaves and adventitious stems are initiated), bud of secondary inflorescence stem, and floral organs of primary inflorescence stem. The message level in young seedlings (Lane 3 from Fig. 3-8) resulted an average message level from roots and shoots (Lanes 1 and 2 from Fig. 3-8). The tissue-specific expression pattern of *IAA17* from Northern analysis correlated well with the GUS expression patterns from *pIAA17*::*GUS* transgenic plants. *IAA19* showed the highest message levels in floral organs (Lanes 10 and 11), followed by root (Lane 1), young seedling (Lane 3), stem base (Lane 8), and young leaves (Lane 4, Fig. 3-8). Lane 10 (secondary inflorescence floral buds) represents a much younger stage than Lane 11 (flower and bud from primary inflorescences) in terms of flower development. Therefore, this indicates that the *IAA19* gene is primarily expressed in the early stage of flower development and in the root.

IAA17 was slightly auxin-responsive in light-grown seedlings, and the level of expression was reduced under dark conditions compared with light-grown seedlings (Fig. 3-3). *IAA19* was strongly auxin-induced in light-grown seedlings, and the level of expression was much greater in etiolated seedlings than in light grown plants (Fig. 3-3). Lane 12 and 13 of Figure 3-8 represents auxin- responsiveness of etiolated seedlings for *IAA17* and *IAA19*. Both *IAA17* and *IAA19* were auxin-responsive in etiolated seedlings. The level of the response of *IAA19* appears to be lower in etiolated seedlings than in light-grown seedlings. In general, based on the Northern data, *IAA17* and *IAA19* were expressed at high levels in roots and were auxin-responsive. These data demonstrate some substantial differences in relative levels and patterns of expression of *IAA17* and *IAA19* in Arabidopsis plants.

Root Cell Size Analysis

Root cell size from WT, *axr3-1*, *axr3-1R4*, and *IAA17K* was measured with both confocal and scanning electron microscopy (SEM). There were two reasons to measure root cell size: 1) the IAA17 gene was expressed primarily in the root based on both Northern analyses and the GUS staining pattern from *pIAA17*::*GUS* transgenic plants, and 2) *axr3-1R4* and *IAA17K* showed somewhat shorter root length compared to WT at various auxin concentrations. The middle of the mature region of the root was used to measure cell size for both confocal and SEM images. SEM was first employed to measure epidermal root cell size. A cryoprep method, which is relatively faster and easier than the classical dehydration method, was used to prepare samples. Generally, WT showed longer root cell length than *axr3-1*, *axr3-1R4*, and *IAA17K* in SEM observations (Fig. 3-9A). WT, *axr3-1R4*, and *IAA17K* showed relatively straight root cell files

compared to *axr3-1* in which the cell files were twisted. Due to the cost, labor intensity, and resolution limitations of SEM, a switch to confocal microscopy was made.

Propidium iodide was used to stain the nucleus, but it also stained plant cell walls. Figures 9B and C show examples of cell images from confocal microscopy. It was possible to observe epidermal, cortex, and vasculature regions with confocal microscopy with propidium iodide staining. WT roots had somewhat longer epidermal and cortical cells than *axr3-1*, *axr3-1R4*, and *IAA17K* (Fig. 3-9 middle and bottom). Root cell lengths were measured with confocal microscopy from at least 100 cells from at least 20 different plants for each genotype (Table 3-1). WT showed longer epidermal root cell length with an average value of 222 (as relative root length) compared to 171 and 172 for *axr3-1R4* and *IAA17K*, respectively, consistent with the somewhat shorter roots relative to WT. However, there was some overlap of standard deviation ranges from WT to *IAA17K*. In retrospect, by increasing the sample size, statistical significance most likely would have been achieved based on viewing a large enough sample size. Although reduced root cell number may also contribute, counting total cell number in the root was not possible in this case.

IAA17K/*IAA19K* Double Mutant Analysis

There was a reduced message level of most auxin up-regulated genes including Aux/IAA genes in the *axr3-1* gain-of-function mutant (Northern data from Chapter II), suggesting that the phenotypes of *axr3-1* may result from the reduced level of Aux/IAAs and/or other auxin up-regulated gene products as well as increased levels of the axr3-1 protein. For this reason, it was difficult to get functionality information of IAA17 from the gain-of-function mutant. *IAA17K* and *IAA19K* were crossed and then selfed. F2 plants were screened with touchdown PCR in

order to isolate the double-knockout mutant of *IAA17* and *IAA19*. Figure 3-10 shows phenotypes of various genotypes including the double knockout mutant. *IAA17K* and *IAA19K* plants did not show distinct phenotypes during growth and development (data not shown). The revertant, *axr3-1R4*, and *IAA17K* showed slightly shorter root length than WT, whereas *IAA19K* showed similar root length compared to WT. The double mutant, however, showed much shorter root length compared to WT, *IAA17K*, and *IAA19K* implying that the double knockout was synergistic in effect relative to single mutant, *IAA17K* and *IAA19K*, plants.

Since a major effect of IAA17 and the relevant mutants is observed on root growth and development, roots were studied further. Leyser et al. (1996) reported that *axr3-1* roots had very few root hairs. Figure 3-11B shows a SEM micrograph of an *axr3-1* root section. The *axr3-1* root forms bulges and appears to initiate root hairs, but further growth of root hairs (i.e., tip growth) was impaired. Figure 3-11 shows that the root hair patterns from *axr3-1R4*, *IAA17K*, and WT are similar. *IAA19K* showed slightly longer root hairs at the top of root, but otherwise root hair differences cannot be distinguished among WT, *axr3-1R4*, *IAA17K*, and *IAA19K*. The double-knockout mutant showed higher root hair density and longer root hairs compared to WT, *IAA17K*, and *IAA19K*. The double mutant also did not show ectopic root hairs. The root cell size of the region where the double mutant showed higher density and longer root hairs was observed with confocal microscopy (Fig. 3-11). The double mutant of four day-old seedlings had much shorter epidermal and cortical root cells compared to WT, *IAA17K*, and *IAA19K*. The higher density of root hairs probably results, at least part, from the much shorter root cell size. However, the pattern of higher root hair density of the double mutant was transitional in that after four days, older seedlings of the double mutant have a WT-like root hair pattern in new root

growth (data not shown). Based on this observation of the double mutant, IAA19 seems to act during early stages of root growth.

Discussion

The *IAA17* gene and mutant forms, *axr3*-1, *axr3-1R4*, and *IAA17K* were selected for the studies reported here based on the fact that *axr3-1* was a well characterized mutant relative to auxin-responsiveness and resulting phenotypic changes. *Axr3-1* showed various auxin-related phenotypes such as agravitropic and short roots, no lateral roots, very few root hairs, short and strong apical dominant inflorescence stem, and upcurled and small-sized leaves. The axr3-1 protein from this gain-of-function mutant was 7 to 20 times more stable than the WT IAA17 (Gary et al., 2001; Ouellet et al., 2001). The mutant protein had similar protein-protein interaction properties to IAA17 such that it interacted with IAA17, some other Aux/IAAs, and ARF1 in yeast two-hybrid assays (Chapter II). Axr3-1R4, however, did not interact with IAA17, other Aux/IAAs, and ARF1 in yeast two-hybrid assays and did not show mutant phenotypes (Chapter II). Thus, it is concluded that *axr3-1R4* is a loss-of-function mutant. Since *axr3-1* is a gain-of-function mutant and showed reduced message levels of most auxin-responsive genes such as Aux/IAAs, GH3, SAUR, and GST, it was difficult to study the specific function(s) of IAA17/AXR3 from *axr3-1* in auxin-related plant growth and development. *Axr3-1R4* and *IAA17K* were used to analyze the function of IAA17. *Axr3-1R4* was backcrossed twice (Chapter II) and *IAA17K* was backcrossed to WT once to clean their respective genetic backgrounds. Spatial and temporal expression patterns were studied using Northern analysis and by GUS expression of *pIAA17*::*GUS* in transgenic plants to obtain insight into the phenotypes of *axr3-1R4* and *IAA17K* in more detail.

The promoter and terminator of *IAA17* were cloned up- and downstream of *GUS* to make as natural as possible an *IAA17* expression indicator. GUS expression was highest from the elongation zone to the root and hypocotyl junction (Fig. 3-6). The lateral root staining pattern was similar to that of the primary root. *DR5*::*GUS* transgenic plants showed very high auxin responsiveness (Ulmasov et al., 1997b). It was interesting to compare GUS expression pattern with *DR5*::*GUS* since GUS was strongly expressed in these transgenic plants in regions known to have high auxin concentrations such as meristems. *DR5*::*GUS* showed very strong expression in primary and lateral root tips including pre-emerging lateral roots, suggesting that the staining starts before cell differentiation. The staining patterns of *DR5* and *pIAA17*::*GUS* showed slight overlaps in the elongation zone of root. In the shoot, *pIAA17*::*GUS* was expressed at the edge of cotyledons, the vicinity of mid-vein of leaves, below the shoot apical meristem, cauline leaves, stem, and in the various floral organs as well as in mature siliques (Fig. 3-7). The staining patterns in the floral organs were very interesting since these parts are actively growing and differentiating (Fig. 3-7). GUS expression was confined to the base of flowers at early stages, and it remained in the base of young siliques, but expression subsequently extended throughout the whole silique at later stages of silique maturation. Based on GUS expression patterns, it is proposed that IAA17 may be involved after organogenesis and may be involved in cell expansion and organ maturation in the case of flowers and silique parts.

Wyatt et al. (1993) studied lacZ expression using of the 0.6 kb promoter from *AtAux2-11*(*IAA4*) (*pAtAux2-11*::*LacZ*) in Arabidopsis; this gene was expressed at relatively high constitutive levels and was auxin-inducible. This is one of the most thorough localization studies of expression pattern of an Aux/IAA gene. The *AtAux2-11* promoter was active in root tips, lateral root initiation zone, elongation zone of roots, elongation side of hypocotyls undergoing

gravitropic curvature, etiolated hypocotyls, anther filaments, and tissues undergoing lignification (e.g., xylem, trichomes). The expression seems to correlate with areas of high auxin concentration where organogenesis, cell expansion, and differentiation take place, and to be more similar to *DR5*::*GUS* staining pattern than *pIAA17*::*GUS*. The expression pattern of several auxin-responsive genes is summarized in Table 3-3. Most expression pattern studies emphasized relatively young seedlings of Arabidopsis except studies of Wong et al. (1996) which used *Ps-IAA4/5* and *Ps-IAA6* promoters in tobacco. *PIAA17*::*GUS* expression showed substantial similarity to patterns of IAA7, IAA14, and IAA28, with some overlap among Aux/IAA promoter activities. *IAA28* is not auxin-responsive in 5 day-old etiolated seedlings, and the message level is not reduced in *axr3-1* (Chapter II). *IAA28* message is slightly reduced in *iaa28-1*, a Domain II gain-of-function mutant; the phenotypes of *iaa28-1* show bushy plants with gravitropic roots (Table 3-2 and Rogg et al., 2001). The *IAA7/AXR2* gene showed reduced message levels in *axr3-1* and *axr2-1* Domain II mutants, but *axr2-1* showed reduced apical dominance and agravitropic roots (Napal et al., 2000). *IAA14/slr* showed auxin-responsiveness and a reduced message level in *axr3-1* plants (Chapter II). However, the roots of the *slr-1* Domain II gain-of-function mutant responded gravitropically in contrast to *axr3-1* that had agravitropic roots, but *slr-1* had fewer lateral roots and strong apical dominance similar to *axr3-1*. *IAA17/axr3-1* is closer to *IAA14/slr-1* than other Domain II gain-of-function mutants in terms of phenotypes, but it is more similar to *IAA28* in terms of GUS expression patterns. GUS expression patterns and Domain II mutant phenotypes of Aux/IAAs show that Aux/IAAs have multiple and varied expression patterns and that their regulation varies during plant growth and differentiation. This would indicate that even though the Aux/IAAs have great similarity in terms of the four conserved functional domains there is both redundancy of function as well as independent action

based on auxin-responsiveness, tissue/organ-specific expression, and selective interactions in heterodimer formation.

Leyser et al. (1996) showed that *axr3-1* is 500-fold less sensitive (more resistant) to auxin in terms of root growth inhibition. It was of interest to examine the auxin sensitivity of the revertant, *axr3-1R4*, and *IAA17K* because these plants have WT-like phenotype; also Northern data showed that *axr3-1* had reduced message levels of auxin up-regulated genes while *axr3-1R4* recovered WT message levels of auxin up-regulated genes. The data presented here show that *axr3-1* was 100-fold less auxin sensitive than the WT to auxin inhibition of root growth. NAA was used as the auxin source in this study instead of IAA as in the Leyser's study (1996) because it is more stable and more permeable to plants than IAA. The fold difference may have resulted from the difference in auxin source (IAA vs NAA), but also, and seemingly more likely, from differences in method of measurement of root length since *axr3-1* has tangled and twisted roots so that measurement of total primary root length is difficult. Scion Image software was used in this study to measure the root length. The software gives accurate root length because when one draws the shape of the root, the software automatically calculates the total primary root length. *Axr3-1R4* and *IAA17K* basically showed the same auxin sensitivity in that both were inhibited by 10^{-6} M NAA similar to WT. *Axr3-1* showed the greatest auxin insensitivity of root growth inhibition among the Aux/IAA mutants used in this work. *Axr2-1*, *bdl*, and *slr-1* also showed reduced auxin sensitivity, but *shy2-1* is an exception in that it showed a WT response to auxin in root growth studies (see Table 3-2).

Based on the spatial and temporal expression patterns of IAA17 and the shorter root lengths of *axr3-1R4* and *IAA17K*, root cell length of *axr3-1R4* and *IAA17K* were also studied. The general trends of root cell sizes of *axr3-1R4* and *IAA17K* were shorter than WT, but

significant differences of root cell size were not clear. Root cell size of Arabidopsis varied especially in different cell files. Measurement of only 100 cells from the middle region of the mature root zone of 20 different plants was not sufficient to produce a low standard deviation. It is concluded that IAA17 may be involved in root growth, specially in enhancing root cell elongation. Because of the high conservation of the protein structure and similar (or overlapping) expression patterns of Aux/IAAs (Abel et al., 1995b; Abel and Theologis, 1996; see Table 3-3), IAA17 may play a role in root cell elongation with other Aux/IAAs causing subtle phenotypic variation in roots as well as in shoot parts.

In order to search for more distinct phenotypes, double knockouts of *IAA17* and *IAA19* were made. Based on gain-of-function phenotypes and expression pattern, an *IAA14/slr* knockout (or loss-of-function mutant) was the most appropriate choice for their studies, but this knockout was not available, so the *IAA19* knockout was selected. A Domain II dominant mutant (gain-of-function) of *IAA19*, *msg2-1*, had a nucleotide change predicted to cause a replacement of Pro to Ser at position 69 (Tatematsu et al., 1999). Hypocotyls of *msg2-1* plants were completely agravitropic, and *msg2-1* also showed weaker phototropic and weaker hypocotyl hook formation responses. Hypocotyls of *msg2-1* were resistant to auxin (2,4-Dichlorophenoxy-acetic acid). Tatematsu et al. (1999) concluded that products of the mutated *IAA19/Msg2* gene might interact with ARF7/NPH4 in a dominant-negative manner suggesting that Aux/IAAs play a central role in differential growth responses of hypocotyls since the phenotype of *msg2* was very similar to that of *nph4/msg1*. Tissue-specific Northern analysis showed that IAA19 message levels were high in the stem, flower, and root (Fig. 3-8). Based on a higher message level in dark grown seedlings than in light grown seedlings and the agravitropic hypocotyls of this Domain II gain-of-function mutant, an IAA19 loss-of-function mutant and/or a null mutant

caused by T-DNA insertion was used to find abnormal phenotypes such as short and/or agravitropic hypocotyls. However, *IAA19K* did not show distinct phenotypes when grown in either dark or light (data not shown).

ARFs are a multigene family of transcriptional regulators, consisting of 23 members in Arabidopsis (reviewed by Liscum and Reed, 2002). ARFs interact with Aux/IAAs through Domain III and IV (Kim et al., 1997; Ulmasov et al., 1997b; Chapter II). In gel shift assays, ARFs showed a preference for forming homodimers in binding to synthetic palindromic AuxREs (Ulmasov et al., 1999b). Arabidopsis contains at least 23 Aux/IAA genes (Appendix A; reviewed by Liscum and Reed, 2002). Aux/IAAs are defined on the basis of four conserved domains independent of auxin-responsiveness, even though the class was originally defined as auxin-responsive genes (Chapter II; Appendix A). Six members of the putative Aux/IAAs which were classified as Aux/IAAs by Liscum and Reed (2002) do not contain Domains I and II but do contain Domains III and IV (Appendix A); some do contain an LxLxLx motif as a partial and apparently functional Domain I (Tiwari et al., 2004). These are renamed here as Aux/IAA-Related Proteins (ARP). The role of these six members (ARPs) is not clear. Since these ARPs were auxin-responsive (Appendix B) and contain Domains III and IV, they also may be also involved in auxin signaling through protein-protein interactions with ARFs and/or other Aux/IAAs. Each Aux/IAA protein shows some preference in forming dimers with other Aux/IAAs and/or ARFs at least in yeast two-hybrid analyses. AUX22, for example, showed strong interactions with other Aux/IAAs, while AUX28 showed a strong preference to form homodimers in yeast-two hybrid analyses (Table 3-3 of Chapter II; unpublished data of O'Grady et al.). Interaction strength between IAA17 and itself was weaker than between IAA17 and AUX22. When AUX28 was used as the bait protein to screen interaction proteins in the yeast

two-hybrid system, only certain Aux/IAAs and ARFs were isolated repeatedly (O'Grady et al., unpublished data). Taken together, the data imply that Aux/IAA and ARF have strong preferences in forming homo- and/or heterodimers. If this proves to be universally true, the dimerization (or interaction) preference among Aux/IAAs and ARFs may be one important factor in regulation of auxin signaling and developmental regulation in addition to protein stability and tissue/organ-specific expression patterns. Severe auxin-related pleiotropic phenotypes of Domain II gain-of-function mutants of Aux/IAAs and phenotypic recovery of revertants with an additional mutation in Domain IV (and to a lesser extent Domain I) demonstrate the importance of protein-protein interaction through Domain III and IV with other Aux/IAAs, ARFs, and/or other unknown interaction proteins in auxin signaling (see Chapter 1 and Introduction section for details). Increased protein stability of Domain II mutants, protein-protein interactions with a degree of preference among Aux/IAAs and ARFs, and phenotypes of gain- and loss-of-function mutants (revertants) of Aux/IAAs, imply that one area of auxin signaling is mediated by protein-protein interactions through Domains III and IV with Aux/IAAs and/or ARFs in a concentration-dependent manner. A model is suggested for *axr3-1* whereby the more stable Aux/IAA caused by the Domain II mutation might interfere with the normal protein-protein interaction cycles through Domains III and IV with other Aux/IAAs, ARFs, and/or other proteins, resulting in down-regulating some auxin up-regulated genes (Chapter II; Tian et al., 2002). This may result in many changes in gene expression including transcription factors, enzymes involved in metabolism, and other auxin-related genes (Chapter IV; Tian et al., 2002), which may result in abnormal responses to auxin, e.g., auxin insensitivity (Figs. 3-4 and 3-5), and auxin-related abnormal phenotypes (i.e., showing severe pleiotropic phenotypes). However, the intragenic revertants (*axr3-1R4*, *axr3-1R2, and shy2-22*) negate the effect of

protein stability by not interacting through Domains III and IV with other Aux/IAAs, ARFs, and/or other proteins. This phenomenon may be applied to other Domain II gain-of-function mutants such as *shy2-2*, *msg2-1*, etc., which show various auxin-related pleiotropic phenotypes (Table 3-2).

IAA17K and *IAA19K* did not show distinct phenotypes, suggestive of redundancy among Aux/IAAs. The possible combinations of protein-protein interactions among Aux/IAAs, ARFs, and ARPs in terms of regulatory signals by forming heterodimers with other groups (e.g., Aux/IAA with ARF or ARP, etc) are 3,174 (= 23 x 23 x 6). However, the possible combinations would be 2^n (where n is the number of family members, in this case 52) since they can also form homodimers. These combinations suggest the complexity of at least one area of auxin signaling in plant growth and development. This very large number would surely be reduced many-fold based on insufficient interaction (or no interaction) and on tissue/organ-specific patterns; other factors might also reduce the redundant number of interactions. However, there are certain redundancies among Aux/IAAs and ARFs in that knockouts of some Aux/IAAs and ARFs do not show distinct phenotypes (reviewed by Liscum and Reed, 2002; this study).

The double knockout of IAA17 and IAA19 showed a synergistic effect of the two genes in reducing root size. *IAA19K* showed normal primary root length with longer root hairs at the top of root. The double knockout showed much shorter root length, longer root hairs, and a higher density of root hairs than WT, *IAA17K*, or *IAA19K* (Fig. 3-11). The higher density of root hairs resulted from the much shorter root cell length (both cortex and epidermis). The synergistic effect, however, seemed to be transient because the double knockout showed a normal (or WT and *IAA17K*) root hair pattern similar to WT after three days under light

conditions (data not shown). The root hair pattern of the double-knockout mutant was very similar to that of *eto1-1*, an ethylene over-producing mutant (Pitts et al., 1998).

After fertilization and during silique growth, the sepals, petals, and stamen must mature and dry up in order to separate from siliques. Ethylene-regulated carpel senescence (Orzaez and Garnell, 1997) and ethylene biosynthesis in the abscission zone is regulated by auxin acting as a suppressor of the ethylene effect at an optimal concentration (Taiz and Zeiger, 1998). Ethylene is a key regulator of fruit ripening (Taiz and Zeiger, 1998). One aspect of *axr3-1* is that the seed pod (carpel) never opens without external mechanical disruption. The seed pods of the loss-of-function mutant, *axr3-1R*, seem to open slightly earlier than WT (data not shown), suggesting a possible relationship of IAA17 (or Aux/IAAs) and ethylene in fruit development and maturation. 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS), which converts *S*-adenosyl-Met to ACC, is the key regulator of the rate-limiting step in ethylene biosynthesis (reviewed by Wang et al., 2002). *Eto* (ethylene-overproducing) mutants have10- to 40-fold higher levels of ethylene than WT in etiolated seedlings. The *eto1* mutation, a recessive mutation, was found to act by increasing the stability of ACS5 (Woeste et al., 1999; Chae et al., 2003). *ACS4* is an auxin primary-response gene (Abel et al., 1995a), and some ACS mRNAs were induced with cycloheximide treatment (Abel et al., 1995a; reviewed by Guilfoyle, 1998). Ethylene is also implicated in the production of root hairs, the development of which is a precise process limited to specialized epidermal cells called hair cells or trichoblasts. Exogenous ethylene or its precursor ACC can stimulate ectopic root hair formation in cells that are normally unable to produce root hairs (Pitts et al., 1998). However, *eto1-1* did not show ectopic root hair patterns suggesting that the higher density of root hairs resulted from shorter epidermal cell length similar to that of the *IAA17* and *IAA19* double-knockout mutant. The relationship between this double

mutant and *eto1-1* is not clear. *ACS4*, a primary auxin-responsive gene, contained an AuxRE in its promoter and was induced by cycloheximide treatment (Abel et al., 1995a), indicating that their transcription is repressed by a short half-life protein such as an Aux/IAA. WT IAA19 and IAA17 may negatively regulate ACS gene transcription by interacting with ARFs. Therefore, an ACS gene in the absence of IAA17 and IAA19 may have high constitutive activity, leading to a higher ethylene level in cells (tissues). This may result in an *eto1-1*-like phenotype as does the double knockout of IAA17 and IAA19 in root hair formation. Measurement of the message level of the ACS genes in the double-mutant background would be informative because of the above model and the phenotypic similarity in the root.

In general, auxin signaling by Aux/IAAs is effected by levels of Aux/IAAA proteins, by auxin-dependent degradation of Aux/IAA proteins, and by protein-protein interactions through Domains III and IV. Data presented here identify additional factors that affect auxin signaling, including the spatial and temporal specificity of Aux/IAA promoters and the potential synergism of various Aux/IAAs, ARFs, and/or putative unknown proteins (?). There are additional levels of complexity of auxin regulation including "cross-talk" between auxin and ethylene in plant process, auxin-light interactions, and auxin-cytokinin interactions to list a few of the more dominant and well studied phenomena (see Swarup et al., 2002), and it seems at this point based on mutant studies that Aux/IAAs and ARFs are centrally involved in these interactions.

Table 3-1. Relative Root Cell Length Analysis of WT, *axr3-1*, *axr3-1R4*, and *IAA17K*

	WT	*Axr3-1*	*Axr3-1R4*	*IAA17K*
Epidermal	222 ± 26	176 ± 22	171 ± 20	172 ± 22
Cortex	175 ± 21	135 ± 23	147 ± 19	138 ± 21

Cell size was measured by confocal microscopy with propidium iodide staining. At least 100 cells were measured from at least 18 different plants from each genotype.

Table 3-2. Mutant Phenotypes of Auxin-Related Genes

Aux/IAA genes					
Axr2	IAA7	DomainII (axr2-1) Dominant	Agravitropic root and normal root growth rate, reduced auxin sensitivity, more lateral roots than WT and fewer adventitious roots fewer root hairs, wavy leaves and agravitropic stem, short hypocotyl in dark and leaf form in dark	*Axr2-1r3* and *axr2-1r4* more auxin sensitive *axr2-5* (knockout) WT-like, slightly slow hypocotyl growth in ligh grown seedlings, show the same auxin sensitivity as WT	Nagpal et all. (2000)
Axr3	IAA17	Domain II (axr3-1) Dominant	Extreme agravitropic and short roots, very reduced auxin sensitivity, more adventitious roots, fewer root hairs, upcurled leaves, short hypocotyls in dark, formation of leaves in dark	*Axr3-1R* slightly short root	Leyser et al. (1996), Rouse et al. (1998)
Short Hypocotyl 2 (SHY2)	IAA3	Shy2-2; Domain II Dominant	Slight agravitropic root, reduced growth in roots, normal response to auxin in root, fewer lateral and adventitious root, normal root hairs, upcurled leaves, short hypocotyls and formation of leaves in dark	*Shy2-24* (truncated before Domain II): shorter root length than shy2-2, more lateral roots than WT and shy2-2, WT-like adventitious roots and seedling, Shy2-22 (change in domain IV): shy2-22-like and WT like, both revertants shows slightly slow gravity response	Tian and Reed (1999)
Bodenlos	IAA12	Dominant	Primary root meristem defects; post-embryonic roots are normal, upcurled leaves and reduced apical dominance, reduced auxin sensitive root, normal root hairs and lateral root, short inflorescence stem		Hamann et al. (1999)
SLR1	IAA14	Dominant	Fewer root hairs (normal root hair junction between root and hypocotyl), slight gravitropic response, no lateral roots, strong apical dominance, small leaves, short and thin inflorescence stem, reduced auxin sensitivity	*Slr-1r1* additional mutation within Domain I (D --> N)- fewer lateral roots	Fukaki et al. (2002)
MSG2	IAA19	Dominant	*Msg2-1* hypocotyls lost gravitropism completely. msg2 showed weaker phototropism and weaker hook formation in hypocotyls, hypocotyls were resistant to 2,4-D		Tatematsu et al. (1999)
Iaa28-1	IAA28	Dominant	Few lateral roots, fewer root hairs, less apical dominance, slight auxin insensitive root	IAA28 is preferentially expressed in roots and inflorescence stem	Roogs and Bartel (2001)
Auxin Response Factors (ARFs)					
Monopteros (MP)	ARF5	Recessive	Formation of vascular system in embryo, lack of provascular cells within a basal domain resulting in short (or lacking) hypocotyls and primary root; root meristem not formed in embryo (normal if initiated), Very few flowers, disconnected vascular strand, defective cotyledons; WT gene - mediating embryo axis formation and vascular development and promote cell axialization through development	No null alleles, stronger alleles in the middle of protein containing helix-loop-helix, two alleles located C-terminal region (domain III & IV) show the most weak alleles	Hardtke and Berleth (1998)
Ettin (ETT)	ARF3- lack of Domains III & IV	Recessive	Flowering mutant (vegetative growth looks normal) : more sepals and petals, fewer stamens and abnormal carpels, Trumpet-shape, reduced valve; WT gene function seems to establish boundaries for proper patterning in flower	Most strong allele: T-DNA insertion in exon 2 (null)	Session et al. (1997)
Non-phototropic hypocotyl 4 (NPH4), BIPOSTO	ARF7	Recessive	Altered phototropic response, altered stem gravitropism, phytochrome-dependent stem curvature, apical hook maintenance, and abaxial/adxial leaf blade expansion	The most strong allele: N-terminal truncated protein of after DNA binding domain, C-terminal truncated alleles shows less severe phenotypes	Harper et al. (2000)

Other Auxin-Responsive Mutants					
Far-rad-insensitive219 (FIN219)	GH3 like	Slight semi-dominant	A downstream regulator of COP1(isolated by suppressor screening), cytoplasmic, long hypocotyls under continuous far-red light,	GH3-like protein: 64KD, 47% identity and 66% similarity	Hsieh et al (2000),
Dwarf in light 1 (DFL1-D)	GH3 like	Dominant mutant	Has a shorter hypocotyl under blue, red and far-red light, but not in darkness. Inhibition of cell elongation in shoots caused an exaggerated dwarf phenotype in the adult plant. The lateral root growth was inhibited without any reduction of primary root length. The dfl1-D phenotype was confirmed by over-expression of the gene in the wild-type plant.	The dfl1-D showed resistance to exogenous auxin treatment. Moreover, over-expression of antisense DFL1 resulted in larger shoots and an increase in the number of lateral roots. These results indicate that the gene product of DFL1 is involved in auxin signal transduction, and inhibits shoot and hypocotyl cell elongation and lateral root cell differentiation in light.	Nakazawa et al. (2001)
Genes Involved in Auxin Transport					
Auxin resistant (AUX1)			Aerial portions of plant similar in appearance to wild type; slight increase in root elongation and altered geotropic response; resistant to ethylene and auxin, Auxin influx carrier in root	Auxin resistant4 (similar to aux1-7); narrow, irregular rosette leaves, slightly curled around leaf axis; roots elongate on auxin-containing medium; defective root gravitropism; reduced number of lateral roots - greater reduction than for either single mutant; dwarf, bushy plants; reduced plant height; ethylene resistant; reduced fertility	Hobbie and Estelle (1995) Bennet et al. (1996) Marchant et al. (1999)
Agrovitropic (Agr)	Wavy6, ethylene-insensitive root1 (eir1), At-pin2		69 KD, 10 transmembrane domain: function as auxin efflux carrier		Luschnig et al (1998), Muller et al.(1998) Chen et al. (1998)
Pinformed 1 (AtPin1)			Pin-formed and naked inflorescence with no or defective flowers (no cauline leaves): efflux carrier in stem, contains TM domain, about 40% homology with agr1		Gälweiler et al. (1998)
Gene Involved in Auxin Homeostasis					
Superroot, Rooty, Hookless3	Similar to aminotransfer-ase	Recessive	Elevated level of free IAA (1.5 times to 3.7), along with an increase in bound IAA		Boerjan et al. (1995)
Others Including Auxin Signal Transduction					
Axr1	N-terminal half of E1 ubiqitination enzyme in Yeast	Recessive	Irregular rosette leaves, tend to curl upward; short petioles; slightly reduced plant height; increased number of lateral branches, reduced fertility		Lincoln et al. (1990)
Auxin transport inhibitor resistant (TIR1)	Encodes a protein that contains an F-box domain and leucine-	Semi-dominant mutation	Resistant to auxin inhibition of root elongation; deficient in auxin-regulated growth processes including: reduced lateral root formation, reduced temperature -induced hypocotyl elongation, and a modest reduction in apical dominance	Tir1 gene may involve protein ubiquitin-associate process	Ruegger et al. (1998)

	rich repeats				
Root curl in NPA (Rcn 1)	Phosphatase IIA -∝ subunit		Reduced root elongation, reduced hypocotyls elongation, defective hypocotyls hook formation, NPA sensitive		Garbers et al. (1996)
Auxin transport inhibitor resistant 3 (TIR3)			Reduced elongation of inflorescences, roots, pedicels, and siliques, decreased apical dominance, great reduction in lateral root formation, both auxin binding activity and NPA biding activity were dramatically reduced		Ruegger et al (1997)
Pinoid (Pid)	Ser-thr-protein kinase	recessive	A pin-like inflorescence,		Christensen et al. (2000)

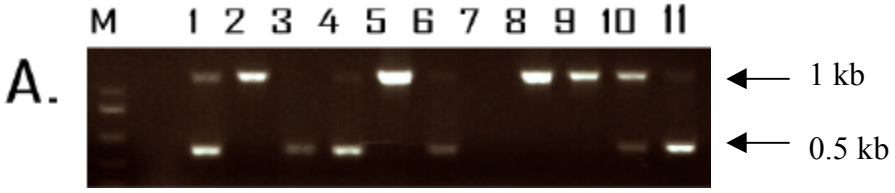
Table 3-3. Summary of Expression Patterns of Auxin-responsive Genes

SAUR-AC1	Root tip, stem, midvein, elongation zone of hypocotyl from etiolated seedling, stem has highest GUS staining pattern (Gil et al., 1997)
Ps-IAA4/5 *Ps-IAA6*	Both promoter activities showed in root meristem, lateral root inintiation, rapid elongation zone of hypocotyls of tobaco. Ps-IAA4/5 especically expressed root vascular tissue, guard cell, apical hook Ps-IAA6 - glandular trichomes, gravitropic stimulation zones. (Wong et al., 1996)
AtAux2-11	Root tip, lateral root inintiation zone, elongation zone of root, elongation side of hypocotyls undergoing gravitropic curvature, etiolated hypocotyl, and anther filaments, lignification tissue (Xylem, Trichomes) (Wyatt et al., 1993)
IAA8	Vascular cells in the root tip far in advance of cell differentiation; however, the signal fade out to older portion of root. Young leaf showed staining on vasculatures, but older mature leaf did not show the staining (Groover et al., 2003)
IAA7/axr2	Mainly elongation zone of root, below shoot apical meristem, lateral root initiation (tip) (Tian et al., 2002)
IAA3/shy2	Mainly hypocotyl and cotyledon (no root signal, however, in Northern very low signal detected in root) (Tian et al., 2002; Hamann et al., 2002)
IAA14/slr	Root elongation zone, vascular tissue of primary root, lateral root staining observed especially in dividing cells (Fukaki et al., 2002)
IAA28	Northern analysis- high in root, significant in inflorescence stem, minor in leaves, siliques, and flower. GUS staining extended from elongation zone to the root-hypocotyl junction (no staining in root and lateral root primordia) (Rogg et al., 2001)
IAA12/bdl	Expressed on early embryogenesis, root and shoot part also showed GUS staining (strong at the junction of root and hypocotyl and root tip) (Hamann et al., 2002)

Figure 3-1. Examples of T-DNA Screening and Structure of T-DNA Insertion.

A. Example of IAA17 knockout screening. DNA was extracted and then amplified by touch-down PCR with three primers (two gene-specific primers and one LB primer from T-DNA). M, marker. Upper band is generated from IAA17 primer and T-DNA LB primer, and bottom band is generated from IAA17 primers.

B. The structure of T-DNA insertion from IAA17 and IAA19 allele (not exact scale) Garlic_1233_C09 line from Syngenta for IAA17. Salk_000337 line from Arabidopsis Biological Resource Center for IAA19. Arrows indicate translational start or stop positions and triangles indicate T-DNA insertion positions on IAA17 and IAA19 gene, respectively. Blocks represent exons and lines represent introns.


M 1 2 3 4 5 6 7 8 9 10 11
A.
1 kb
0.5 kb

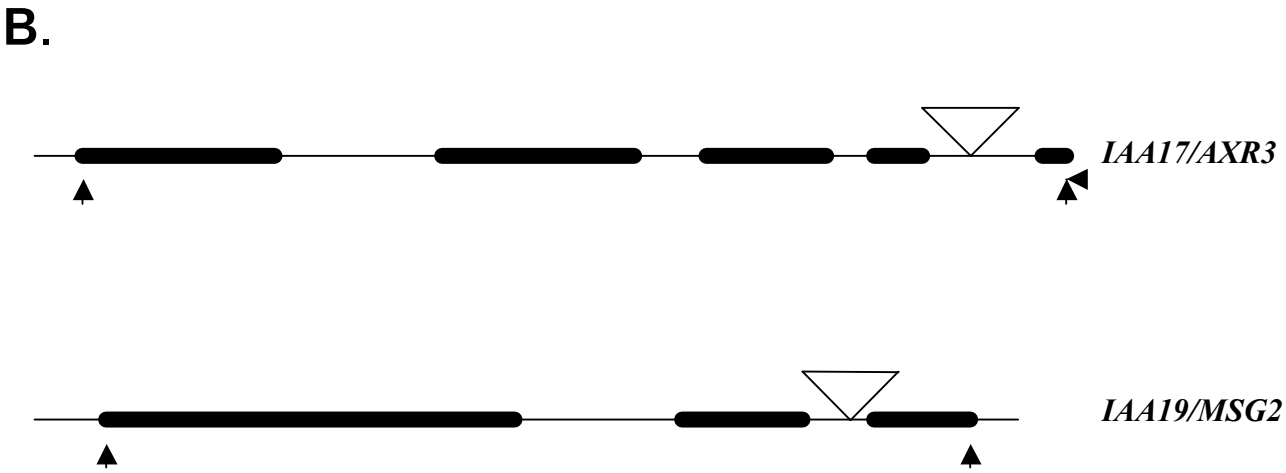

B.
IAA17/AXR3
IAA19/MSG2

Figure 3-2. Sourthern Analysis of *IAA17* and *IAA19* Knockouts.

Lane 1, WT DNA digested with ApaL I. Lane 2, *IAA17K* genomic DNA digested with ApaL I. Lane 3, *IAA17K* genomic DNA digested with Hpa I. Lane 4, *LPLK* genomic DNA digested ApaL I. Lane 5, *LPLK* digested with Hpa I. Lane 6, WT DNA digested with Hpa I. Lane 7, *IAA19K* genomic DNA digested with ApaL I. Lane 8, *IAA19K* genomic DNA digested with Hpa I. *LPLK* and *IAA17K* were obtained from Syngenta, so that the two knockouts generated common band from Hpa I digest (▶) when hybridized with T-DNA LB fragment. Five µg of total genomic DNA was loaded, and the blot was hybridized overnight with T-DNA LB region, and then exposured to X-ray film.

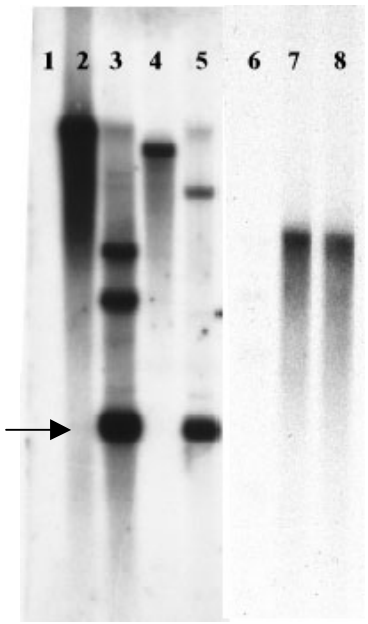

1 2 3 4 5 6 7 8

Figure 3-3. Northern Analysis of *IAA17* and *IAA19* Transcripts.

Five day-old plants were harvested for Northern analysis. Lane 1, WT green plant; Lane 2, WT green with 2hr IAA treatment; Lane 3, Etiolated WT plant; Lane 4, Etiolated knockouts of *IAA17K* and *IAA19K*

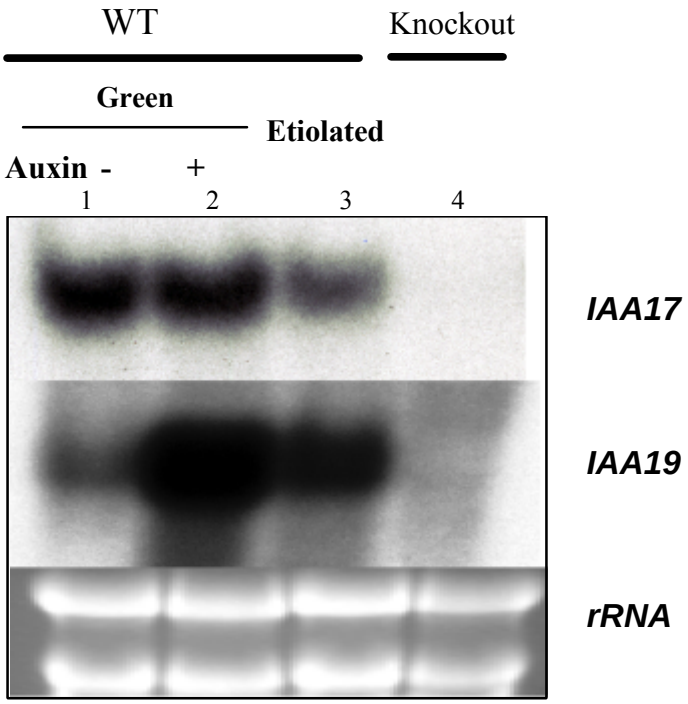

WT
Knockout
Green
Etiolated
Auxin -
+
1
2
3
4
IAA17
IAA19
rRNA

Figure 3-4. Relative Root Growth Inhibition Ratio by NAA Concentrations from Four Different Genotypes.

Plants were germinated for 2 days and then transferred onto 0.5X MS/0.8% agar plates containing 0.2% sucrose and the indicated concentrations of NAA. Plants were grown vertically with a ligh:dark (18hr:6hr). After 4 days plants were photographed, and then the root lengths were measured with Scion Image (HIH, MD) software. Error bars represent standard deviation. At least 35 plants from each genotype were measured.

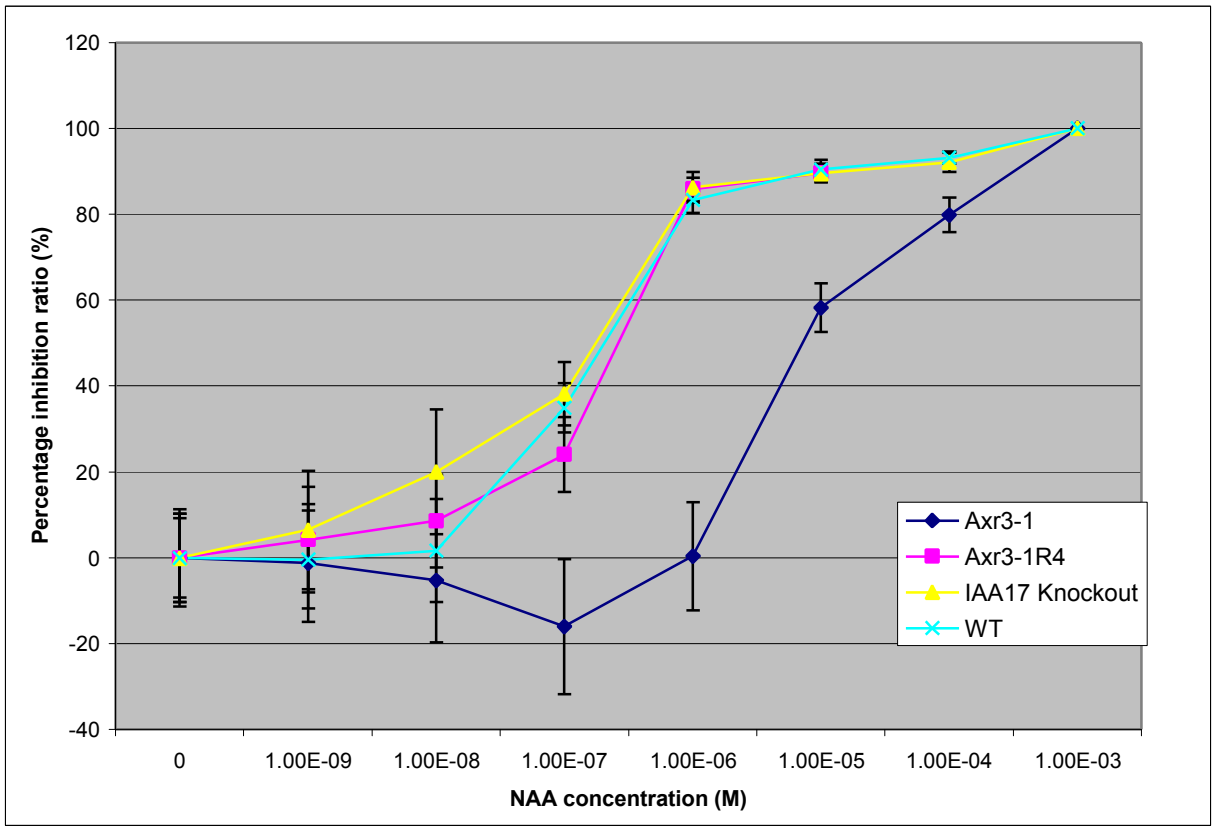
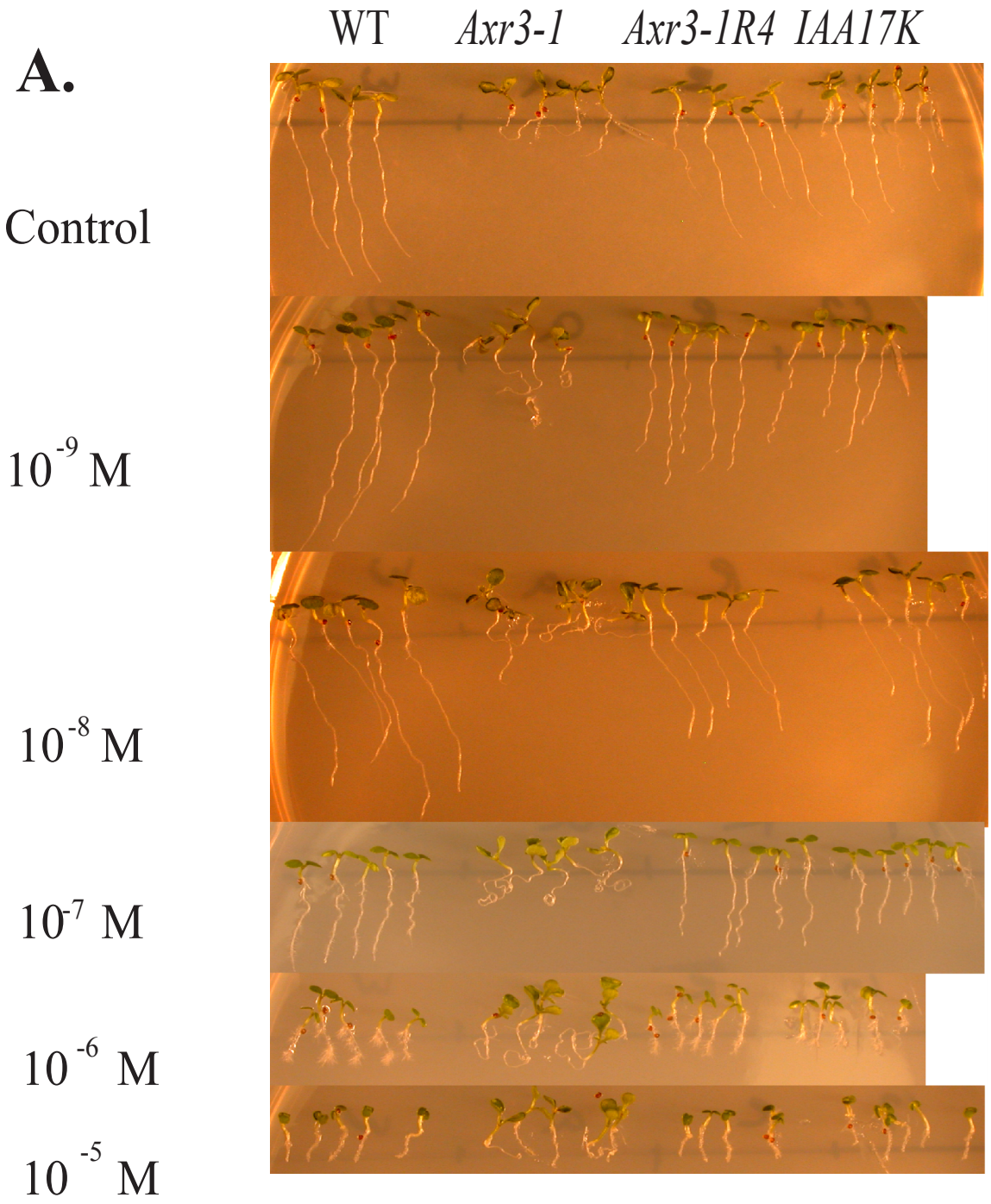
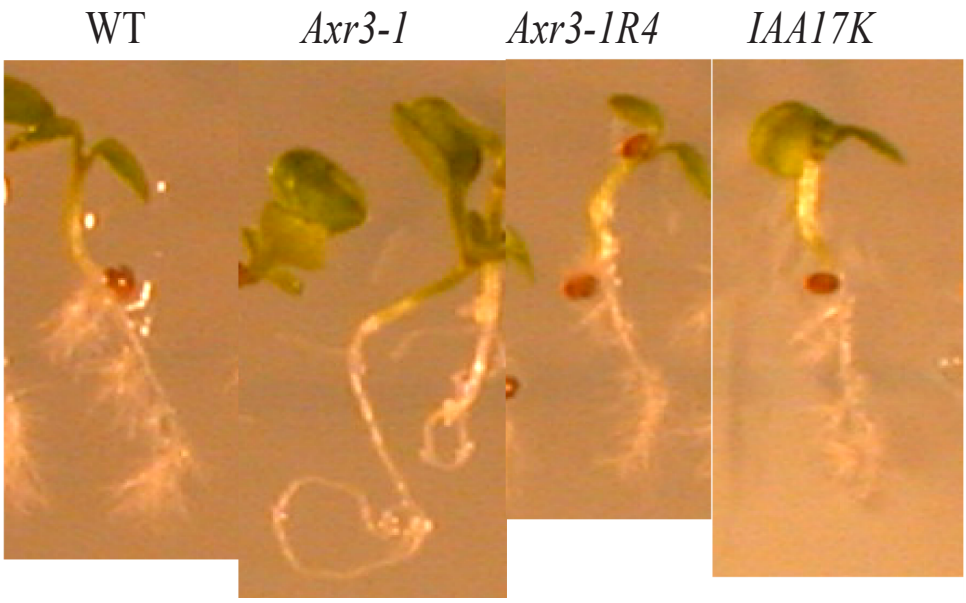

120
100
80
60
40
20
0
-20
-40
Percentage inhibition ratio (%)
0
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
1.00E-04
1.00E-03
NAA concentration (M)
Axr3-1
Axr3-1R4
IAA17 Knockout
WT

Figure 3-5. Relative Root Length of WT and Other Mutant Genotypes at Various NAA Concentrations.

A, a photograph of actual data; B, the magnification of plants grown on the 10^{-6} M NAA; C, measurement of plant root lengths on various NAA concentrations. Plants were germinated for two days and then transferred into 0.5X MS/0.8% agar plate containing 0.2% sucrose and various NAA concentrations. Plants were grown vertically with an 18hr light:6hr dark cycle. After four days, plants were photographed, and then the root lengths were measured with Scon Image (HIH, MD) software. Error bar represents standard deviation. At least 35 plants from each genotype were measured.


A.
WT
Axr3-1
Axr3-1R4
IAA17K
Control
10-9 M
10-8 M
10-7 M
10-6 M
10-5 M

B.


WT
Axr3-1
Axr3-1R4
IAA17K

C.

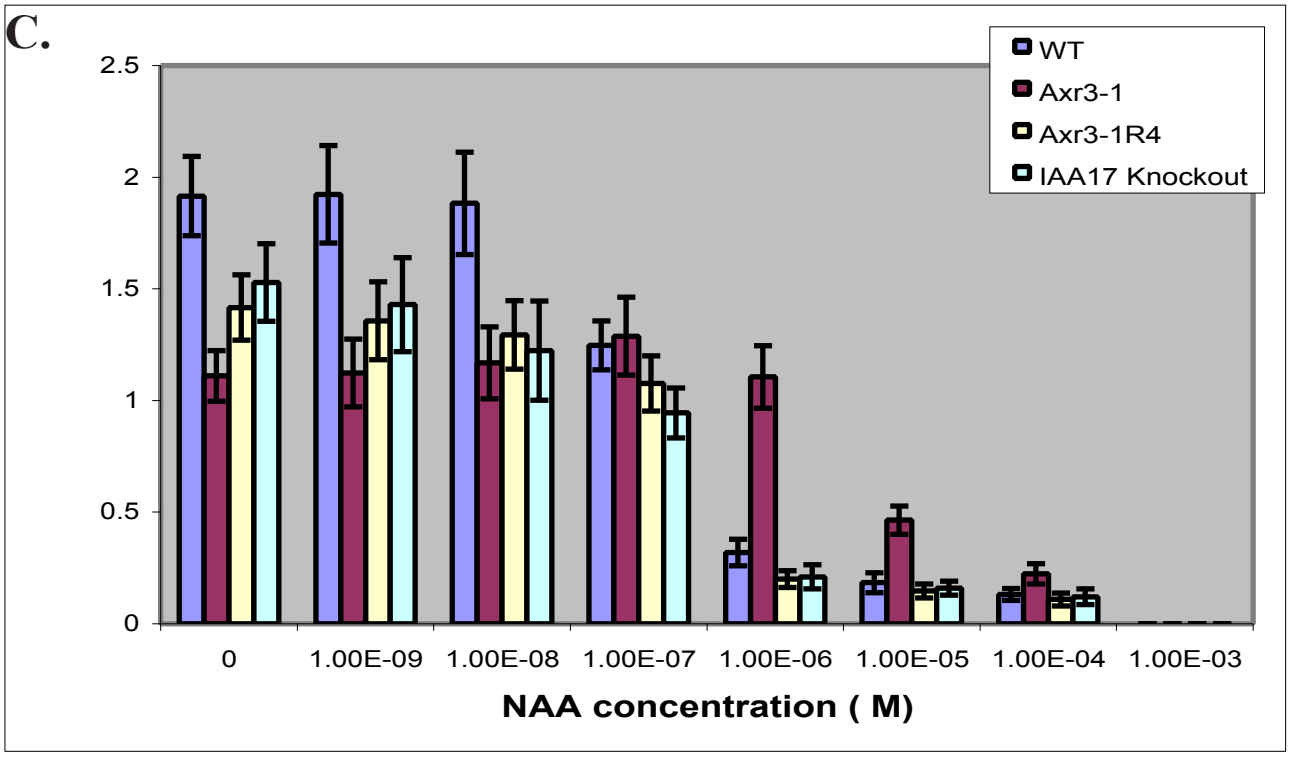

WT
Axr3-1
Axr3-1R4
IAA17 Knockout
2.5
2
1.5
1
0.5
0
0
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
1.00E-04
1.00E-03
NAA concentration (M)

Figure 3-6. IAA17 Expression Patterns of Various Ages of the Transgenic Plants

Plants were stained in the GUS staining solution for 2 to 3 hrs. A to G, *pIAA17*::*GUS* transgenic plant; H and I, *DR5*::*GUS* transgenic plants. *DR5* contans a synthetic auxin-responsive element. A, GUS staining in root especially where root is bent; B, Rosette leaves, *IAA17* expressed strongly only on the main midvein in mature leaves. C, Cotylendons, *IAA17* expressed on the margin of cotyledon, but not in the veins. D, IAA17 is not expressed in root hair. E, Whole seedling of a transgenic plant, staining occurs below the shoot apical meristem, the margin of cotyledons, and root. F and G, Emerging lateral roots, staining occurs in late elongation-mature zone, but not in root tip (root apical meristem). Primary root also shows the same pattern (A). H and I, *DR5*::*GUS* highly expressed in root tip and lateral root tip region as well as in putative lateral root inintiation zone.

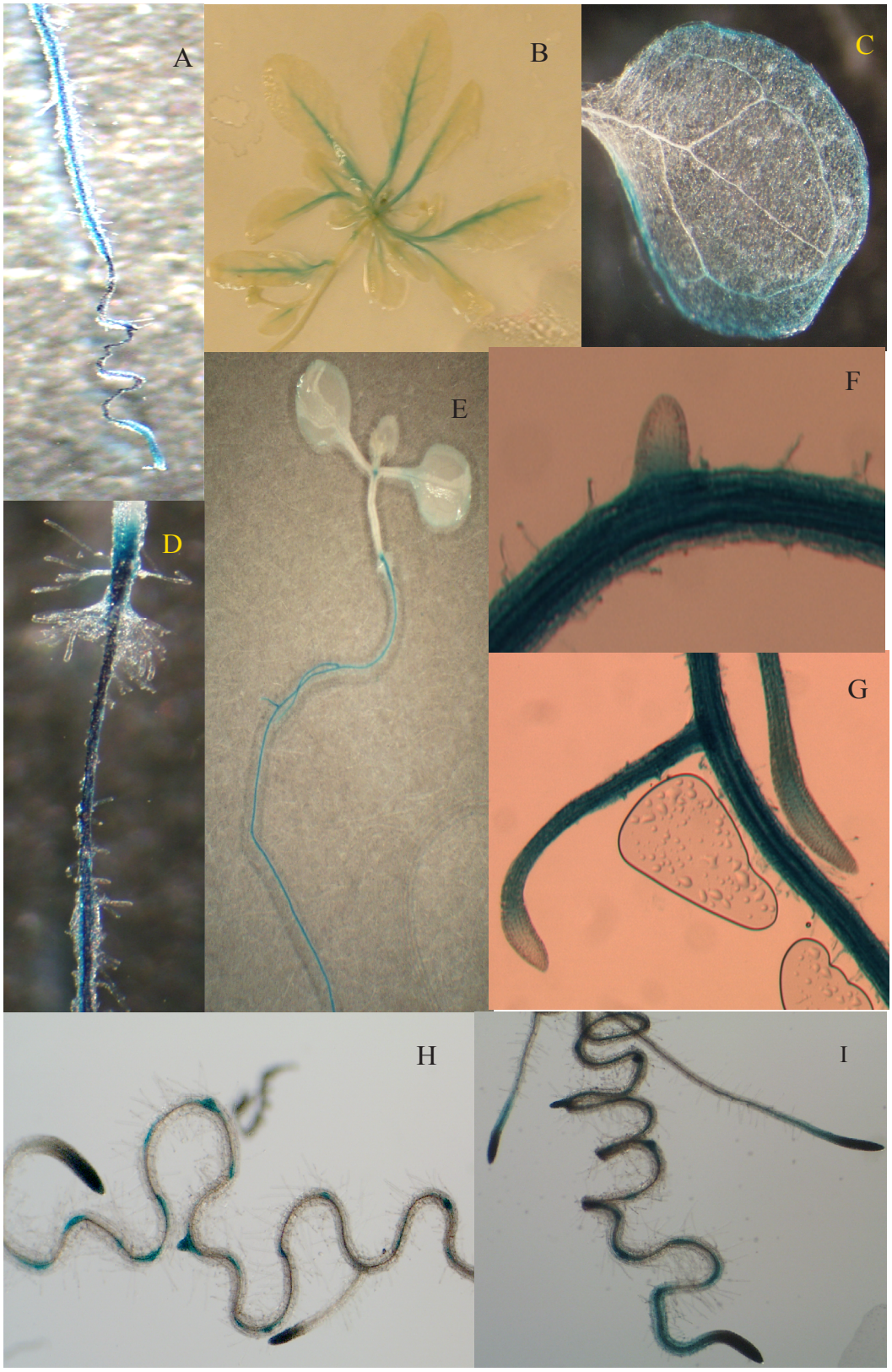

A
B
C
D
E
F
G
H
I

Figure 3-7. GUS Staining Pattern of *pIAA17*::*GUS*

Transgenic *pIAA17*::*GUS* plants were stained with the GUS staining solution for 3 hours and then color-bleached for 3 days with changing 3 to 4 times with 75% ethanol. A to G, Expression pattern of IAA17 gene on various floral parts; H, IAA17 expression pattern on the shoot part; I, IAA17 is expressed just below of shoot apical meristem. Stain can be seen in cauline leaves, flower (sepal and base of flower), and siliques. Only the cut area of the stem stains because the staining solution cannot penetrate the cutin layer of stem.

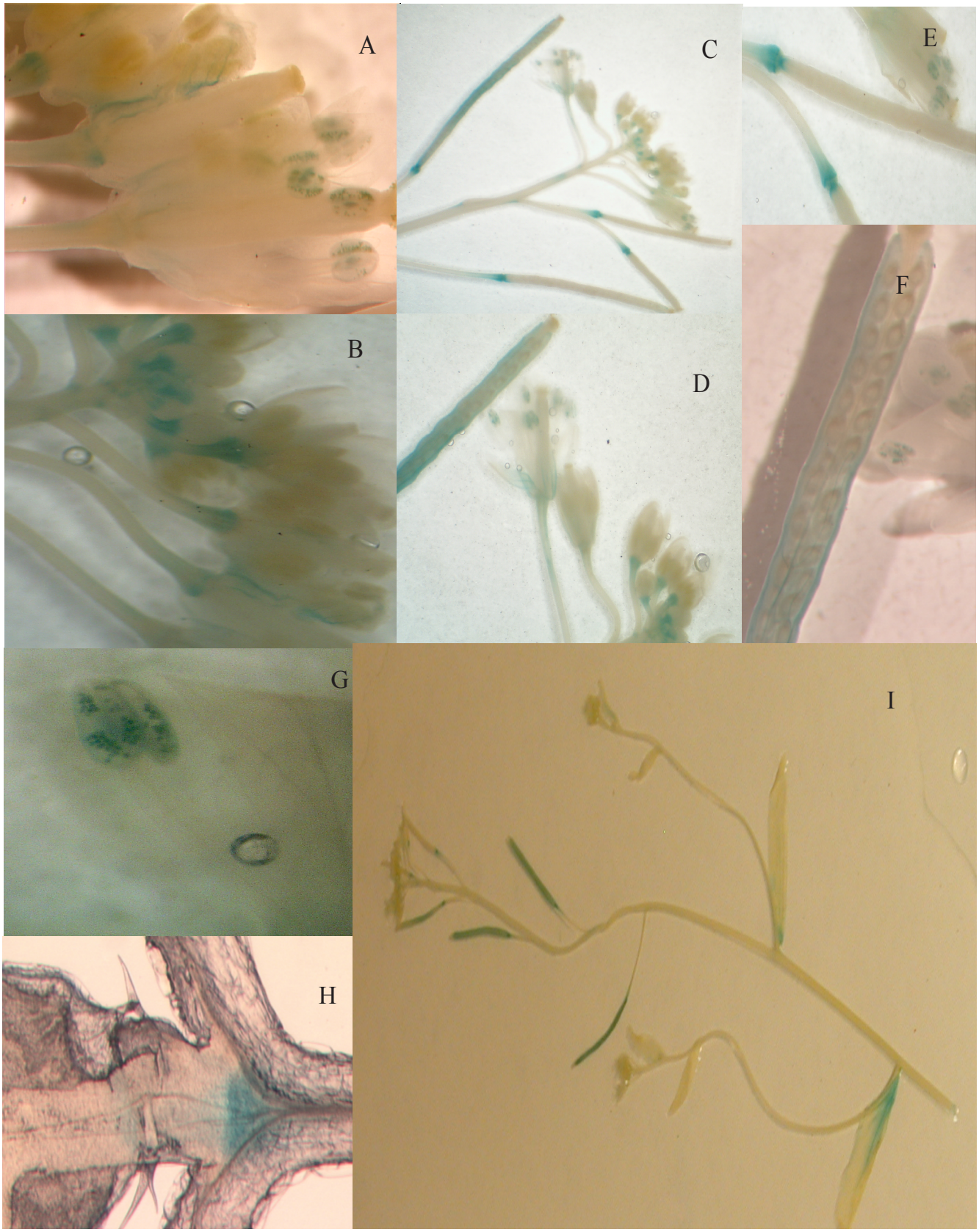

A
C
E
B
D
F
G
I
H

Figure 3-8. Northern Analysis of *IAA17* and *IAA19* Transcripts on Various Plant Parts from WT

Lane 1, Root; Lane 2, Shoot (upper part, 6 day-old); Lane 3, Whole seedling from 7 day-old; Lane 4, Young leaves from 12 day-old plant; Lane 5, Leaves from 16 day-old plant; Lane 6, Expanded and mature leaves; Lane 7, Base part of hypocotyl and primary inflorescence stem junction with axilary bud (contains very
small bud); Lane 8, Primary inflorescence stem; Lane 9, Cauline leaves; Lane 10, Floral buds from secondary inflorescences; Lane 11, Floral bud and flower from primary inflorescence; Lane 12, 5 day-old etiolated whole seedlings; Lane 13, 5 day-old etiolated whole seedling with 2 hr IAA treatment. Fifteen micrograms of total RNA was separated by formaldehyde gel electrophoresis. Blot was hybridized with full lengh IAA17 and IAA19 unique coding region overnight and then exposed to X-ray film**.**

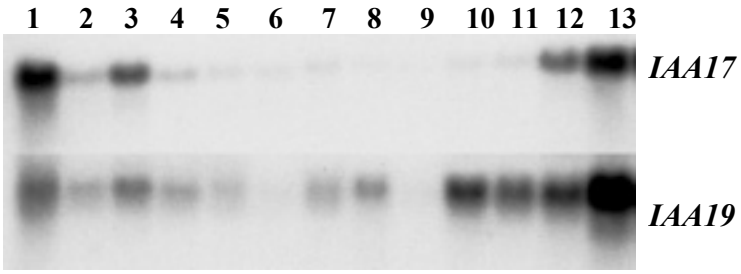

1 2 3 4 5 6 7 8 9 10 11 12 13
IAA17
IAA19

Figure 3-9. Root Cell Size from WT, *axr3-1*, *axr3-1R4*, and *IAA17K*.

Top, SEM images; Middle and Bottom, Confocal images
Four day-old plants from each genotype were used for SEM and confocal microscopy. *Axr3-1* root shows twisted patterns with lack of root hairs (SEM images). Nucleus can be seen as a white dot on confocal images. Plants were grown on vertical plate (0.5X MS/0.2% sucross) for four days in light condition (16 hrs light:8 hrs dark) and harvested. Confocal images were taken after propidium iodide staining.

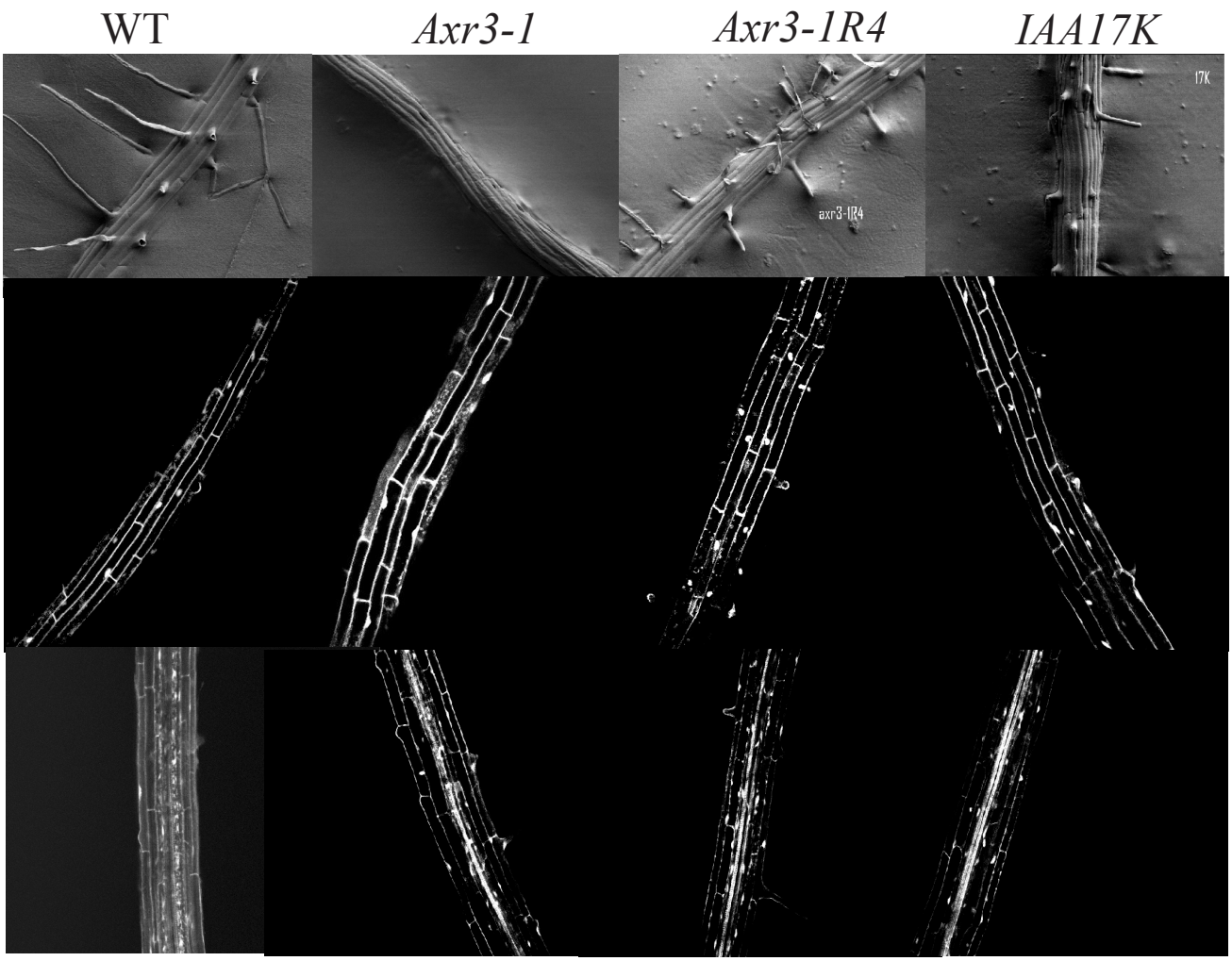

WT
Axr3-1
Axr3-1R4
IAA17K
axr3-1R4
17K

Figure 3-10. Phenotypes of Four Day-Old from WT, *Axr3-1*, *Axr3-1R4*, *IAA17K*, *IAA19K*, and Double Mutant of *IAA17K* and *IAA19K*.

The double knockout showed shorter roots compared to other plants

WT ***Axr3-1*** ***Axr3-1R4*** ***IAA17K*** ***IAA19K*** ***IAA17K/IAA19K***

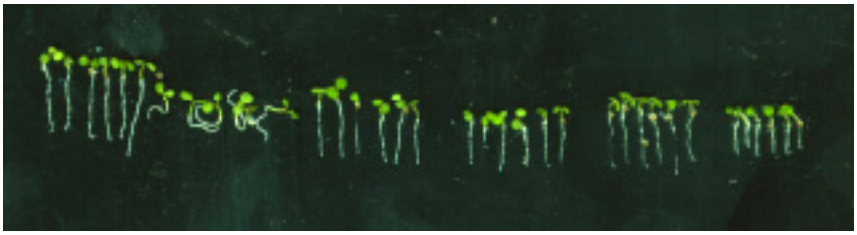
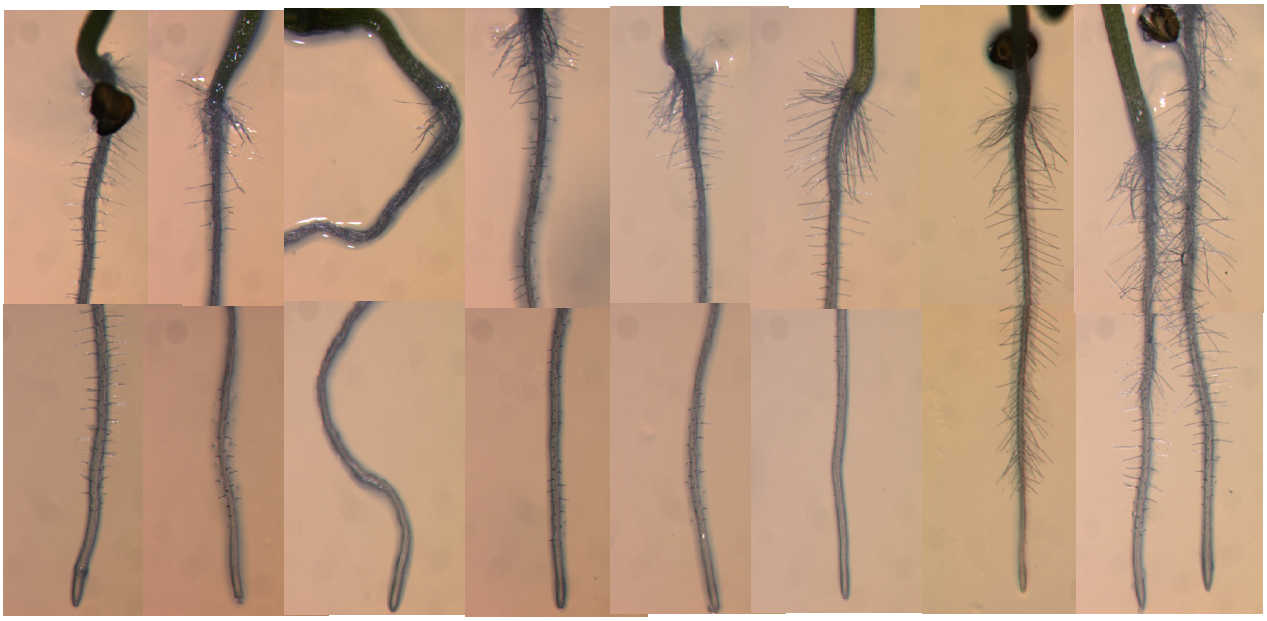


Figure 3-11. Phenotypes of Various Mutants.

A, Root hair patterns of WT, *axr3-1*, *axr3-1R4*, *IAA17K*, *IAA19K*, and Double mutant of *IAA17K/IAA19K*.

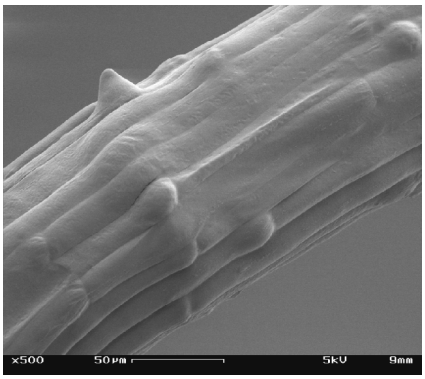
B, SEM of *axr3-1* root hair, showing root hair initiation, but no elongation; C, Confocal images of WT and Double mutant of *IAA17K* and *IAA19K*. Plants were grown on a vertial plate (0.5X MS/0.2% sucrose) for four days in light condition (16 hrs light:8hrs dark) and harvested. Confocal images were taken after propidium iodide staining.

A

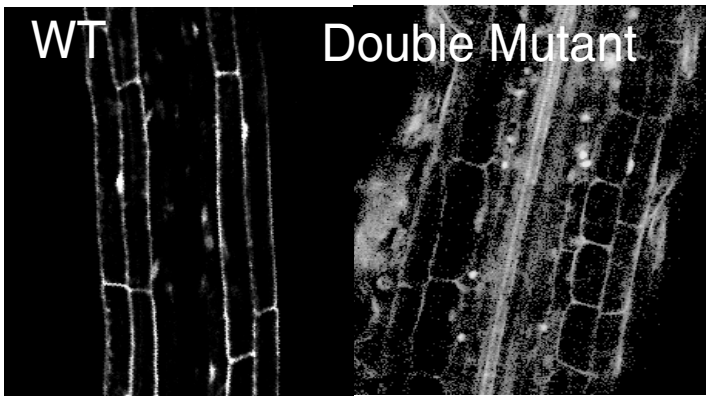


WT *Axr3-1* *Axr3-1R4* *IAA17K* *IAA19K* *IAA17/IAA19 Double Knockout*

B



C



References

Abel S, **Nguyen D**, **Chow W**, **Theologis A** (1995a). *ASC4*, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in *Arabidopsis thalian*: structural characterization, expression in Escherichia coli, and expression charactreristics in response to auxin. J Biol Chem **270:**19093-19099

Abel S, Nguyen MD, Theologis A (1995b). The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. J. Mol. Biol. 251, 533-549

Abel S, Theologis A (1996). Early genes and auxin action. Plant Physiol. **111**, 9-17

Baulcombe D, Giorgini J, Key JL (1980). The effect of auxin on the polyadenylated RNA of soybean hypocotyls *In* Nato Advanced Studies Institute Published in Genome Organization and Expression in Plants edited by CJ Leaver Plenum Press, pp 175-185

Baulcombe DC, Key JL (1980). Polyadenylated RNA sequences which are reduced in concentration following auxin treatment of soybean hypocotyls. J. Biol. Chem. 255:8907-8913

Bennett M J, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B Feldmann KA (1996). *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. Science **273**:948-950

Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Onckelen HV, Montagu MV, Inzé D (1995). *Superroot*, a recessive mutation in *Arabidopsis*, confers auxin overproduction. Plant Cell **7**:1405-1419.

Cao X, Linstead P, Berger F, Kieber J, Dolan L (1999). Differential ethylene sensitivity of epidermal cells is involved in the establishment of cell pattern in the *Arabidopsis* root. Physiologia Plantarum **106**:311-317

Carland FM, McHale NA (1996). *LOP1* : a gene involved in auxin transport and vascular patterning in *Arabidopsis*. Development **122:**1811-1819

Cernac A, Lincoln C, Lammer D, Estelle M (1997). The *SAR1* gene of *Arabidopsis* acts downstream of the *AXR1* auxin response. Development **124:**1583-1591

Chae HS, Faure F, Kieber J (2003). The eto1, eto2, and eto3 mutations and cytokitin treatment increase ethylene biocynythesis in Arabidopsis by increasing the stability of ACS protein. Plant Cell **15**: 545-559

Chen J, Ullah H, Young JC, Sussman MR, Jones M (2001). ABP1 is required for organized cell elongation and division in *Arabidopsis* embryogenesis. Genes Dev. **15**:902 –911

Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH (1998). The Arabidopsis thaliana AGRAVITROPIC1 gene encodes a component of the polar auxin-transport efflux carrier. Proc. Natl. Acad. Sci. USA **95**:15112-15117

Christensen S, Dagenais N, Chory J, Weigel D (2000). Regulation of auxin response by the protein kinase PINOID. Cell **100**: 469-478

Clough SJ, Bent AF (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J. **16:**735 –743

DeLong A, Mockaitis K, Christen S (2002). Protein phosphorylation in the delivery of and response to auxin signal. Plant Mol. Biol. **49**:285-303

Fukaki , Tameda S, Masuda H, Tasaka M (2002). Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsi*s. Plant J. **29**:153-168

Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov K, Palme K (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science **282**:2226-2230

Garbers C, DeLong A, Deruere J, Bernasconi P, Soll D (1996). A mutation protein phosphatase 2A regulatory subunit A effects auxin transport in Arabidopsis. EMBO J. **15**:2115-2124

Gil P, Green P (1997). Regulatory activity exerted by the SAUR-AC1 promoter region in transgenic plants. Plant Mol. Biol. **34 :**803-808

Gray WM, Estelle M (2000). Function of the ubiquitin-proteasome pathway in auxin response. Trends Biochem. Sci. **25:**133-138

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001). Auxin regulates SCF^{TIR1}-dependent degradation of Aux/IAA proteins. Nature **414:**271 -276

Groover AT, Pattishall A, Jones AM (2003). IAA8 expression during vascular cell differentiation. Plant Mol. Biol. **51**: 427-435

Guilforyle T (1999). Auxin-regulated genes and promoters. *In* Biochemistry and Molecular Biology of Plant Hormones edited by Hooykaas P, Hall M, and Libbenga K. Elsevier Science B.V. pp423-459.

Hellmann H, Hobbie L, Chapman A, Dharmasiri S, Dharmasiri N, del Pozo C, Reinhardt D, Estelle M (2003). Arabidopsis AXR6 encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis**.** EMBO J. **22**:3314-3325

Hamann T, Benkova E, Baurle I, Kientz M, Jurgens G (2002). The *Arabidopsis* BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS –mediated embryo patterning. Genes Dev. **16:**1610 -1615

Hardtke CS, Berleth T (1998). The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J. **17:**1405-1411

Harper RM, Stowe-Evans EL, Luesse DR, Muto H, Tatematsu K, Watahiki MK, Yamamoto K, Liscum E (2000). The NPH4 locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial Arabidopsis tissue. Plant Cell **12:** 757–770

Hellmann H, Hobbie L, Chapman A, Dharmasiri S, Dharmasiri N, del Pozo C, Reinhardt D, Estelle M (2003). Arabidopsis AXR6 encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis. EMBO J. **22**: 3314-3325

Hsieh HL, Okamoto H, Wang M, Ang LH, Matsui M, Goodman H, Deng XW (2000). FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development. Genes Dev. **14**:1958-1970

Kepinski S, Leyser O (2002). Ubiquitination and auxin signaling: a degrading story. Plant Cell **14:**S81 - S95

Kim BC, Soh MS, Kang BJ, Furuya M, Nam HG (1996). Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2*. Plant J. **9:**441-456

Kim J, Harter K, Theologis A (1997). Protein-protein interactions among the Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **94**:11786-11791

Leyser O, Pickett FB, Dharmasiri S, Estelle M (1996). Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. Plant J. **10**: 403-413

Lincoln C, Britton JH, Estelle M (1990). Growth and development of the axr1 mutants of Arabidopsis. Plant Cell **2:**1071–1080

Liscum M, Reed J (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol. Biol. **49**:387-400

Luschnig C, Gaxiola R, Grisafi P, Fink G (1998). EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in Arabidopsis thaliana. Genes Dev **12**: 2175-2187

Marchant A., J Kargul, ST May, P Muller, A Delbarre, C Perrot-Rechenmann, MJ Bennett (1999). AUX1 regulates root gravitropism in Arabidopsis by facilitating uptake within root apical tissues. EMBO J. **18**: 2066-2073

Martin T, Wohner R, Hummel S, Willmitzer L, Frommer W (1992). The GUS reporter system as a tool to study plant gene expression. *In* GUS Protocol:Using the GUS Gene as a Reporter of Gene Expression Academic Press, Inc. pp 23-43

Muller A, Guan C, Galweiler L, Tanzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K (1998). *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. EMBO J. **17**:6903-6911

Nagpal P, Walker LM, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW (2000). *AXR2* encodes a member of the Aux/IAA protein family. Plant Physiol. **123**:563-574

Nakazawa M, Yabe N, Ichikawa T, Yamamoto YY, Yoshizumi T, Hasunuma K, Matsui M (2001). DFL1, an auxin-responsive GH3 gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. Plant J. **25** :213-221

Orzaez D, Granell A (1997). DNA fragmentation is regulated by ethylene during carpel senescence in *Pisum sativum*. Plant J. **11**:137-144

Ouellet F, Overvoorde PJ, Theologis A (2001). IAA17/AXR3: Biochemical insight into an auxin mutant phenotype. Plant Cell **13:**829-842

Pitts RJ, Cernac A, Estelle M (1998). Auxin and ethylene promote root hair elongation in *Arabidopsis*. Plant J. **16:**553 -560

Ramos JA, Zenser N, Leyser O, Callis J (2001). Rapid degradation of Auxin/Indoleacetic Acid proteins requires conserved amino acids of Domain II and is proteasome dependent. Plant Cell **13:**2349 -2360

Reed JW, Elumalai RP, Chory J (1998). Suppressors of an *Arabidopsis thaliana phyB* mutation identify genes that control light signalling and hypocotyl elongation. Genetics **148:**1295 –1310

Reed JW (2001). Roles and activities of Aux/IAA proteins in Arabidopsis. Trends Plant Sci. **6 :**420–425

Rogg LE, Lasswell J, Bartel B (2001). A gain-of-function mutation in *IAA28* suppresses lateral root development. Plant Cell **13**:465-480

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O (1998). Changes in auxin response from mutations in an *AUX/IAA* gene. Science **279**:1371-1373

Ruegger M, Dewey E, Hobbie L, Brown D, Bernasconi P, Turner J, Muday G, Estelle M (1997). Reduced naphthylphthalamic acid binding in the *tir3* mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. Plant Cell **9**:745-757

Ruegger M, Dewey E, Gray W, Hobbie L, Turner J, Estelle M (1998). The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grr1p. Genes Dev. **12**:198-207

Sambrook J, Fritsch EF, Maniatis T (1992). Molecular cloning: a laboratory manual, second edition Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sessions A, Nemhauser JL, McColl A, Roe JL, Feldmann KA, Zambryski PC (1997). *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. Development **124**: 4481-4491

Taiz L, Zeiger (1998). Ethylene *In* Plant Physiology, Sinauer Associates, Inc. Sunderland, Massachusetts, pp 651 - 670

Tatematsu K, Watahiki K, Yamamoto K (1999). Evidences for a dominant mutation of IAA19 that disrupts hypocotyl growth curvature responses and alters auxin sensitivity. *In* 10th International Conference on Arabidopsis Research (Melbourne, Australia). Abstract No. 8-39

Tian Q, Reed JW (1999). Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. Development **126**:711-721

Tian Q, Uhlir JU, Reed JW (2002). Arabidopsis SHY2/IAA3 inhibits auxin-regulated gene expression. Plant Cell **14**: 301-319

Tiwari S, Wang WJ, Hagen G, Guilfoyle T (2001). AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell **13**:2809-2822

Tiwari S, Hagen G, Guilfoyle T (2003). The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell **15**:533-543

Ulmasov T, Liu ZB, Hagen G, Guilfoyle TJ (1995). Composite structure of auxin response elements. Plant Cell **7**:1611-1623

Ulmasov T, Hagen G, and Guilfoyle TJ (1997a). ARF1, a transcription factor that binds auxin response elements. Science **276**:1865-1868

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997b). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9**: 1963-1971

Ulmasov T, Hagen G, Guilfoyle TJ (1999a). Activation and repression of transcription by auxin response factors. Proc. Natl. Acad. Sci. USA **96**:5844-5849

Ulmasov T, Hagen G, Guilfoyle TJ (1999b). Dimerization and DNA binding of auxin response factors. Plant J. **19**:309-319

Wang K, Li H, Ecker J (2002). Ethylene Biosynthesis and Signaling Networks. Plant Cell S131-151

Woeste K, **Ye C**, **Kieber J** (1999). Two Arabidopsis mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. Plant Physiol. **119**: 521-530

Wong LM, Abel S, Shen N, de la Foata M, Mall Y, Theologis A (1996). Differential activation of the primary auxin response genes, PS-IAA4/5 and PS-IAA6, during early plant development. Plant J. **9** : 587-599

Worley CK, Zenser N, Ramos J, Rouse D, Leyser O, Theologis A, Callis J (2000). Degradation of Aux/IAA proteins is essential for normal auxin signalling. Plant J. **21**:553-562

Wyatt R, Ainley W, Nagao R, Conner T, Key J (1993). Expression of the Arabidopsis AtAux2-11 Auxin-Responsive Gene in Transgenic Plants. Plant Mol. Biol. **22**:731-749

Zenser N, Ellsmore A, Leasure C, Callis J (2001). Auxin modulates the degradation rate of Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **98**:11795–11800

Zurfluh LL, Guilfoyle TJ (1980). Auxin-induced changes in the patterns of protein synthesis in soybean hypocotyls. Proc. Natl. Acad. Sci. USA **77**:357-361

Zurfluh LL, Guilfoyle TJ (1982). Auxin-induced changes in the population of translatable messenger RNA in elongationg sections of soybean hypocotyl. Plant Physiol. **69**:332-337

CHAPTER IV

THE EFFECT OF IAA17/AXR3 MUTATION ON GLOBAL TRANSCRIPTIONAL PROFILES[3]

[3] Lee CM, Nagao RT, Key J To be submitted to Plant Physiology

Introduction

Auxin, the first identified plant hormone, affects plant growth and development in multiple ways such as inhibition of primary root growth, promotion of root hair formation as well as adventitious and lateral root formation, hypocotyl and stem elongation, mediation of root and stem tropisms, vascular tissue differentiation, apical dominance, and phyllotaxy (Thimann, 1977; Guilfoyle, 1999). At the cellular level, auxin modulates cell expansion, division, and differentiation by regulating ion transporters and gene expression. (Thimann, 1977; Guilfoyle, 1999; Chen et al., 2001).

The expression of a number of families of genes are up-regulated specifically by auxin, including most notably the Aux/IAAs (Walker and Key, 1982; Hagen et al., 1984; Walker et al.,1985; Theologis et al., 1985; Conner et al., 1990), SAURs (Small Auxin Up-Regulated RNAs, McClure and Guilfoyle, 1987), GH3 (Hagen et al., 1984), and other less defined individual genes or groups of genes that may respond to auxin and a number of unrelated compounds (reviewed by Guilfoyle, 1999). Some genes are also down-regulated by auxin based on both cDNA cloning (Baulcombe and Key, 1980) and *in vitro* translation of poly-A RNA (mRNA) and 2-D gel analysis of the translation products (Baulcombe et al., 1980; Zurfluh and Guilfoyle, 1980, 1982).

Among the auxin up-regulated genes, Aux/IAAs are the most thoroughly studied family. Arabidopsis contains at least 23 Aux/IAA genes with four highly conserved domains, suggesting the biological importance of those domains. However, the function of Aux/IAAs was not known until mutant characterization of the genes in combination with biochemical analyses were made in recent years. *Axr3-1*, a gain-of-function mutation with a Pro to Leu change within Domain II, was the first characterized Aux/IAA mutant gene and encodes a modified IAA17 protein (Rouse

et al., 1998). Currently nine gain-of-function mutants have been characterized from the Aux/IAA family: *shy1-1* (*IAA6*, Kim et al., 1996), *shy2-2* (*IAA3*, Tian and Reed, 1999), *axr2-1* (*IAA7*, Nagpal et al., 2000), *bdl* (*IAA12*, Hamann et al., 2002), *slr* (*IAA14*, Fukaki et al., 2002), *axr3-1*(*IAA17*, Leyser et al., 1996; Rouse et al., 1998), *iaa18-1* (*IAA18*, Reed, 2001), *msg2-1* (*IAA19*, Tatematsu et al., 1999), and *iaa28-1* (*IAA28*, Rogg et al., 2001). All the above mutants have mutations within Domain II, with a single amino acid change centered within a core GWPPV motif (reviewed by Kepinski and Leyser, 2002). These mutants show auxin-related pleiotropic (semi-) dominant phenotypes, demonstrating the importance of Domain II and its critical role in auxin signaling. Mutation in Domain II of Aux/IAAs seems to increase protein stability by 7- to 20-fold (Ramos et al., 2001; Ouellet et al., 2001; Gray et al., 2001). Domain II-mediated protein degradation is facilitated by auxin (Zenser et al., 2001; Tiwari et al., 2001, 2003; Gary et al., 2001).

Studies using yeast two-hybrid analyses demonstrate that Domains III and IV serve as protein-protein interaction domains with Aux/IAAs and/or Auxin Response Factors (ARFs) in homo- and to a lesser extent heterodimer formation (Chapter II ; Kim et al., 1997; Ulmasov et al., 1999b; Ouellet et al., 2001; Tatematsu et al., 2004). After EMS mutagenesis of *axr3-1,* screens were done in search of suppressor(s). From this screen revertant, *axr3-1R4*, was isolated (Rouse et al., 1998). *Axr3-1R4* has an additional mutation within Domain IV resulting in the loss of half of conserved Domain IV; axr3-1R4 shows WT-like phenotypes. In addition, the revertant protein did not show protein-protein interactions in yeast two-hybrid assays whereas the WT and axr3-1 proteins did interact as expected, indicating the importance of Domains III and IV as interaction domains with Aux/IAAs and/or ARFs (Chapter II). Phenotypic recovery by loss of protein-protein interaction through Domains III and IV suggests their critical role in

auxin signaling in plant growth and development. The *axr3-1* allele shows the most severe phenotype of the Aux/IAA mutants such as agravitropic and short roots, very few root hairs, strong apical dominance, short hypocotyls in the dark, small sized upcurled-leaves, small plants, etc. (Leyser et al., 1996; Chapter III). A T-DNA knockout mutant, *IAA17K,* was isolated as a null mutant (Chapter III). The phenotypes of *IAA17K* and *axr3-1R4* were WT-like except for slightly shorter primary roots in the seedling stage (Chapter III). *Axr3-1* is a gain-of-function mutant that caused down-regulation of most auxin-responsive genes such as Aux/IAAs, GH3, SAURs, and GST (Chapter II) and showed auxin-resistant primary root growth by 100- (Chapter III) to 500-fold (Leyser et al., 1996), while *axr3-1R4* and *IAA17K* did not show differences compared to WT (Chapter III).

Microarray technology is a powerful tool to examine global transcription profiles. Microarrays, specially cDNA-spotted arrays, have been used in many studies, including plant-pathogen interactions (Reymond et al., 2000), cold and drought stress (Seki et al., 2001), seed development (Girke et al., 2000), nutrient response (Wang et al., 2003), mutant analysis (Perez-Amador et al., 2001), and light-regulated gene expression (Ma et al., 2001), etc. Oligonucleotide microarrays from Affymetrix for *Arabidopsis thaliana*, have been used in many studies (Puthoff et al., 2003). Affymetrix designed a new *Arabidopsis* GeneChip (ATH1) containing > 22,000 probes covering the entire Arabidopsis genome on the chip. The new generation GeneChip has been used in a variety of studies in Arabidopsis (Borevitz et al., 2003; Monroe-Augustus et al., 2003; Mussig et al., 2003; op den Camp et al., 2003; Rhee et al., 2003; Rizhsky et al., 2003; Wang et al., 2003; Ulm et al., 2004). These GeneChips experiments showed reliability and reproducibility from array to array as well as hybridization to hybridization (Carson et al., 2002; Chen et al., 2002).

Here, global transcriptional patterns from WT, *ax3-1*, *axr3-1R4,* and *IAA17K* were compared to assess possible relationships among their phenotypes and possible related changes in the transcriptional patterns. Depending upon the specific change in the transcriptional patterns, possible molecular/biochemical action(s)/function(s) of the mutations might be assessed in terms of increased protein stability and loss of protein-protein interactions. It was predicted that *axr3-1* might show many more gene expression changes than those of *axr3-1R4* and *IAA17K* based on the severity of phenotypic changes of *axr3-1* compared to the WT-like phenotypes of *axr3-1R4* and *IAA17K*. Up- and down-regulated genes compared to WT could be sorted into different groups based on their behavior in the four different genetic backgrounds: 1) Genes up- and down-regulated only in the mutant may be correlated with the axr3-1 phenotype. These genes may be common downstream genes of Aux/IAA genes (and/or a cascade of Aux/IAA signaling) since *axr3-1* showed reduced message levels of Aux/IAAs (Chapter II). In addition, the phenotypes of Aux/IAA Domain II mutants show similarities, and the null mutant of IAA17 (*IAA17K*) showed WT-like phenotypes. 2) Common up- and down-regulated genes in both the mutant and revertant (and/or *IAA17K*) may not be correlated with the phenotype of *axr3-1* since they are transcribed similarly in both the *axr3-1* mutant and the revertant. In addition, these gene expression changes may result from the Domain II mutation (*axr3-1* allele) because the common mutation between *axr3-1* and *axr3-1R4* is in Domain II. 3) Genes up- and down-regulated only in the revertant may result from the lack of Domain IV-mediated protein-protein interactions. 4) Genes up- and down-regulated only in *IAA17K* may result from the lack of IAA17 protein (or from some unrelated T-DNA insertion into the genome). Tiwari et al. (2004) recently suggested that the conserved Domain I functions as a general repressor motif (LxLxLx motif). These gene expression changes may have some relationship with Domain I. The third and fourth groups of

genes are suggested to be downstream of the IAA17 function.

Light is an important factor for plants as an energy source for photosynthesis and as a signal to modulate developmental processes. Light regulates various aspects of plant growth and development such as seed germination, apical hook opening, stem elongation, phototropism, chloroplast development, and floral timing (Tian and Reed, 2001). Many light effects interact with and/or overlap with auxin in plant growth and development. Light and auxin signaling networks also seem to be closely related. Phototropic curvature of stems involves light-mediated movement of auxin (polar auxin transport) resulting in asymmetric distribution of auxin. The asymmetric distribution of auxin results in enhanced elongation on the dark or high-auxin side, resulting in bending toward the light source (Firn, 1994). Hypocotyl and root elongation and gravitropism were strongly inhibited by 1-naphthylphthalamic acid (NPA), a polar auxin transport inhibitor, in light-grown *Arabidopsis* seedlings; NPA also disrupted the gravitropic response but did not affect hypocotyl elongation in the dark-grown seedlings, suggesting that auxin has a more important role in hypocotyl elongation responses in light-grown than in dark-grown seedlings (Jensen et al., 1998). Light is first perceived by photoreceptors such as phytochromes for red/far-red light, cryptochromes and phototropins for blue light, and unknown UV light photoreceptors (Halliday and Fankhauser, 2002). Light regulates expression of many genes including early auxin-responsive genes such as Aux/IAA, SAUR, and GH3 (Chapter III; Abel et al., 1995; Gil and Green, 1997; Tanaka et al., 2002). Tanaka et al. (2002) suggested that phytochrome B regulates the expression of *AtGH3a* genes by altering the levels of auxin. Domain II gain-of-function mutants of Aux/IAAs have light-related phenotypes and can form true leaves in the dark-grown state (Leyser et al., 1996; Kim et al., 1996; Nagpal et al., 2000; Tian and Reed, 1999). *Shy2-2*, a Domain II mutant of *IAA3/SHY2* has a short hypocotyl and

expanded cotyledons in the dark (Tian and Reed, 1999). *Shy2-2* showed elevated levels of *CAB* and other light-regulated genes (Tian et al., 2002). The *IAA19* gene was highly auxin-induced in light- and dark-grown seedlings and was repressed by light relative to expression in etiolated seedlings, while the I*AA17* gene was more highly expressed in light-grown seedlings compared to the level in etiolated seedlings (Chapter III). Because *axr3*-1 plants can form true leaves and floral organs in the dark, it was of interest to compare the global transcriptional patterns with respect to light response of WT and *axr3-1*.

In addition to studying the global transcriptional patterns of the four different genetic backgrounds, auxin and light effects on the global transcriptional patterns of WT and *axr3-1* were also studied.

Materials and Method

Plant Materials and Auxin Treatment

Arabidopsis WT(Co.), *axr3-1*, *axr3-1R4*, and *IAA17K* plants were grown on 0.7% Phytoagar (Gibco, Grand Island, NY)/0.5 X MS (Gibco, Carlsbad, CA)/0.2% sucrose for five days either under light (17 hr light:7 hr dark) or dark conditions. For auxin treatment, five day-old etiolated WT and *axr3-1* plants were treated with 0.5X MS(mock) or 20 μM IAA/0.5X MS for 2 hrs.

Microarray Experiments

The gene chip data were generated from the analysis of three independent RNA extractions, labelings, and hybridizations. Plants were grown under either light (17 hr light:7 hr dark) or dark conditions. RNA was extracted using the Trizol method (http://afgc.stanford.edu/

afgc_html/site2Rna.htm#trizol). Total RNA was purified by Qiagen RNeazy column (Qiagene, Valencia, CA). Fourteen micrograms of purified total RNA was used for first strand cDNA synthesis. All labeling was performed following Affymetrix's Eukaryotic Sample and Array Processing Manual (Affymetrix, Sunnyvale, CA). First- and double-strand cDNA was synthesized with the SuperScript™ Double-Stranded cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) using a T7-$(dT)_{24}$V primer (GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGC GG -$(dT)_{24}$-A, G, or C; synthesized and HPLC-purified by IDT Inc., Coralville, IA). The cDNA reactions were purified using GeneChip Cleanup Module (Affymetrix, Santa Clara, CA), and eluted from the cleanup module column with 30 µl cDNA elution buffer. Biotinylated cRNA then was generated from 8 µl purified cDNA elute using the Enzo BioArray High Yield RNA Transcript Kit (Affymetrix). The isolated cRNA was fragmented in fragmentation buffer (1X fragmentation buffer is 40 mM Tris acetate, pH 8.1, 100 mM KOAc, and 30 mM MgOAc) at 94°C for 35 min before chip hybridization. First-strand cDNA, double-strand cDNA, amplified biotin-labeled cRNA, and fragmented biotin-labeled cRNA were separated by 1% agarose gel electrophoresis to check the sizes of the products as one of the quality control steps. Fifteen micrograms of labeled and fragmented cRNA was sent to The University of Pennsylvania Microarray Facility (http://www.med.upenn.edu/microarr) for hybridization with Arabidopsis ATH1 Genome Array **(**Affymetrix) which contains more than 22,500 probe sets representing approximately 24,000 genes. Affymetrix® Microarray Suite software 5.0 (MAS 5.0) was used for basic analyses.

Data Analysis

Analysis of GeneChip gene expression data was started with Affymetrix® Microarray Suite software (MAS 5.0). This software tool manages both the acquisition and processing of GeneChip-generated data, providing a seamless transition from assay performance to data analysis (Affymetrix). MAS 5.0 provides indicators of sample integrity with raw number (hybridization intensity) and p-value with detection call as Present, Marginal, or Absent. The algorithm identifies and removes the contributions of stray hybridization signals, and combines the results from probes that interrogate different fragments of a transcript (see Probe Selection and Array Design in Affymetrix website at www.affymetrix.com). The statistical significance of each detection call is indicated by an associated p-value.

Global normalization of Genechip data was done using MAS 5.0 as follows. The output (raw number hybridization) from all GeneChip hybridizations was scaled globally such that its average intensity from a chip was equal to an arbitrary target intensity of 150. Because all experiments were scaled to the same target intensity, comparison among GeneChips was possible.

After global normalization, two approaches were carried out to remove false changes. The first approach was pair-wise comparison by using MAS 5.0. For example, to identify the gene changes between two genotypes, all nine possible pair-wise comparisons were obtained from two samples (different genotypes, i.e. WT and *axr3-1*) with three replicates. Each sample (GeneChip hybridization intensity) was compared with another with MAS 5.0. MAS 5.0 generated a detection call for each probe (P for Present, A for Absent, or M for Marginal), signal log ratio (as $\log_2$) for changes between two samples, and change call (NC for no change, I for induced, or D for down) with p-value of the change call. A Change P-Value measures the

probability that the expression levels of a probe set in two different arrays are the same. A P-value close to 0.5 indicates that they are likely to be the same. When the p-value is close to 1, the expression level in the experiment arrays is lower than that of the baseline, indicating that the probe is changed, either I or D compared to that of base-line samples. These values were transferred to Microsoft Excel spread sheets, and then data were filtered and sorted.

A second approach used average numbers of hybridization intensities from three independent repeat experiments. After global normalization from MAS 5.0, only Present calls from all three replicates were selected, and the intensities from three replicates were averaged. Probes (genes) were removed as inconsistent replicates if the value (=(Standard Deviation x 100)/Average) was greater than 50. The average values were normalized again by using Cluster software (Eisen et al., 1998) with first log-transformation of data, and normalized genes, and then median genes, and then repeating these steps five times as described in the manual of the software. After the final step, positive and/or negative numbers were generated with respect to average values. The values were then uploaded into various clustering programs.

The EPCLUST program (http://ep.ebi.ac.uk/EP/EPCLUST/) was selected for clustering of data. Various hierarchical and K-means clustering patterns were generated from EPCLUST using various distance-calculating methods. After various approaches, two data sets were compared to evaluate the data sets and to select an appropriate method of analysis.

Results

Phenotypes of Five Day-Old Seedlings

Seedlings (WT, the revertant *axr3-1R4*, and *IAA17K*) grown under light conditions did not show morphological differences (Fig. 4-1A). *Axr3-1* plants, however, showed agravitropic

roots. The root lengths of *axr3-1R4* and *IAA17K* plants were slightly shorter than that of WT (Fig. 4-1A; Chapter III). Global transcriptional patterns among the four different genetic background plants may show transcriptional differences correlated with their phenotypes. DNA microarray experiments were carried out using five day-old light-grown seedlings from three independent replicates to reduce variation among chips and hybridizations and to reduce biological variation.

Data Analysis with P-value Criteria by Microarray Suit 5.0 and EP Clustering

One of the first steps to verify credibility of experimental results was to analyze data by scatter plot analysis. Raw hybridization signal intensities for each genotype were generated from MAS 5.0 software and then plotted against those of the other genotypes. Figure 4-2 presents typical scatter plots. WT hybridization intensities of each gene were plotted with respect to hybridization intensities of the same gene from *axr3-1*, *axr3-1R4*, and *IAA17K* (Fig. 4-2). Red dots represent probes having a Present detection call from MAS 5.0, and yellow and blue represent probes having Absence and Marginal detection calls in terms of gene transcription, respectively. The scatter plots of WT versus *axr3-1R4* and WT versus *IAA17K* showed very narrow scatter forms with the exact slope 1, while the scatter plot of WT versus *axr3-1* showed more widely dispersed scatter also with a slope 1. Most of the expressed genes (having Present call, red dots) from WT versus *axr3-1R4* and *IAA17K* showed changes within a range of 1-fold as $\log_2$ ratio. The internal controls and spike controls showed similar results from chip to chip (Data not shown). Since the scatter plot was narrow and showed a slope of one, experimental results were considered to be 'reliable-quality' data. The phenotypic differences among WT, *axr3-1R4*, and *IAA17K* correlated with the results of scatter plots compared to that of WT versus

axr3-1. Most of the gene expression changes were within a linear two-fold range when WT vs *axr3-1* were compared in the scatter plot (Fig. 4-2).

Data from microarray experiments were normalized globally such that the average intensity from a chip was equal to an arbitrary target intensity of 150. After global normalization, two approaches were used to remove false changes. The first was a P-value criterion approach of pair-wise comparisons by using MAS 5.0. For example, to identify gene expression changes between two genotypes, all nine possible pair-wise comparisons were obtained from two samples (different genotypes, i.e., WT and *axr3-1*) with three replicates. Those values were transferred to a spread sheet in Microsoft Excel, and then data were filtered and sorted.

The second approach used average signal intensities from three independent repeats. After global normalization using MAS 5.0, only Present calls from all three replicates were selected by using Microsoft Excel, and the intensities from three replicates were averaged. Probes (genes) were removed as 'inconsistent replicates' if the value derived from the equation (standard deviation X 100)/average) was greater than 50. From 22,000 genes, 11,113 genes showed Present detection calls in MAS from three replicates consisting of 50.5 %. Of the 11,113 genes, 10,338 (or 93%) showed less than 50 (standard deviation x 100)/average) values from Present calls (Data not shown). The remaining 7%, consisting of 775 genes, were removed from further analysis as inconsistent replicates. These variations may be generated from technical and/or biological variations. The 10,338 genes were used for cluster analysis. The data were again normalized and then uploaded to EPCLUST (see Materials and Methods section for details of normalization method). Various hierarchical and K-mean clusters were generated from EPCLUST with various input methods. After the two basic approaches were done, the two data

sets were compared to evaluate the two approaches and to select the more appropriate method for further analysis.

Figure 4-3 shows hierarchical and K-means clustering maps generated by EPCLUST from the four different genetic backgrounds (WT, *axr3-1*, *axr3-1R4*, and *IAA17K*). As seen in Fig. 4-3A (hierarchical clustering), expression of many genes changed very much in *axr3-1R4* and *IAA17K* (green and red in the two samples). It was difficult to extract useful clusters, and correlations appeared very complicated using hierarchical clustering, so K-means cluster analysis was used to simplify clusters. Fig. 4-3B shows one example of K-means clustering, showing that 447 genes were up-regulated in *axr3-1* and down-regulated in *IAA17K* compared to WT and *axr3-1R4*. Greater changes in gene expression were anticipated in *axr3-1* than in *axr3-1R4* and *IAA17K* compared to WT based on two facts; first, phenotype changes of *axr3-1* are more dramatic than the phenotypes of *axr3-1R4* and *IAA17K*, which appear WT-like, and second, the scatter plot for WT vs *axr3*-1 was much more dispersed compared to the plots for WT vs. *axr3-1R4* and for WT vs *IAA17K* (Fig. 4-2). In the hierarchical cluster, more than half of the genes (from 10,338 genes) appeared to change in *axr3-1R4* and *IAA17K* as seen in red and green. However, the P-value criteria method supports the more reasonable interpretation that the expression of more genes changed in *axr3-1* than in *axr3-1R4* and *IAA17K* (Tables 1 to 4). Since the P-value criteria method provided more readily interpretable results with very high stringency, all additional data analyses were done by this method.

Transcriptome Changes between WT and *Axr3-1*

Most auxin signal transduction studies have concentrated on the upstream events of the Aux/IAA gene family, because downstream events have not been identified. Microarray

experiments provide a tool to possibly identify downstream events in a global scale. Results of all microarray experiments were compared by analyzing mRNA populations and expression patterns (transcriptional profiling) from five day-old light-grown seedlings among WT, *axr3-1*, *axr3-1R4*, and *IAA17K*. Since *axr3-1* shows severe phenotypic differences from WT (Chapter III and Fig. 4-1), one should be able to identify gene expression changes that may relate to the phenotypic differences by comparing expression profiles between WT and *axr3-1*. The morphological characteristics of *axr3-1* plants include shorter and agravitropic roots, lack of root hairs, shorter length of dark grown hypocotyls, and upward-curling of leaves with reduced leaf size. Physiologically, the mutant shows 100- to 500-fold less sensitivity to auxin than WT (Chapter III and Leyser et al., 1996). The mutation in Domain II of IAA17 resulted in increased protein stability of the axr3-1 protein compared to WT IAA17 by 7- to 20-fold (Ramos et al., 2001; Ouellet et al., 2001; Gray et al., 2001). However, the axr3-1 protein did not show alterations of protein-protein interactions and subcellular localization (Ouellet et al., 2002; Chapter II; personal communication, J. Nairn).

Tables 4-1 and 4-2 show some selected lists of up- and down-regulated genes for *axr3-1* compared to WT, respectively. The lists of up- and down-regulated genes between WT and *axr3-1* were generated using the P-value criteria method. A total of 231 genes were up-regulated and 292 genes were down-regulated. The magnitude of change of up- and down-regulated genes varied several-fold such that the average fold change of up- and down-regulated genes in *axr3-1* ranged from 0.5 to 3.37 and -0.5 to -6.53 fold on a $\log_2$ scale, respectively. One can identify downstream genes that may be involved in biological pathways such as signal transduction or metabolic pathways by examining functionality of these genes. Up- and down-regulated genes between WT and *axr3-1* (resulting from the mutation of *axr3-1*) are likely to be genes

functioning downstream of *IAA17* in auxin signaling. These up- and down-regulated genes (231 and 292 genes) were sorted based on their functionality in the Affymetrix Gene Ontology Mining Tool related to molecular function, cellular component, and biological process (Table 4-3). However, the output ontology maps didn't show distinct trends, and the genes were distributed over a broad range of functionalities (data not shown). Various up- and down-regulated genes included transcription factors, kinases, hormone response elements, and genes related in some way to hormones (e.g., auxin, ethylene, jasmonate, etc.; Tables 4-1 and 4-2; for full list of genes, see Appendix C and D).

Most of the up- and down-regulated genes in *axr3-1* were *axr3-1*-specific based on comparative expression in WT, *axr3-1R4*, and *IAA17K*, indicating that expression of these genes at least correlates with the *axr3-1* phenotypes, perhaps as the result of more stable axr3-1 protein, thus resulting in stronger protein-protein interactions through Domains III and IV (e.g., longer interaction times with their interaction partners such as Aux/IAAs and ARFs). More genes were down-regulated with greater fold changes than were up-regulated in *axr3-1*. Since *axr3-1* shows agravitropic and shorter root phenotypes, it was interesting to find cell wall-related proteins among the changes; for examples, extensin-like protein (At5g35190), proline-rich protein (At3g62680), and xyloglucan endotransglycosylase (At5g57530) were down-regulated many-fold (-3.88 to -6.53). ATH1 GeneChip contains 35 extensin or extensin-like genes. Most extensin genes were down-regulated in *axr3-1* when they were sorted by average fold change (i.e., average signal intensities, data not shown); but only the At5g35190 extensin-like gene showed a consistent pattern with P-value criteria. Among up-regulated genes, four pEARLI 1 or pEARLI 1–like genes were up-regulated with relatively high fold ranges of 1.79 to 2.37. pEARLI 1 belongs to a highly conserved, Pro-rich hydrophobic protein family of unknown

function (Richards et al., 1998). The ATH1 chip contained a total of 6 pEARLI 1 or pEARLI 1–like genes. Among them, one pEARLI 1-like (At4g12520) gene was down-regulated by 1.6-fold and another (At3g03190) gene was not changed (see Appendix C and D for details). However, two pEARLI 1 and pEARLI 1-like genes (At4g12480 and At4g12470) were also up-regulated in *axr3-1R4* and *IAA17K*, but the level of fold induction was not as high as that of *axr3-1*. pEARLI 1 and pEARLI 1-like genes are also induced by aluminum-induced oxidative stress (Richards et al., 1998). Glutathione S-transferases (GST, At1g02930 and At1g49860) and pathogenesis-related proteins (PR protein, At2g19970 and At2g43620) showed relatively high levels of induced expression in *axr3-1*. PR proteins are induced by many factors such as stress, ethylene, pathogens, etc. (Park and Lee, 1992). GSTs are reported to be induced with infection, in response to treatment with ozone, hydrogen peroxide, glutathione and biotic elicitors, plant hormones including auxin, heavy metals, heat shock, dehydration, and wounding and senescence (reviewed by Marrs, 1996). Induction of GST and PR proteins including pEARLI 1 suggests that the Domain II mutation of IAA17 may result in stressful conditions for the plant. Interestingly, eight ribosomal proteins (AtCg00750, AtCg00760, AtCg00770, AtCg00780, AtCg00790, AtCg00800, AtCg00810, and AtCg00050) of the chloroplast genome were up-regulated in *axr3-1* plant compared to WT green seedlings (Table 4-1), while the gene expression of these genes were not changed in *axr3-1 etiolated* seedlings compared with WT etiolated seedlings. This suggests a relationship between Aux/IAA proteins (or auxin) and chloroplast gene expression.

It was interesting to examine the transcript levels of the Aux/IAAs and other auxin up-regulated genes in this experiment since these genes were down-regulated in *axr3-1* plants based on Northern analysis (Chapter II). In microarray experiments, *IAA17* and a GH3 (At1g28130)

gene were found to be down-regulated in *axr3-1*. Other Aux/IAAs and auxin up-regulated genes were not affected consistently based on P-value criteria. This discrepancy may result from sample-to-sample variation and the low level of expression of some of the Aux/IAAs and other genes. For example, *IAA5* and *IAA20* were not expressed sufficiently to warrant a 'Present' call in MAS 5.0 (e.g., the level of expression was not enough, or the signals were not consistent among the 11 probe sequences representing one gene on the chip). Since the Northern analyses in Chapter II were generated from five day-old etiolated seedlings, the data cannot be compared directly with this experiment where the RNA samples were prepared from five day-old light-grown seedlings. In addition, light is known to reduce the level of expression of at least some Aux/IAA genes Chapter III).

Here, expression of a total of 525 genes was identified to change in *axr3-1* compared to WT in light-grown seedlings. Among them, expression of over 95% of these genes was specifically changed only in *axr3-1* compared to WT and the revertant (i.e., *axr3-1*-specific changes). The expression of these genes thus correlates with *axr3-1* phenotypes, and many of these genes have regulatory function (Tables 4-1 and 4-2).

Transcriptome Changes in *Axr3-1R4* and *IAA17K* Compared to WT

Phenotypes of *IAA17K* and *axr3-1R4* after two additional backcrosses were WT-like and cannot be distinguished from WT (Chapter III). *IAA17K* did not produce detectable *IAA17* transcript and was assumed to be a null knockout. However, both revertant and knockout had slightly shorter roots than WT in five day-old plants (Fig. 4-IA and Chapter III). *Axr3-1R4* has the additional mutation within Domain IV resulting in loss of half of conserved Domain IV. In addition, the axr3-1R4 protein did not show protein-protein interaction with other putative

interaction proteins (e.g., ARFs and Aux/IAAs) (Chapter III). The lack of protein-protein interaction may be responsible for the WT-like *axr3-1R4* phenotypes and for changes in expression of downstream genes. Thus, the Domain IV mutation effectively overrides the *axr3-1* mutation. Likewise, the altered expression of genes in *IAAA17K* may result also from the lack of protein-protein interaction due to the lack of IAA17 protein (null mutation).

There were fewer changes in gene expression in *axr3-1R4* and *IAA17K* than in *axr3-1* relative to WT as seen in scatter plots (Fig. 4-2). The expression data from *axr3-1R4* and *IAA17K* were compared to base-line expression data of WT, and expression values (e.g., I for induced, D for down-regulated, and NC for no-change) were sorted as previously described. These gene expression changes are listed in Tables 4-4 and 4-5. Only six were up-regulated in *axr3-1R4* compared to WT (Table 4-4). However, these six genes were also up-regulated in both *axr3-1* and *IAA17K* compared with WT. Since one common phenotype among the mutant, revertant, and knockout of five day-old seedlings is shorter (for *axr3-1*) and slightly shorter (for *axr3-1R4* and *IAA17K*) primary root length, these genes may be responsible for a portion of the root length phenotype. Since the axr3-1 and axr3-1R4 protein-protein interaction data are opposite, it suggests that these genes may not be direct downstream genes, but be indirect and/or effect common downstream events after Aux/IAA function. A total of 29 genes were up-regulated in *IAA17K* (Table 4-5). Among them, six genes were also up-regulated in *axr3-1,* and 18 genes were *IAA17K*-specific. A total of 13 and 23 genes were down-regulated in *axr3-1R4* and *IAA17K*, respectively (Table 4-5). Among the 13 down-regulated genes in *axr3-1R4*, seven genes belong to the chloroplast genome (Table 4-4). Specific gene expression changes in only *axr3-1R4* may have resulted from the lack of protein-protein interaction. It is not clear how the lack of protein-protein interaction would down-regulate some chloroplast genes, although there

are known nuclear effects on chloroplast gene expression (reviewed by Barkan and Goldschmidt-Clermont, 2000). However, two phytochrome-associated proteins were isolated by yeast two-hybrid screening, and both were identified as Aux/IAA proteins, IAA26 and IAA27 (Lee et al., unpublished data; Genbank accession numbers of the genes are AF088281 and AF087936, respectively). Phytochrome (phy A) can phosphorylate Aux/IAA proteins *in vitro* (Colon-Carmona et al., 2000). These researchers suggest relationships between Aux/IAA proteins and phytochrome.

Among the 23 down-regulated genes in *IAA17K*, 14 genes were knockout-specific and nine genes were somehow related to *axr3-1* and *axr3-1R4*. The knockout-specific up- and down-regulated genes may have resulted from the T-DNA insertion in the genome (i.e., T-DNA disturbance) and/or the lack of IAA17 protein. Tiwari et al., (2004) suggested that the conserved Domain I (including the LxLxLx motif) of Aux/IAAs functions as a general repressor. The expression of these genes may result from the lack of general repression by IAA17 protein, assumming this is a null mutant. The largest gene expression change of down-regulated genes in *IAA17K* compared to WT in GeneChip experiments was the *IAA17* transcript, correlating with the Northern data of Chapter III, suggesting that *IAA17K* can be assumed a null mutant. This is another confirmation that this knockout is a null mutant.

In general, the gene expression changes based on fold ratio in *axr3-1R4* and *IAA17K* were not large compared to those in *axr3-1*. In addition, expression of about 50 genes (less than 10% of genes from *axr3-1*) in *axr3-1R4* and *IAA17K* changed compared to *axr3-1* correlating with their relative phenotypic severity.

Auxin-Responsive Genes in WT and *Axr3-1*

Auxin mediates multiple developmental and physiological processes. One approach to understand the molecular action of auxin is to identify auxin-responsive genes. Experiments were designed to evaluate how the auxin up- and down-regulated genes responded to light treatment in *axr3-1* backgrounds, and whether these genes are auxin-responsive in the mutant with auxin treatment as well as to identify additional auxin-responsive genes in the entire genome. *Axr3-1* plants showed 100- to 500-fold auxin-resistant primary root growth, and most of the known auxin up-regulated genes were down-regulated in *axr3-1* based on Northern data (Chapter II). Etiolated seedlings were chosen because of the large number of studies previously done using this material (Abel et al., 1995; Chapter II). Five day-old etiolated WT and *axr3-1* seedlings were treated with auxin and without auxin (mock) for two hours as describe in Materials and Methods.

Table 4-6 shows selected auxin up-regulated genes. The baseline expression in this comparison (Table 4-6) used RNA from a WT etiolated seedling sample. A total of 169 genes were up-regulated (see Appendix E for full list of genes) including known classical auxin up-regulated genes such as Aux/IAAs, SAUR, GH3, ACC synthase, etc. The Arabidopsis chip (ATH1) contains at least 28 putative Aux/IAA genes; among them, nine Aux/IAAs were up-regulated by auxin in WT etiolated seedlings with a range of 0.63 to 2.53 fold increase. Seven out of the nine Aux/IAAs showed reduced levels of expression in etiolated *axr3-1* seedlings. However, the nine genes responded to auxin in *axr3-1 etiolated* seedlings, but the expression levels of *IAA5* and *IAA19* in *axr3-1 etiolated* seedlings with auxin treatment were lower than those in WT etiolated seedlings without auxin treatment (e.g., the value in WT etiolated seedlings is 0; Table 4-6). GH3 (At1g59500) showed the highest auxin responsiveness (3.6-fold

induction by auxin). About 55 genes among the 169 genes have an identified regulatory function such as kinase (receptor kinase, etc.), transcriptional regulators (Aux/IAAs, bZip, AtB2, etc.), related to protein degradation (Ring-H2 finger, NAC, etc.; Xie et al., 2002; Greve et al., 2003), etc. A total of 54 genes encode unknown proteins such as hypothetical, expressed, putative, etc. (Table 4-6; Appendix E). A total of eight genes are related to other hormones such as brassinosteroid receptor kinase, cytokinin oxidase, etc (Appendix F). The remaining genes (about 50) have miscellaneous functions such as pathogen-related proteins, ß-1,3-glucanase, an embryo-abundant protein, etc. (Appendix E).

Auxin down-regulated genes are relatively less studied than auxin up-regulated genes, and their responses to auxin also are relatively slower than for auxin up-regulated genes that respond within minutes (reviewed by Abel and Theologis, 1996 and Guilfoyle, 1999). In this experiment, etiolated seedlings were treated with auxin for two hours which is relatively short compared to previous studies (Baulcombe and Key, 1980; reviewed by Guilfoyle, 1999). Table 4-7 shows selected auxin down-regulated genes using expression data from the RNA of WT etiolated seedlings as the base line (in Table 4-7; WTe_I, e and I stand for etiolated state and auxin treatment, respectively). A total of 142 genes were down-regulated (see Appendix F for full list) in the range of -0.6 to -1.7 (i.e., 30 to 69 % compared to the original level in WT etiolated seedlings). Most of these genes were not known to respond to auxin from previous studies. A total of eight genes from 142 genes are regulatory proteins such as Myb DNA binding protein, bHLH transcriptional factor, etc. Interestingly, a total of eight genes from the 142 genes are related to photosynthesis such as chlorophyll a/b-binding protein, photosystem I subunit, etc. (Table 4-7). In addition, 11 genes from the 142 genes are likely related to cell wall genes, such

as xyloglucanase, expansin, putative pectinacetylesterase, etc. Ten genes among the 142 genes seem to relate to lipid metabolism, such as lipase, GDSL-motif lipase, lipid transfer protein, etc.

To follow the trends of auxin up- and down-regulated genes in *axr3-1*, these auxin-responsive genes were plotted by fold change in *axr3-1*e and auxin-treated *axr3-1*e. Most auxin up-regulated genes were down-regulated in *axr3-1*, and responded to auxin treatment (Fig. 4-4). However, some genes (all unknowns; At4g37740, At1g29480, At2g22880) did not respond to auxin in *axr3-1* (Fig. 4-4; Table 4-6). The trend of auxin down-regulated genes in *axr3-1* was compared to the base line expression of WTe (Fig. 4-5). However, in this case, it was difficult to find distinct trends with just fold changes. In addition, many of these genes were even up-regulated in *axr3-1* with auxin treatment. To clear these trends, the P-value criterion method (pair-wise comparison) was applied. No gene was up-regulated based on P-value criteria, while only 16 genes were shown to be down-regulated in auxin-treated *axr3-1* (but 57 genes were up-regulated in auxin-treated *axr3-1*, data not shown).

In *axr3-1 etiolated* seedlings, a total of 85 and 21 genes were up- and down-regulated by auxin treatment, respectively (data not shown), which is relatively fewer than those of WT etiolated seedlings. Forty-nine genes among the 85 genes were also up-regulated, and six genes among 21 genes were down-regulated in WT etiolated seedlings by auxin treatment. It was interesting that many fewer genes were up- and down-regulated in *axr3-1 etiolated* seedlings by auxin treatment than in WT etiolated seedlings.

In general, *axr3-1* showed reduced transcript levels of auxin up-regulated genes compared to WT etiolated seedlings, but these genes responded to auxin in *axr3-1*e seedlings even though the level of responsiveness was less than in WT etiolated seedlings. These data correlate well with the Northern data for auxin-responsive genes (Chapter II).

Light-Regulated Genes

For the purpose of evaluating the influence of light on auxin-responsive gene expression, global transcriptional profiles were examined from both five day-old WT green (17 hrs light:7 hrs dark) and WT etiolated seedlings and from both *axr3-1* green and etiolated seedlings. Based on sorting genes by P-value criteria and an average fold change of 0.8 as an artificial cut-off value, a total of 1208 and 1300 genes were up- and down-regulated by light, respectively (see Appendix G and H for the full list of genes). Sixty-two genes were related to photosynthesis such as light photosystem I subunit X precursor, putative chloroplast inner envelope photosystem II reaction center 6.1kD protein, etc. among the light up-regulated genes, while five gene were related to photosynthesis among the genes down-regulated by light. Only four Aux/IAA genes were repressed by light among 28 Aux/IAAs in five day-old etiolated seedlings compared to light-grown seedlings. Since *axr3-1* seedlings can form true leaves and even floral organs in the etiolated state and show greatly reduced hypocotyl elongation in the dark (Fig. 4-1B; Leyser et al., 1996), it was of interest to investigate whether the light response(s) was different between WT and *axr3-1* at the level of gene expression. Global transcriptional profiles were analyzed between WT light-grown and WT etiolated seedlings, and between *axr3-1* light-grown and *axr3-1* etiolated seedlings. In *axr3-1*, 1493 and 1505 genes were up- and down-regulated, respectively, in etiolated seedlings compared to *axr3-1* light-grown seedlings (Fig. 4-6). There were 789 and 910 common genes which were up- and down-regulated in WT and *axr3-1* by light, respectively. However, seven and two genes responded in the opposite way in WT and mutant (Fig. 4-6).

To focus on auxin and light regulation, auxin up- and down-regulated genes were sorted and then compared to the level of expression in light (Table 4-8). Among 168 auxin up-

regulated genes in WTe, 57 genes were repressed by light, and only five genes were induced by light in WT seedlings. However, among 142 auxin down-regulated genes in WTe, 25 genes were repressed by light, while 45 genes were induced by light in WT seedlings. These data indicate an opposite effect (or action mode) between auxin and light. Four Aux/IAAs (*IAA2*, *IAA4*, *IAA13*, *IAA19*) were repressed by light among nine auxin up-regulated Aux/IAAs in WT etiolated seedlings, while six Aux/IAAs from 28 Aux/IAAs on the ATH1 chip were repressed by light. Also, homeobox-leucine zipper and zinc-finger transcription factors (At5g47370 and At4g29190, respectively), NAM/CUC2-like protein (At5g39610), GH3-like (At4g27260), glycine-rich RNA-binding protein AtGRP2 (At4g13850), putative kinase (At3g15540), etc. belonged to this group (Auxin Up- and Light Down-Regulated Genes in Table 4-8).

In general, auxin up-regulated genes were repressed by light and auxin down-regulated genes were induced by light, suggesting additional evidence of a close and interactive relationship between auxin and light in plant growth and development. In general, these data suggest that auxin and light act in opposite directions in terms of expression of auxin- and light-responsive genes.

Discussion

Transcriptional Profiles in WT, *axr3-1*, *Axr3-1R4*, and *IAA17K*

Using Affymetrix ATH1 GeneChips, global transcriptional profiles of four different genetic backgrounds of *Arabidopsis thaliana*, WT, *axr3-1*, *axr3-1R4*, and *IAA17K* have been determined. It was interesting to examine the mRNA alterations among the four genotypes since *axr3-1* showed 100- to 500-fold reduced sensitivity to auxin inhibition of primary root growth (Chapter III; Leyser et al., 1996) while *axr3-1R4* and *IAA17K* showed similar auxin sensitivities

compared to WT (Chapter III). In addition, the gain-of-function mutant (*axr3-1*) showed reduced steady state mRNA levels of auxin up-regulated genes, but the intragenic revertant of *axr3-1* (*axr3-1R4)* showed mRNA levels similar to those of WT (Chapter II). The phenotype of *axr3-1* shows various auxin-related altered plant growth responses such as agravitropic and shorter roots with lack of root hairs, smaller sized leaves, etc. (Leyser at al., 1996; Chapter III). However, the *axr3-1R4* and *IAA17K* phenotypes are WT-like except for slightly shorter primary root length (Chapter III). The mutation within Domain II in *axr3-1* resulted in increased protein stability by 7- to 20-fold compared to WT IAA17 protein (Gray et al., 2001; Ouellet et al., 2001), but axr3-1 protein showed similar protein-protein interaction behavior as WT protein displayed in yeast two-hybrid assays (Chapter II). The axr3-1R4 revertant protein lacked these protein-protein interactions.

For the above reasons, especially the phenotype differences of *axr3-1* and *axr3-1R4*, more transcriptional changes might be expected in *axr3-1* compared to *axr3-1R4* and *IAA17K*. A total of 231 genes were up-regulated and 293 genes were down-regulated in *axr3-1* compared to WT. Most genes that differed in expression in *axr3-1* vs WT did not respond to auxin treatment in WT etiolated seedlings even though *axr3-1* showed auxin-related severe phenotypes. Most Domain II gain-of-function mutants of Aux/IAAs showed reduced levels of transcripts of that particular mRNA compared to WT (Chapter III; Tian and Reed, 1999). *Axr3-1* showed a two-fold decrease of *IAA17* transcript (Table 4-2). Surprisingly, only three auxin up-regulated genes (*IAA14*, *IAA17,* and GH3 or At1g28130) were down-regulated when the level of gene expression in *axr3-1* was compared to that of WT in green seedlings using the GeneChip technology; many more auxin up-regulated genes showed lower expression in *axr3-1* based on Northern analysis (ChapterII). *Shy2-2* has a similar Domain II mutation and showed altered

auxin-related phenotypes such as slower root gravitropic response, short hypocotyls, enlarged cotyledons, etc. (Tian and Reed, 1999). Shy2-2 protein has a higher stability level than IAA3/SHY2 protein (Colón-Carmona et al., 2000). Tian et al. (2002) examined the transcriptional profiles of auxin-responsive genes in six day-old light-grown WT, *shy2-2,* and the intragenic revertant *shy2-24*. *Shy2-2* plants showed reduced levels of *IAA2*, *IAA3*, *IAA7*, *IAA11*, *IAA18*, and *IAA20*, while the revertant (*shy2-24*) maintained transcript levels similar to those of WT (Tian et al., 2002). In terms of root phenotypes, *axr3-1* showed a dramatically more severe auxin-related phenotype than *shy2-2*. The experiment of Tian et al. (2002) was done with a single chip from an Affymetrix Arabidopsis Genome Array (containing 8,000 genes) while our experiments used ATH1 Arabidopsis GeneChips containing over 22,000 genes and were done with three independent replicate experiments. For the experiments reported here, genes were sorted based on P-value criteria from three independent pair-wise comparisons. This method provided higher stringency than in sorting gene expression changes by average signal difference. Based on methodology differences it is difficult to compare directly the results of Tian et al. (2002) with those of this research even though the mutations are located within Domain II of Aux/IAAs. However, these data from *axr3-1* etiolated seedlings can be compared with the Northern data in Chapter II in that the Northern data were generated from five day-old etiolated seedlings and the same mutant. *IAA3*, *IAA6*, *IAA7*, *IAA9*, *IAA17*, *IAA19*, and GH3/*dfl* were down-regulated in etiolated *axr3-1* without auxin treatment compared to etiolated WT (Chapter II). However, in light-grown seedlings (in this experiment), only GH3, *IAA14*, and *IAA17* were down-regulated in *axr3-1*. This presumably relates to differences in seedling state (i.e., green vs etiolated), and in technical analysis, i.e., direct observation on Northern blots vs. programmed analysis of gene chip data using selected high stringency criteria as noted.

Since *axr3-1* plants have agravitropic and shorter roots than WT, it was interesting to identify expression changes in cell wall-related proteins. Extensin-like protein (At5g35190), proline-rich protein (At3g62680), and xyloglucan endotransglycosylase (At5g57530) were down-regulated many fold (-3.88 to -6.53). Arabinogalactan proteins (AGPs) are abundant plant proteoglycans involved in mediating plant growth and development (reviewed by Showalter, 2001; Zhang et al., 2003). Eight AGP were down-regulated in *axr3-1* with in the range of –2.3 to –0.93. Four pEARLI 1 or pEARLI 1-like genes were up-regulated with relatively high fold range. pEARLI 1 belongs to a highly conserved, Pro-rich hydrophobic protein family of unknown function (Richards et al., 1998). Two pEARLI 1 and pEARLI 1-like genes (At4g12480 and At4g12470) were also up-regulated in *axr3-1R4* and *IAA17K*, but the level of fold induction was not as high as those of *axr3-1*.

The altered gene expression in *axr3-1R4* may be responsible for the phenotype of the revertant at five day-old plants. In terms of physiological and molecular aspects, the gene expression changes in *axr3-1R4* and *IAAA17K* may result from the lack of protein-protein interaction and lack of protein (null mutation), respectively. Relatively fewer gene expression changes occurred in *axr3-1R4* and *IAA17K* compared to *axr3-1*, correlating with the scatter plots and phenotypes of the revertant and the knockout. Interestingly, *axr3-1R4* showed a total of only six up-regulated genes that were also up-regulated in *axr3-1* (Table 4-4). Among 13 down-regulated genes specific to ax*r3-1R4*, seven gene products originated from the chloroplast genome. In contrast, nine different genes (most genes were ribosomal proteins) from the chloroplast genome were up-regulated in *axr3-1* (Table 4-1). The *axr3-1R4*-specific gene expression changes may result from the lack of protein-protein interaction through Domains III and IV, while *axr3-1*-specific gene expression changes may result from the stronger/longer (or

more stable) protein-protein interactions. It is not clear how the protein-protein interaction alterations between axr3-1 and axr3-1R4 proteins cause up- and down-regulation of some chloroplast genes, although there are many examples showing that nuclear factors affect chloroplast gene expression (reviewed by Barkan and Goldschmidt-Clermont, 2000). A nuclear s factor protein (SigB) has been shown to be translocated into chloroplasts and to regulate expression of certain genes involved in chloroplast development (Shirano et al., 2000). It seems that at least one downstream gene product of IAA17 resulted from lack of protein-protein interaction through Domains III and IV altered signals (i.e., either signal is removed or is transferred abnormally) that resulted in down-regulation of certain chloroplast genes. The knockout-specific up- and down-regulated genes might be the result of the lack of the IAA17 protein and/or T-DNA insertion into the genome (i.e., T-DNA disturbance). Tiwari et al., (2004) suggested that the conserved Domain I (the core LxLxLx motif) of Aux/IAAs functions as a general transcription repression domain. The expression of a total of 37 *IAA17K*-specific genes may result from the lack of general repression by IAA17 protein in the null mutant, IAA17K.

The phenotypes of *axr3-1R4* and *IAA17K* are WT-like except for slightly shorter roots. The gene expression changes in these two genetic backgrounds were small compared to those in *axr3-1*. Here, there appears to be a correlation between phenotypes and gene changes in *axr3-1* and its intragenic revertant as well as in its null mutant.

Auxin and Light Responses in WT and *Axr3-1* Plants

In this research, the global transcriptional patterns of WT and *axr3-1* with respect to auxin and light treatments were examined to assess whether there is a correlation between auxin and light in effects on gene expression and growth responses to each. *Axr3-1* was included in

this study based on the following: 1) *axr3-1* can tolerate 100- to 500-fold higher concentrations of auxin before primary root growth is inhibited (auxin resistance) than WT, and has various auxin-related phenotypes, suggesting that this mutant may have altered levels of auxin-related gene expression; 2) *axr3-1* can form true leaves and floral organs in the dark, suggesting that this mutant overcomes dark-repressed photomorphogenesis (e.g., de-etiolation process in the dark). By analyzing transcriptional profiles of WT and *axr3-1* with or without auxin and light treatment, gene expression changes correlated in multiple parameters may be identified which cause the *axr3-1* phenotypes. Thus, one may identify auxin-responsive genes that are common with genes related to light-dark-related photomorphogenesis. Light and auxin are related in many ways in plant growth and development. For example, light inhibits hypocotyl elongation compared to dark-grown plants, while auxin promotes elongation, suggesting the simple model that light may inhibit auxin action or decrease auxin levels or auxin sensitivity. Red light decreased auxin levels in epidermal cells of both elongating maize mesocotyl and pea epicotyl (Behringer and Davies, 1992; Jones et al., 1991). Phototropism is mediated by light and auxin. Auxin seems to function down-stream of light in phototropism. Light regulation in elongating stems might result from changes in auxin transport into or out of the expanding cells (Jensen et al., 1998).

A total of 169 genes were induced by auxin treatment in etiolated WT seedlings (Table 4-6). In this experiment, many known auxin up-regulated genes were identified such as GH3, Aux/IAAs, SAUR, ACC synthase, etc. Several kinases and transcription factors were newly identified as auxin response genes. Pufky et al. (2003) studied auxin-induced transcription changes in etiolated seedlings after 20, 40, and 60 minutes of auxin treatment by using an Agilent microarray (contains over 20,000 genes, each represented by 60 nt oligomers). They

identified several auxin-induced genes such as receptor kinases, homeobox factors, a zinc-finger transcription factor, etc. Both results presented here and those of Pufky et al. (2003) identified several common and novel auxin-induced genes such as homeobox factors (At5g47370) and a F-box containing protein (At1g78100), etc. Results from the experiments reported here show that auxin-induced gene expression changes were generally repressed by light, suggesting a somewhat opposing mode of action between light and auxin. Auxin is known to modulate the degradation of Aux/IAA proteins by facilitating interactions between SCF^{TIR1} and Aux/IAA proteins through Domain II of Aux/IAAs (Gray et al., 2001; Zenser et al., 2001, 2003). Several proteins related to protein degradation such as Ring-H2 finger (Xie et al., 2002), NAC (Greve et al., 2003) and F-box containing proteins responded to auxin in these studies (Table 4-6). However, it is not clear if there is a direct relationship between SCF^{TIR1} and these protein degradation-related proteins in terms of interaction of SCF^{TIR1} with Aux/IAA proteins.

The soybean SAUR (Small Auxin-Up RNA) genes are transcriptionally induced by exogenous auxins within a few minutes, primarily in epidermal and cortical cells within the elongation zone of hypocotyls and epicotyls after hormone application (Gee et al., 1991). The results presented here identified genes putatively involved in auxin-dependent hypocotyl elongation: retrotransposon-like protein (At4g37890), auxin-induced protein 15A (a SAUR, At4g36110), GH3-like protein (At4g27260), glycosyl hydrolase family 17 (At4g18340), glycine-rich RNA-binding protein AtGRP2 (At4g13850), bHLH protein (At3g25710), homocysteine S-methyltransferase AtHMT-1 (At3g25900), putative protein kinase (At3g14370), early auxin-induced protein (IAA19, At3g15540), putative senescence-associated protein 12 (At2g17840), and other putative proteins (At2g186other90, At5g67060, At5g53660, At3g59900, At4g37740, At4g35720). These genes were selected based on the criteria that the expression of these genes

was induced by auxin, repressed in *axr3-1* etiolated seedlings compared with WT etiolated seedlings, and repressed by light in WT but did not respond to light in *axr3-1* seedlings. Hypocotyl elongation in the dark occurs mainly through cell expansion by loosening the structures of cellulose and xyloglucan fibers and pectin layer by the regulation of gene expressions (Nemhauser and Chory, 2002). Putative candidate genes involved in this loosening may be hydrolases, xyloglucan endo-transglycosylases, expansins, etc. However, in this study, it was difficult to relate these enzymes to the selected gene list mentioned above which assigned possible function to hypocotyl elongation.

Light (specially red/far-red) is perceived by phytochromes, and phytochromes are then transported from the cytoplasm into the nucleus where they can interact with downstream signaling components including transcriptional factors such as Phytochrome Interaction Factor3 (PIF3), LONG HYPOCOTYL 5 (HY5), ATHB-2, etc. (reviewed by Tian and Reed, 2001). ATHB-2 is a homeodomain-leucine zipper protein and seems to be a negative regulator of gene expression (Steindler et al., 1999). ATHB-2 enhances cell elongation and inhibits secondary vascular cell proliferation and lateral root formation (Steindler et al., 1999). The ATHB-2 gene (At1g75390, Table 4-6, and Tian et al., 2002) and similar homeobox-leucine zipper proteins (At5g47370, Table 4-6, and Pufky et al., 2003) were induced by auxin treatment, suggesting a link between light and auxin at the molecular level. An interesting part of interaction between light and auxin at the molecular level is protein degradation. Axr3-1 protein has increased protein stability, and the Domain II mutation abolished the interaction of IAA17 mutant protein with SCF^{TIR1} (Gray et al., 2001), and the mutant plants showed de-etiolated phenotypes in dark. Other Domain II mutants of Aux/IAAs (*axr2-1* and *shy2-2*) also showed de-etiolated phenotypes. The SCF^{TIR1} complex has E3 ubiquitin ligase activity, and Aux/IAA proteins are among its

substrates (Gray et al., 2001; reviewed by von Arnim, 2003). Constitutive Photomorphogenesis 9 (COP9) Signalosome is able to modify the cullin subunit of E3-ubiquitin-ligase complex (like SCF^{TIR1}) by cleaving off the covalently coupled peptide (Nedd8) (reviewed by von Arnim, 2003). Any mutation in a component in the COP9 Signalosome destabilizes the entire complex and yields a similar phenotype (reviewed by von Arnim, 2003). One of the partial loss-of-function mutants, *csn5*, of the COP9 Signalosome showed reduced levels of Aux/IAA gene expression by auxin treatment and showed higher reporter gene activity of *PsIAA6LUC* in *csn5* transgenic line than in WT transgenic line (Schwechheimer et al., 2001); this response is similar to that of the gain-of-function Domain II Aux/IAA mutants. This indicates that the COP9 Signalosome may be involved in mediating auxin responses and suggests that one function of *csn5* is to reduce auxin signaling.

Here, putative common or independent genes between light and auxin involved in photomorphogenesis were identified, and they seem to work downstream of protein degradation steps, which are key regulatory steps in both light and auxin signaling. It would appear from the data presented above and the known strong interactions of auxin and light in the control of growth and development that further analysis of this interaction at the gene expression level would be in order along with further analysis of whether the COP9 Signalosome plays a central role in regulating the balance of, for example, phytochrome and AUX/IAAs as they relate to growth, development, and photomorphogenesis. Additional microarray experiments might also provide some additional insights into light and auxin interactions at the gene level.

Table 4-1. Up-Regulated Genes in *Axr3-1* Compared to Five Day-Old WT Green Seedlings

Affy ID	TAIR ID	Description	Avg $\log_2$ ratio	Stdv
Possible regulatory proteins and chloroplast genes				
258155_at	At3g18130	protein kinase C-receptor/G-protein	0.50	0.13
249874_at	At5g23070	putative thymidine kinase	0.62	0.23
254250_at	At4g23290	serine/threonine kinase	0.68	0.17
254251_at	At4g23300	serine/threonine kinase - like protein KI domain interacting kinase 1	0.72	0.10
248607_at	At5g49480	NaCl-inducible Ca2+-binding protein-like; calmodulin-like	0.48	0.11
246307_at	At3g51800	putative nuclear DNA-binding protein	0.58	0.13
256678_at	At3g52380	RNA-binding protein (cp33)	0.63	0.17
262094_at	At1g56110	SAR DNA binding protein	0.68	0.18
259311_at	At3g05060	putative SAR DNA-binding protein-1	0.78	0.10
267076_at	At2g41090	calcium binding protein (CaBP-22)	0.79	0.35
266801_at	At2g22870	putative nucleotide-binding protein	0.83	0.30
264121_at	At1g02280	putative GTP-binding protein	0.86	0.26
246932_at	At5g25190	ethylene-responsive element - like protein	1.56	0.30
247549_at	At5g61420	myb-related transcription factor(mixta)	0.83	0.22
248801_at	At5g47370	homeobox-leucine zipper protein-like	0.87	0.30
249916_at	At5g22880	histone H2B like protein	0.87	0.26
251311_at	At3g61140	COP9 complex subunit	1.03	0.54
257008_at	At3g26920	F-box protein	1.7	0.45
251690_at	At3g56510	putative TATA-binding protein-binding protein	0.93	0.16
251951_s_at	At3g53600	zinc finger - like protein	0.90	0.32
258434_at	At3g16770	AP2 domain protein RAP2.3	1.23	0.33
254654_at	At4g18040	translation initiation factor eIF4E	0.80	0.22
254910_at	At4g11175	translation initiation factor IF-1, putative	0.83	0.32
244979_at	AtCg00750	ribosomal protein S11	0.97	0.57
244980_at	AtCg00760	ribosomal protein L36	1.20	0.78
244981_at	AtCg00770	ribosomal protein S8	1.17	0.72
244982_at	AtCg00780	ribosomal protein L14	1.47	0.64
244983_at	AtCg00790	ribosomal protein L16	1.13	0.67
244984_at	AtCg00800	ribosomal protein S3	1.03	0.71
244985_at	AtCg00810	ribosomal protein L22	1.23	0.91
244993_s_at	AtCg01000	hypothetical protein	0.83	0.67
245049_at	AtCg00050	ribosomal protein S16	0.67	0.23
Genes based on fold change as criteria				
258419_at	At3g16670	unknown protein	1.67	0.23
251438_s_at	At5g33355	putative protein	1.71	0.46
257008_at	At3g26920	myrosinase-associated protein	1.72	0.43
252612_at	At3g45160	hypothetical protein	1.73	0.50
247327_at	At5g64120	peroxidase	1.77	0.17
257891_at	At3g17170	hypothetical protein	1.78	0.30
254818_at	At4g12470	pEARLI 1-like protein	1.79	0.12
249894_at	At5g22580	unknown protein	1.83	0.43
254832_at	At4g12490	pEARLI 1-like	1.99	0.50
252170_at	At3g50480	hypothetical protein	2.04	0.18
254889_at	At4g11650	osmotin precursor	2.22	0.17
254805_at	At4g12480	pEARLI 1	2.23	1.06

260556_at	At2g43620	putative endochitinase	2.31	0.36
254819_at	At4g12500	pEARLI 1-like protein	2.37	1.06
259813_at	At1g49860	glutathione S-transferase	2.56	0.56
262119_s_at	At1g02930	glutathione S-transferase	2.74	0.57
247252_at	At5g64770	unknown protein	2.84	1.76
265588_at	At2g19970	putative pathogenesis-related protein	3.37	1.40

A total of 231 genes were up-regulated in *axr3-1* based on P-value criteria compared with five day-old WT green seedlings. Above genes were selected for possible involvement in auxin signaling and signal transduction, or fold change with an arbitrary cut-off value of 1.6 as $\log_2$ ratio. For full list of genes, see Appendix C. Average $\log_2$ ratio as a fold change is the average fold change value from three independent replicate experiments as $\log_2$ ratio (two in $\log_2$ means four-fold difference)

Table 4-2. Down-Regulated Genes in *Axr3-1* Compared to Five Day-Old WT Green Seedlings

Affy ID	TAIR ID	Description	Avg $\log_2$ ratio	Stdv
Possible regulatory proteins and genes involved in auxin signaling				
263664_at	At1g04430	IAA17/AXR3-1	-0.98	0.24
245593_at	At4g14550	IAA14/slr	-0.80	0.29
259596_at	At1g28130	auxin-regulated GH3 protein	-0.76	0.28
246375_at	At1g51830	light repressible receptor protein kinase	-3.08	1.00
246374_at	At1g51840	light repressible receptor protein kinase	-2.83	1.15
247170_at	At5g65530	putative protein similar to lectin-like protein kinase	-1.90	1.18
245711_at	At5g04340	putative c2h2 zinc finger transcription factor	-1.33	0.43
245731_at	At1g73500	similar to MAP kinase kinase 5	-1.53	0.31
246913_at	At5g25830	GATA zinc finger protein	-0.83	0.42
247655_at	At5g59820	zinc finger protein Zat12	-1.70	0.23
247708_at	At5g59550	putative COP1-interacting protein CIP8	-1.17	0.33
252278_at	At3g49530	NAC2-like protein	-0.8	0.42
258436_at	At3g16720	similar to RING-H2 zinc finger protein.	-0.83	0.27
260230_at	At1g74370	putative RING zinc finger protein; contains C3HC4 type	-1.27	0.22
263379_at	At2g40140	putative CCCH-type zinc finger protein	-1.83	0.58
267456_at	At2g33770	E2, ubiquitin-conjugating enzyme, putative	-0.93	0.38
261713_at	At1g32640	putative identical to bHLH protein	-1.36	0.38
265031_at	At1g61610	serine/threonine protein kinase	-1.23	0.24
250277_at	At5g12940	leucine rich repeat protein family	-1.57	0.13
253786_at	At4g28650	receptor protein kinase-like protein	-0.87	0.25
258207_at	At3g14050	putative GTP pyrophosphokinase	-0.86	0.30
264348_at	At1g12040	putative NPK1-related protein kinase 2	-0.52	0.13
245250_at	At4g17490	ethylene responsive element binding factor-like protein (AtERF6)	-3.56	0.77
251857_at	At3g54770	RNA binding protein - like SEB4 protein, Mus musculus	-2.84	0.71
254075_at	At4g25470	DREB1C involved in low-temperature-responsive gene expression	-2.01	0.84
260230_at	At1g74370	putative DNA-binding protein	-1.92	0.57
259328_at	At3g16440	similar to jasmonate inducible protein GB:Y11483	-1.70	0.42
257053_at	At3g15210	ethylene responsive element binding factor 4 (AtERF4)	-1.57	0.64
267028_at	At2g38470	putative WRKY-type DNA binding protein	-1.41	0.64
249626_at	At5g37540	putative protein nucleoide DNA-binding protein cnd41	-1.07	0.44
246099_at	At5g20230	blue copper binding protein	-0.92	0.25
245139_at	At2g45430	putative AT-hook DNA-binding protein	-0.72	0.23
Genes based on fold change as criteria				
246652_at	At5g35190	extensin -like protein extensin, soybean, PIR:T06782	-6.53	0.82
251226_at	At3g62680	proline-rich protein proline-rich protein	-5.20	0.41
252238_at	At3g49960	peroxidase ATP21a	-5.03	0.53
262045_at	At1g80240	hypothetical protein predicted by genemark.hmm	-4.06	0.87
247871_at	At5g57530	xyloglucan endotransglycosylase	-3.83	0.35
258745_at	At3g05920	unknown protein	-3.77	1.10
248636_at	At5g49050	putative protein similar to unknown protein	-3.76	1.32
259291_at	At3g11520	unknown protein similar to unknown protein	-3.49	0.52
246991_at	At5g67400	peroxidase (emb|CAA66967.1)	-3.43	1.88
265102_at	At1g31010	similar to cationic peroxidase	-3.41	0.72
258145_at	At3g18200	integral membrane protein	-3.23	0.23
261691_at	At1g50060	branched-chain amino acid aminotransferase	-3.16	1.17

246229_at	At4g37160	pectinesterase like protein	-3.06	1.08
258498_at	At3g02480	unknown protein similar to pollen coat protein	-3.06	1.30
261985_at	At1g33750	terpene synthase	-3.01	0.50
260553_at	At2g41800	unknown protein	-2.98	0.99
265049_at	At1g52060	jasmonate inducible protein	-2.88	0.49
265050_at	At1g52070	jasmonate inducible protein	-2.86	0.49
250469_at	At5g10130	pollen allergen -like protein SAH7 protein	-2.84	1.11
253998_at	At4g26010	putative peroxidase peroxidase ATP13a	-2.83	0.53
267457_at	At2g33790	putative proline-rich protein	-2.82	0.17
266514_at	At2g47890	putative zinc-finger protein (B-box zinc finger domain)	-2.80	0.58
247604_at	At5g60950	putative phytochelatin synthetase	-2.77	0.61
261562_at	At1g01750	actin depolymerizing factor	-2.70	1.10
255814_at	At1g19900	unknown protein	-2.63	1.35
254915s_at	At4g11290	cysteine proteinase	-2.52	0.50
253259_at	At4g34410	putative ethylene-responsive element binding protein	-2.46	0.99
248178_at	At5g54370	root cap protein 2-like protein	-2.44	0.38
249675_at	At5g35940	putative protein myrosinase-binding protein-like	-2.38	0.35
254044_at	At4g25820	putative xyloglucan endo-1,4-beta-D-glucanase	-2.37	0.44
264567s_at	At1g05250	putative peroxidase	-2.32	0.42
248252_at	At5g53250	putative protein	-2.32	0.45
247094_at	At5g66280	GDP-D-mannose 4,6-dehydratase	-2.31	0.27
250916_at	At5g03630	monodehydroascorbate reductase (NADH) - like protein	-2.29	0.31
253643_at	At4g29780	hypothetical protein	-2.21	0.95
255695_at	At4g00080	putative protein	-2.21	0.25
250778_at	At5g05500	unknown protein	-2.14	0.26
254718_at	At4g13570	putative protein disease resistance response protein 206-d	-2.13	0.50
261648_at	At1g27730	salt-tolerance zinc finger protein	-2.12	0.91
254120_at	At4g24780	putative mitochondrial uncoupling protein	-2.12	0.80
251668_at	At3g57010	putative protein strictosidine synthase (EC 4.3.3.2)	-2.03	0.39
256589_at	At3g28740	cytochrome P450	-2.02	0.31

A total of 292 genes were down-regulated in *axr3-1* based on P-value criteria compared with five day-old WT green seedlings. Above genes were selected for possible involvement in auxin signalings and signal transductions, or fold change with an arbitrary cut-off value of -2 as $\log_2$ ratio. For full list of genes, see Appendix D. Average $\log_2$ ratio as a fold change is the average fold change value from three independent replicate experiments as $\log_2$ ratio (-2 in $\log_2$ means 1/4 level compared with the original level).

Table 4-3. The Ontology Analysis of Genes Where Expression Changed in *Axr3-1* Compared to WT

Function\ Probe Sets	Down-Regulated Probe Set (292)*	Up-Regulated Probe set (231)*
Molecular Function	164	92
Cellular Component	172	118
Biological Process	81	50

* represents the number of genes where expression changed in *axr3-1* compared to WT.
The total number of genes in the probe set in ATH-1 chip (Affymetrix) is 22,810. Among them, 10,732 probes are annotated for molecular function, 10,732 probes are annotated for cellular component, and 6,181 probe are annotated for biological process, respectively.

Table 4-4. Up-and Down-Regulated Genes in Revertant, *Axr3-1R4* compared to Five Day-Old WT Green Seedlings

Affy ID	TAIR ID	Descriptions	*Axr3-1*	*Axr3-1R4 G*	*IAA17K G*
Up-Regulated Genes					
249866_at	At5g23010	2-isopropylmalate synthase-like	1.07	0.70	0.90
254687_at	At4g13720	cytochrome P450 monooxygenase (CYP83A1)	1.13	0.93	1.20
254805_at	At4g12480	pEARLI 1	2.27	0.80	1.17
257021_at	At3g19710	branched-chain amino acid aminotransferase	1.20	0.83	1.33
257823_at	At3g25190	integral membrane protein	0.53	0.87	0.47
260385_at	At1g74090	putative flavonol sulfotransferase	NC	0.73	0.87
Down-Regulated Genes					
244934_at	AtCg01080	NADH dehydrogenase ND6	NC	-1.13	NC
244977_at	AtCg00730	cytochrome b/f	NC	-1.33	NC
244994_at	AtCg01010	NADH dehydrogenase ND5	NC	-0.97	NC
245008_at	AtCg00360	hypothetical protein	NC	-1.43	NC
245015_at	AtCg00490	large subunit of riblose-1,5-bisphosphate carboxylase	NC	-1.07	NC
245016_at	AtCg00500	carboxytransferase beta subunit	NC	-0.73	NC
245026_at	AtCg00140	ATPase III subunit	NC	-0.83	NC
248564_at	At5g49700	putative similarity to AT-hook DNA-binding protein	NC	-0.50	NC
248877_at	At5g46140	putative protein	NC	-0.57	NC
258905_at	At3g06390	unknown protein	NC	-0.87	D
261569_at	At1g01060	putative similar to DNA binding protein CCA1	NC	-0.63	D
264204_at	At1g22710	putative sucrose transport protein	NC	-0.37	NC
264655_at	At1g09070	unknown protein Similar to Glycine SRC2	NC	-1.33	NC
266719_at	At2g46830	MYB-related transcription factor (CCA1)	NC	-0.47	NC

Genes were sorted based on P-value criteria compared with five day-old WT green seedlings. Average $\log_2$ ratio as a fold change is the average fold change value from three independent replicate experiments as $\log_2$ ratio (-2 in $\log_2$ means 1/4 level compared with the original level). G represents green seedlings. NC, not changed in gene expression by P-value criteria; I, Induced; D, down-regulated.

Table 4-5. Up- and Down-Regulated Genes in *IAA17K* compared to WT

Affy ID	TAIR I.D.	Descriptions	*Axr3-1*G	*Axr3-1R4*G	*IAA17K*G
Up-Regulated Genes					
244944_s_at	AtMg00090	ribosomal protein L16	NC	NC	0.63
244990_s_at	AtCg00870	hypothetical protein	NC	NC	2.17
246040_at	At5g19370	peptidyl-prolyl cis-trans isomerase - like protein	NC	NC	0.40
246099_at	At5g20230	blue copper binding protein	D	NC	1.20
247862_at	At5g58250	similar to unknown protein	NC	NC	0.60
249866_at	At5g23010	2-isopropylmalate synthase-like	I	I	0.90
250895_at	At5g03850	RIBOSOMAL PROTEIN S28- like	NC	NC	0.37
252413_at	At3g47370	40S ribosomal protein S20-like protein	I	NC	0.53
252592_at	At3g45600	mitogen-activated protein kinase 3	NC	NC	0.53
253308_at	At4g33670	putative protein aminotransferase (AspC family)	NC	NC	0.37
253908_at	At4g27260	GH3 like protein GH3 protein	NC	NC	0.50
254687_at	At4g13720	cytochrome P450 monooxygenase (CYP83A1)	I	I	1.20
254805_at	At4g12480	pEARLI 1	I	I	1.17
254818_at	At4g12470	pEARLI 1-like protein	I	NC	0.90
256825_at	At3g22120	similar to cell wall-plasma membrane linker protein	NC	NC	0.70
256940_at	At3g30720	unknown protein	NC	NC	3.23
257021_at	At3g19710	branched-chain amino acid aminotransferase	I	I	1.33
257823_at	At3g25190	integral membrane protein	I	I	0.47
258788_at	At3g11780	unknown protein	NC	NC	0.40
258900_at	At3g05590	putative 60S ribosomal protein	NC	NC	0.37
259013_at	At3g07430	unknown protein	I	NC	0.67
260385_at	At1g74090	putative flavonol sulfotransferase	NC	I	0.87
260429_at	At1g72450	unknown protein	I	NC	0.43
260847_s_at	At1g17290	alanine aminotransferase, putative	NC	NC	0.43
262119_s_at	At1g02930	glutathione S-transferase, putative	I	NC	1.03
266395_at	At2g43100	3-isopropylmalate dehydratase, small subunit	NC	NC	0.67
266587_at	At2g14880	unknown protein	I	NC	0.47
267564_at	At2g30740	putative protein kinase	NC	NC	0.47
Down-Regulated genes					
245889_at	At5g09480	PEE-rich protein	NC	NC	-0.83
246860_at	At5g25840	Putative protein	NC	NC	-0.50
248282_at	At5g52900	unknown protein	I	NC	-0.70
248790_at	At5g47450	membrane channel protein-like	D	NC	-0.73
249045_at	At5g44380	berberine bridge enzyme-like protein	D	NC	-0.63
249307_s_at	At5g41370	DNA excision repair cross-complementing protein	NC	NC	-0.87
250824_at	At5g05180	Putative protein	NC	NC	-1.20
253024_at	At4g38080	Putative protein	D	D	-0.70
253629_at	At4g30450	Glycine-rich cell wall structural protein	NC	NC	-0.47
254889_at	At4g11650	osmotin precursor	I	NC	-0.67
256674_at	At3g52360	unknown protein	NC	NC	-0.50
256994_s_at	At3g25830	putative similar to limonene cyclase	D	NC	-0.63
257506_at	At1g29440	auxin-induced protein	I	NC	-0.80
257790_at	At3g27090	putative similar to gda-1	NC	NC	-0.47
257895_at	At3g16950	dihydrolipoamide dehydrogenase	NC	NC	-0.33
258133_at	At3g24500	ethylene-responsive transcriptional coactivator	NC	NC	-0.93

258497_at	At3g02380	putative flowering-time gene CONSTANS (COL2)	NC	NC	-0.50
259140_at	At3g10230	lycopene beta cyclase	NC	NC	-0.30
260756_at	At1g48970	similar to guanine nucleotide exchange factor, eIF-2B	NC	NC	-0.47
261569_at	At1g01060	DNA-binding protein	NC	D	-1.10
261914_at	At1g65870	dirigent protein	NC	NC	-0.73
262238_at	At1g48300	hypothetical protein	D	NC	-0.43
263664_at	At1g04430	putative auxin-induced protein, IAA17/AXR3-1	D	D	-2.43
263796_at	At2g24540	unknown protein	NC	NC	-0.63
263985_at	At2g42750	unknown protein	NC	NC	-0.63
265817_at	At2g18050	histone H1	NC	NC	-0.73
265892_at	At2g15020	hypothetical protein	NC	NC	-1.20
265990_at	At2g24280	putative prolylcarboxypeptidase	NC	NC	-0.60
266363_at	At2g41250	hypothetical protein	NC	NC	-0.70

Genes were sorted based on P-value criteria compared with five day-old WT green seedlings. Average $\log_2$ ratio as a fold change is the average fold change value from three independent replicate experiments as $\log_2$ ratio (-2 in $\log_2$ means 1/4 level compared with the original level). G represents green seedlings. NC, not changed in gene expression by P-value criteria; I, Induced; D, down-regulated.

Table 4-6. Auxin Up-Regulated Genes in Five Day-Old WT Etiolated Seedlings

Affy ID	TAIR ID	Descriptions	Average log_2 ratio		
			WTe_I	*Axr3-1*e	*Axr3* -1e_I
251246_at	At3g62100	auxin-induced protein IAA30	1.53	0.00	0.97
261766_at	At1g15580	auxin-induced protein IAA5	2.53	-2.90	-0.63
257766_at	At3g23030	auxin-inducible gene (IAA2)	1.10	-0.40	0.40
258399_at	At3g15540	early auxin-induced protein, IAA19	1.10	-2.37	-0.47
255788_at	At2g33310	auxin regulated protein (IAA13)	1.20	-0.47	0.47
249109_at	At5g43700	auxin-induced protein AUX2-11/IAA4	0.63	-0.33	0.00
247148_at	At5g65670	auxin-induced protein IAA9	0.67	-0.23	0.30
245397_at	At4g14560	auxin-responsive protein IAA1	1.57	-0.63	0.07
253791_at	At4g28640	early auxin-inducible protein 11 (IAA11)	1.07	0.00	0.57
262099_s_at	At1g59500	auxin-regulated protein GH3	3.60	-1.07	2.67
253908_at	At4g27260	GH3 like protein GH3 protein	1.60	-1.03	0.97
254685_at	At4g13790	SAUR-AC - like protein	1.00	-2.73	-1.80
252970_at	At4g38850	small auxin up RNA (SAUR-AC1)	0.97	-1.70	-1.07
253103_at	At4g36110	high similarity to auxin-induced protein 15A	0.93	-1.40	-0.53
266611_at	At2g14960	putative auxin-regulated protein	3.20	-1.03	3.50
253066_at	At4g37770	ACC Synthase like	1.57	0.77	1.10
255177_at	At4g08040	ACC Synthase like	1.77	-3.13	-2.60
256981_at	At3g13380	brassinosteroid receptor kinase	1.20	-0.27	1.00
249467_at	At5g39610	NAM / CUC2 - like protein CUC2	1.07	-0.20	0.13
251643_at	At3g57550	guanylate kinase-like protein	0.67	0.03	0.27
247351_at	At5g63790	similarity to NAC-domain protein	0.63	-0.70	-0.50
255959_at	At1g21980	phosphatidylinositol-4-phosphate 5-kinase	0.87	0.03	0.43
250443_at	At5g10520	Pto kinase interactor - like protein	0.93	-0.37	0.17
264025_at	At2g21050	AUX1-like amino acid permease	0.77	0.17	0.80
259773_at	At1g29500	auxin-induced protein	0.67	-2.57	-2.33
264929_at	At1g60730	putative similar to auxin-induced atb2	1.23	-0.07	0.57
248163_at	At5g54510	auxin-responsive-like protein	1.77	-0.77	0.97
254665_at	At4g18340	beta-1,3-glucanase-like protein	0.77	-1.40	-0.90
249983_at	At5g18470	putative S-receptor kinase PK3 precurso	1.17	-0.53	-0.23
245528_at	At4g15530	pyruvate,orthophosphate dikinase	0.63	-0.10	0.03
264537_at	At1g55610	putative similar to CLV1 receptor kinase	0.77	-0.50	0.50
262971_at	At1g75640	receptor-like protein kinase	1.00	0.30	1.03
254409_at	At4g21400	serine/threonine protein kinase - like protein	0.70	0.60	0.53
258367_at	At3g14370	putative protein kinase	1.07	-0.83	-0.03
267083_at	At2g41100	calmodulin-like protein	1.07	-1.33	-0.10
266908_at	At2g34650	putative protein kinase	1.97	-0.13	1.03
267134_at	At2g23450	putative protein kinase	0.63	-0.13	0.10
266663_at	At2g25790	putative receptor-like protein kinase	1.03	-0.20	0.43
265144_at	At1g51170	putative serine/threonine protein kinase	1.37	-0.63	0.43
250820_at	At5g05160	receptor-like protein kinase	0.73	-0.53	0.10
253779_at	At4g28490	receptor-like protein kinase 5 precursor (RLK5)	1.30	-0.17	0.20
264479_at	At1g77280	similar to receptor-like protein kinase	0.70	-0.23	1.23
264788_at	At2g17880	putative DnaJ protein	0.83	-1.17	-0.40
263653_at	At1g04310	putative ethylene receptor (ERS2)	1.10	-0.17	0.13
248713_at	At5g48180	similarity to jasmonate inducible protein	0.63	-0.43	-0.03
261327_at	At1g44830	transcription factor, putative contains AP2 domain	0.70	-0.53	-0.30
261114_at	At1g75390	bZIP transcription factor ATB2	0.80	-0.47	-0.40
257643_at	At3g25730	AP2 domain transcription factor	0.87	-0.93	-0.63
253722_at	At4g29190	putative zinc finger transcription factor	0.80	0.07	0.50

255742_at	At1g25560	DNA-binding protein RAV2	0.83	-0.07	0.03
264788_at	At2g17880	putative DnaJ protein	0.83	-1.17	-0.40
249992_at	At5g18560	AP2 domain -like protein	1.20	-0.20	0.00
248253_at	At5g53290	similarity to PR genes transcriptional activator	1.23	-0.43	0.93
249087_at	At5g44210	DNA binding protein EREBP-3-like protein	0.80	-0.10	0.07
248801_at	At5g47370	homeobox-leucine zipper protein-like	1.90	-0.13	1.37
265084_at	At1g03790	hypothetical Cys3His zinc finger domain	1.10	-0.27	-0.10
255802_s_at	At4g10150	putative RING-H2 finger protein RHA1a	0.90	-1.57	-1.30
262001_at	At1g33790	myrosinase binding protein	1.13	0.20	0.80
258516_at	At3g06490	myb-related protein	0.70	0.13	0.43
253054_at	At4g37580	probable N-acetyltransferase hookless 1	0.70	-0.77	-0.50
259297_at	At3g05360	putative Cf-2 disease resistance protein	0.93	-0.47	-0.37
253722_at	At4g29190	putative zinc finger transcription factor	0.80	0.07	0.50

Plus 54 Unknown Proteins (Putative, Expressed Protein)

Genes were sorted first by P-value criteria from WT etiolated expression as baseline and then selected based on 0.6 average $\log_2$ ratio as a cut-off value from RNA of five day-old WT etiolated seedlings. Possible regulatory and auxin-related genes were selected for a total of 169 auxin up-regulated genes. A total of 54 unknown genes were auxin up-regulated from the total of 169 genes. For a full list of genes, see Appendix E. WTe_I, WT etiolated seedlings with auxin treatment; *axr3-1*e, *axr3-1* etiolated seedlings; *axr3-1*e_I, *axr3-1* etiolated seedlings with auxin treatment.

Table 4-7. Auxin Down-Regulated Genes in Five day-Old WT etiolated Seedlings

Affy ID	TAIR ID	Descriptions	Average log_2 ratio		
			WTe_I	*Axr3-1*e	*Axr3* -1e_I
251977_at	At3g53250	putative auxin-induced protein 6B	-1.43	-0.97	-1.63
251857_at	At3g54770	RNA binding protein - like SEB4 protein	-1.40	-1.17	-1.40
254606_at	At4g19030	nodulin-26 - like protein	-1.37	-0.27	-1.13
245196_at	At1g67750	F12A21.12 similar to pectate lyase like protein	-1.33	-0.50	-0.03
246002_at	At5g20740	ripening-related protein	-1.33	-1.20	-2.00
254820_s_at	At4g12520	pEARLI 1-like protein	-1.27	-0.87	-1.13
265443_at	At2g20750	beta-expansin	-1.27	-0.67	-1.00
250500_at	At5g09530	periaxin - like protein periaxin	-1.23	1.23	1.23
263841_at	At2g36870	putative xyloglucan endo-transglycosylase	-1.23	0.43	-0.07
258003_at	At3g29030	expansin At-EXP5 identical	-1.17	-0.87	-0.73
258321_at	At3g22840	early light-induced protein	-1.17	1.27	1.27
258589_at	At3g04290	putative GDSL-motif lipase/acylhydrolase	-1.13	-0.40	-0.93
260097_at	At1g73220	putative transporter	-1.13	0.23	-0.53
262830_at	At1g14700	purple acid phosphatase	-1.13	-0.23	-0.63
248727_at	At5g47990	cytochrome P450	-1.10	-1.80	-2.53
258239_at	At3g27690	putative chlorophyll A-B binding protein	-1.10	0.20	0.23
262736_at	At1g28570	lipase	-1.03	0.10	-0.50
262733_s_at	At1g28670	lipase	-1.03	-0.23	-0.60
254056_at	At4g25250	putative Group I Pectinesterase	-1.00	-1.17	-1.80
255298_at	At4g04840	putative transcriptional regulator	-1.00	0.13	-0.60
263034_at	At1g24020	pollen allergen-like protein	-1.00	0.80	0.37
248844_s_at	At5g46900	extA	-0.97	-1.47	-1.77
253050_at	At4g37450	putative probable arabinogalactan protein precursor	-0.97	0.00	-0.03
263595_at	At2g01890	putative purple acid phosphatase	-0.97	-1.23	-2.20
250207_at	At5g13930	chalcone synthase (naringenin-chalcone synthase)	-0.93	1.20	1.10
255433_at	At4g03210	putative xyloglucan endotransglycosylase	-0.93	0.80	0.40
252536_at	At3g45700	putative transporter protein	-0.93	-0.20	-0.57
252534_at	At3g46130	Myb DNA binding protein -like	-0.90	-0.20	-1.87
253667_at	At4g30170	peroxidase ATP8a	-0.90	-0.70	-1.17
258181_at	At3g21670	nitrate transporter identical to nitrate transporter	-0.90	0.43	-0.40
260266_at	At1g68520	putative B-box zinc finger protein	-0.90	0.70	0.43
248921_at	At5g45950	GDSL-motif lipase/hydrolase-like protein	-0.87	-0.60	-0.90
260806_at	At1g78260	RNA recognition motif-containing protein	-0.87	-0.60	-0.73
254024_at	At4g25780	putative pathogenesis-related protein	-0.87	0.00	-0.43
253794_at	At4g28720	putative dimethylaniline monooxygenase	-0.83	0.97	0.67
254573_at	At4g19420	putative pectinacetylesterase precursor	-0.83	-0.37	-0.73
259391_s_at	At1g06350	putative similar to delta 9 desaturase	-0.83	-1.47	-1.70
262128_at	At1g52690	late embryogenesis-abundant protein	-0.83	-3.10	-3.43
246481_s_at	At5g15960	cold and ABA inducible protein kin1	-0.80	-0.47	-0.70
247406_at	At5g62920	response regulator 6 (ARR6)	-0.80	-0.10	-1.00
250582_at	At5g07580	ethylene responsive element binding factor 5	-0.80	0.07	-0.37
251814_at	At3g54890	chlorophyll a/b-binding protein	-0.80	-0.13	-0.23
252130_at	At3g50820	putative PSII oxygen-evolving complex	-0.80	0.33	0.43
254644_at	At4g18510	putative CLAVATA3/ESR-Related-2 (CLE2)	-0.80	-1.87	-2.00
256275_at	At3g12110	actin 11 (ACT11) identical to actin 11 (ACT11)	-0.80	-1.10	-1.30

256321_at	At1g55020	lipoxygenase	-0.80	0.17	-0.13
259276_at	At3g01190	putative peroxidase very similar to peroxidase	-0.80	-2.00	-1.93
264501_at	At1g09390	putative lipase Similar to nodulins and lipase	-0.80	-0.23	-0.57
264839_at	At1g03630	putative protochlorophyllide reductase	-0.77	0.33	0.27
245736_at	At1g73330	Dr4(protease inhibitor)	-0.77	0.93	0.97
251714_at	At3g56370	putative leucine-rich receptor-like protein kinase	-0.77	0.13	-0.07
255962_at	At1g22335	glycine-rich RNA-binding protein	-0.77	-0.50	-0.67
258497_at	At3g02380	putative flowering-time gene CONSTANS2	-0.77	0.60	0.43
259840_at	At1g52230	photosystem I subunit VI precursor	-0.77	0.13	0.20
262608_at	At1g14120	dioxygenase-like protein	-0.77	-1.83	-2.10
266790_at	At2g28950	expansin AtEx6	-0.77	-0.03	-0.17
251031_at	At5g02120	one helix protein (OHP)	-0.73	-0.10	-0.07
247246_at	At5g64620	invertase inhibitor homolog	-0.70	0.43	0.20
252363_at	At3g48460	lipase - like protein lipase Arab-1	-0.70	-2.03	-2.20
254119_at	At4g24780	putative pectate lyase pectate lyase	-0.70	-0.17	-0.10
261727_at	At1g76090	S-adenosyl-methionine-sterol-C-methyltransferase	-0.70	-0.70	-0.87
267635_at	At2g42220	rhodanese-like family protein	-0.70	0.43	0.47
253790_at	At4g28660	photosystem II protein W - like	-0.67	0.63	0.60
255127_at	At4g08300	nodulin-like protein nodulin gene	-0.67	0.00	-0.57
257066_at	At3g18280	lipid transfer protein	-0.67	0.90	0.83
261769_at	At1g76100	plastocyanin	-0.67	0.20	0.13
262516_at	At1g17190	putative glutathione transferase	-0.67	-1.23	-1.33
262826_at	At1g13080	putative cytochrome P450 monooxygenase	-0.67	-0.03	-0.67
264857_at	At1g24170	putative glycosyl transferase	-0.67	0.17	-0.13
266873_at	At2g44740	putative PREG1-like negative regulator	-0.67	-0.80	-0.83
245242_at	At1g44446	chlorophyll a oxygenase	-0.63	0.20	-0.03
246011_at	At5g08330	putative auxin-induced basic helix-loop-helix TF	-0.63	-0.47	-0.73
252168_at	At3g50440	putative pir7a protein	-0.63	0.67	0.30
252711_at	At3g43720	lipid-transfer protein-like protein	-0.63	-0.80	-1.07
253362_s_at	At4g33110	putative cyclopropane-fatty-acyl-phospholipid synthase	-0.63	-0.20	-0.40
253684_at	At4g29690	nucleotide pyrophosphatase - like protein	-0.63	-0.63	-0.90
255302_at	At4g04830	putative transcriptional regulator	-0.63	0.37	-0.47
255506_at	At4g02130	predicted glycosyl transferase similar to lgtC	-0.63	-0.03	-0.10
255732_at	At1g25450	fatty acid condensing enzyme CUT1	-0.63	-0.07	-0.37
255942_at	At1g22360	UDP-glucose glucosyltransferase	-0.63	-0.63	-0.90
256309_at	At1g30380	photosystem I subunit X precursor	-0.63	0.23	0.40
259892_at	At1g72610	germin-like protein	-0.63	-0.03	0.00
261768_at	At1g15550	gibberellin 3 beta-hydroxylase	-0.63	-0.23	-0.67
266636_at	At2g35370	glycine decarboxylase complex H-protein	-0.63	0.43	0.43
Plus 59 Unknown proteins					

Genes were sorted first by P-value criteria from WT etiolated expression as baseline and then selected 0.6 average $\log_2$ ratio as a cut-off value from RNA of five day-old WT etiolated seedlings. A total of 59 unknown genes were auxin up-regulated from the 169 genes. For Full list of genes, see Appendix F. WTe_I, WT etiolated seedlings with auxin treatment; *axr3-1*e, *axr3-1* etiolated; *Axr3-1*e_I, *Axr3-1 etiolated* seedlings with auxin treatment.

Table 4-8. Auxin-Responsive Genes Which also Respond to Light

Affy ID	TAIR ID	Discription	Auxin Response [1)]			Light [2)] Response
			WTe_I	*Axr3*-1e	*Axr3*-1e_*I*	WT
Auxin Up- and Light Down-Regulated Genes (Total 57 Genes)						
245233_at	At4g25580	putative low-temperature-induced protein 65	0.93	0.13	0.57	-2.63
245277_at	At4g15550	glucosyltransferase like protein	0.97	-0.40	-0.37	-1.23
245528_at	At4g15530	pyruvate,orthophosphate dikinase	0.63	-0.10	0.03	-1.83
245821_at	At1g26270	similar to putative ubiquitin	0.70	-0.30	-0.07	-1.33
245947_at	At5g19530	spermine synthase (ACL5)	1.00	-0.13	0.53	-1.03
247023_at	At5g67060	unknown protein	1.10	-1.13	-0.87	-3.73
247474_at	At5g62280	putative protein	0.80	-0.20	0.00	-2.37
248162_at	At5g54500	quinone reductase, putative	0.80	-0.77	-0.20	-0.93
248213_at	At5g53660	putative protein	0.67	-0.93	-0.73	-2.63
248563_at	At5g49690	anthocyanidin-3-glucoside rhamnosyltransferase	1.10	-0.83	-0.53	-3.07
248713_at	At5g48180	putative similarity to jasmonate inducible protein	0.63	-0.43	-0.03	-1.70
248801_at	At5g47370	homeobox-leucine zipper protein-like	1.90	-0.13	1.37	-1.23
249065_at	At5g44260	putative protein	1.07	0.00	0.43	-2.77
249109_at	At5g43700	auxin-induced protein AUX2-11/IAA4	0.63	-0.33	0.00	-1.50
249467_at	At5g39610	NAM / CUC2 - like protein	1.07	-0.20	0.13	-1.40
250182_at	At5g14470	putative protein	0.70	0.23	0.60	-3.53
250201_at	At5g14230	ankyrin - like protein	0.93	-0.10	-0.07	-2.10
251144_at	At5g01210	anthranilate N-benzoyltransferase - like protein	0.97	0.00	0.37	-1.53
251436_at	At3g59900	putative protein	0.93	-1.37	-1.00	-1.57
251643_at	At3g57520	glycosyl hydrolase family 36	0.67	0.03	0.27	-0.90
253011_at	At4g37890	putative retrotransposon -like protein	1.10	-1.33	-0.67	-1.37
253054_at	At4g37470	putative beta-ketoadipate enol-lactone hydrolase	0.70	-0.77	-0.50	-2.67
253065_at	At4g37740	putative protein	0.90	-1.37	-1.30	-1.63
253103_at	At4g36110	high similarity to auxin-induced protein 15A	0.93	-1.40	-0.53	-2.47
253155_at	At4g35720	putative protein	0.67	-0.83	-0.50	-4.43
253722_at	At4g29190	putative zinc finger transcription factor	0.80	0.07	0.50	-1.43
253908_at	At4g27260	GH3 like protein	1.60	-1.03	0.97	-1.30
253994_at	At4g26080	protein phosphatase ABI1	1.00	-0.50	-0.33	-0.67
254409_at	At4g21400	serine threonine protein kinase - like	0.70	0.60	0.53	-1.37
254665_at	At4g18340	glycosyl hydrolase family 17	0.77	-1.40	-0.90	-1.30
254685_at	At4g13850	glycine-rich RNA-binding protein AtGRP2	1.00	-2.73	-1.80	-3.10
255028_at	At4g09890	putative protein	0.77	-0.60	-0.07	-1.93
255543_at	At4g01990	expressed protein	0.63	-0.83	-0.50	-0.93
255788_at	At2g33310	auxin regulated protein (IAA13)	1.20	-0.47	0.47	-1.43
257643_at	At3g25710	bHLH protein	0.87	-0.93	-0.63	-1.40
257766_at	At3g23030	auxin-inducible gene (IAA2)	1.10	-0.40	0.40	-1.53
257975_at	At3g20820	disease resistance protein family (LRR) Cf-2.1	1.13	-0.57	-0.07	-0.70
258075_at	At3g25900	homocysteine S-methyltransferase AtHMT-1	0.83	-0.93	-0.47	-1.07
258253_at	At3g26760	putative short chain alcohol dehydrogenase	1.10	-0.33	0.80	-1.17
258367_at	At3g14370	putative protein kinase	1.07	-0.83	-0.03	-1.80
258399_at	At3g15540	early auxin-induced protein, IAA19	1.10	-2.37	-0.47	-2.20
258878_at	At3g03170	expressed protein	0.77	-0.83	0.03	-1.13
260900_s_t	At1g21400	branched-chain alpha keto-acid dehydrogenase	0.83	-0.17	0.03	-2.67
262001_at	At1g33790	myrosinase binding protein, putative	1.13	0.20	0.80	-1.13

262525_at	At1g17060	cytochrome P450, putative	0.83	-0.47	0.47	-1.37
262643_at	At1g62770	expressed protein	0.73	0.87	1.27	-2.37
263653_at	At1g04330	unknown protein	1.10	-0.17	0.13	-1.70
264777_at	At1g08630	similar to L-allo-threonine aldolase	0.63	0.27	0.23	-1.87
264788_at	At2g17840	putative senescence-associated protein 12	0.83	-1.17	-0.40	-1.60
265084_at	At1g03830	hypothetical protein	1.10	-0.27	-0.10	-3.87
266017_at	At2g18690	expressed protein	0.63	-1.63	-1.73	-1.23
266364_at	At2g41230	unknown protein	1.53	-0.47	-0.37	-2.33
266507_at	At2g47860	unknown protein	1.37	-0.80	0.57	-2.63
266974_at	At2g39620	hypothetical protein	2.20	-0.10	1.87	-4.53
267008_at	At2g39350	ABC transporter family protein	0.73	-0.77	-0.27	-2.07
267230_at	At2g44080	unknown protein	0.73	-0.60	-0.43	-1.60
267337_at	At2g19310	putative small heat shock protein	1.20	-0.73	-0.20	-2.73

Auxin Up- and Light Up-Regulated Genes (Total 4 Genes)

248028_at	At5g55630	outward rectifying potassium channel KCO	0.70	0.90	0.90	2.63
248282_at	At5g52900	expressed protein	1.17	0.80	1.70	1.40
250327_at	At5g12050	putative serine rich protein	0.90	0.93	1.67	2.07
256024_at	At1g58340	expressed protein	0.97	0.43	1.77	0.83

Auxin Down- and Light Down-Regulated Genes (Total 25 Genes)

245196_at	At1g67750	polysaccharide lyase family 1 (pectate lyase)	-1.33	-0.50	-0.03	-1.90
245306_at	At4g14690	Expressed protein	-1.10	0.40	0.23	-0.83
245637_at	At1g25230	putative purple acid phosphatase precursor	-0.70	-0.67	-0.73	-1.40
246825_at	At5g26260	putative protein	-0.70	-0.17	-0.17	-0.83
247246_at	At5g64620	invertase inhibitor homolog	-0.70	0.43	0.20	-2.00
247946_at	At5g57180	putative protein	-0.87	0.10	-0.17	-1.53
248727_at	At5g47980	acyltransferase family	-1.10	-1.80	-2.53	-3.57
249750_at	At5g24570	expressed protein	-0.63	0.27	0.10	-1.03
253362_s_t	At4g33110	cyclopropane-fatty-acyl-phospholipid synthase	-0.63	-0.20	-0.40	-0.73
254193_at	At4g23850	acyl-CoA synthetase - like protein	-0.67	-0.83	-0.73	-2.07
254644_at	At4g18510	putative; CLAVATA3/ESR-Related-2 (CLE2)	-0.80	-1.87	-2.00	-2.27
254954_at	At4g10910	expressed protein	-0.87	-0.37	-0.53	-1.43
255127_at	At4g08300	nodulin-like protein	-0.67	0.00	-0.57	-2.17
255942_at	At1g20350	MT inner membrane translocase component	-0.63	-0.63	-0.90	-0.93
256626_at	At3g20015	hypothetical protein	-0.97	-0.70	-1.57	-1.03
257066_at	At3g18280	lipid transfer protein	-0.67	0.90	0.83	-1.33
259391_s_t	At1g06340	hypothetical protein	-0.83	-1.47	-1.70	-2.53
260097_at	At1g73220	putative organic cation transporter 3	-1.13	0.23	-0.53	-5.13
262128_at	At1g52690	late embryogenesis-abundant protein	-0.83	-3.10	-3.43	-2.37
262236_at	At1g48330	similar to hypothetical protein	-1.10	-0.97	-0.77	-1.87
262516_at	At1g17190	glutathione transferase	-0.67	-1.23	-1.33	-1.27
263098_at	At2g16070	expressed protein	-1.03	-1.97	-2.07	-1.87
265067_at	At1g03850	expressed protein	-0.70	-0.77	-0.93	-1.70
265296_at	At2g14060	SAM: carboxyl methyltransferase family	-0.87	-1.83	-2.03	-0.87
267209_at	At2g30930	expressed protein	-0.67	-1.17	-1.20	-1.50

Auxin Down- and Light Up-Regulated Genes (Total 45 Genes)

245242_at	At1g44446	chlorophyll a oxygenase	-0.63	0.20	-0.03	1.43
245304_at	At4g15630	expressed protein	-0.80	-0.07	-0.43	0.73

245736_at	At1g73330	Dr4(protease inhibitor	-0.77	0.93	0.97	1.40
246011_at	At5g08330	putative auxin-induced bHLHtranscription factor	-0.63	-0.47	-0.73	0.80
247899_at	At5g57345	Expressed protein	-0.63	0.60	0.50	1.63
248683_at	At5g48490	putative protein	-1.73	-0.07	-0.27	1.50
248921_at	At5g45950	GDSL-motif lipase/hydrolase-like protein	-0.87	-0.60	-0.90	1.40
249876_at	At5g23060	putative protein	-0.70	1.00	1.07	2.37
250207_at	At5g14040	mitochondrial phosphate translocator	-0.93	1.20	1.10	2.30
250500_at	At5g09530	surface protein PspC-related	-1.23	1.23	1.23	2.33
251031_at	At5g02120	one helix protein (OHP	-0.73	-0.10	-0.07	1.73
251714_at	At3g56140	chloroplast lumen common protein family	-0.77	0.13	-0.07	1.00
251814_at	At3g54890	light-harvesting chlorophyll a/b binding protein	-0.80	-0.13	-0.23	1.40
252130_at	At3g50890	putative protein	-0.80	0.33	0.43	2.43
252711_at	At3g43720	lipid-transfer protein-like protein	-0.63	-0.80	-1.07	0.57
253024_at	At4g38080	extensin related	-1.07	1.73	1.80	2.43
253790_at	At4g28660	photosystem II protein W - like	-0.67	0.63	0.60	1.57
254119_at	At4g24640	Bnm1 like protein	-0.70	-0.17	-0.10	1.17
255298_at	At4g04840	putative protein	-1.00	0.13	-0.60	3.70
256275_at	At3g12110	actin 11 (ACT11)	-0.80	-1.10	-1.30	1.30
256309_at	At1g30380	photosystem I subunit X precursor	-0.63	0.23	0.40	1.03
257204_at	At3g23805	Expressed protein	-0.83	-0.30	-0.57	1.17
257673_at	At3g20290	expressed protein	-0.63	0.10	-0.10	1.70
258003_at	At3g29030	expansin (At-EXP5)	-1.17	-0.87	-0.73	1.43
258181_at	At3g21670	nitrate transporter	-0.90	0.43	-0.40	2.67
258239_at	At3g27690	light harvesting chlorophyll A/B binding protein	-1.10	0.20	0.23	1.63
258497_at	At3g02380	Zinc finger protein CONSTANS-LIKE 2 (COL2	-0.77	0.60	0.43	3.43
259840_at	At1g52230	photosystem I subunit VI precurso	-0.77	0.13	0.20	1.17
259892_at	At1g72610	germin-like protein	-0.63	-0.03	0.00	0.80
260877_at	At1g21500	expressed protein	-0.83	0.20	0.10	2.13
261488_at	At1g14345	Expressed protein	-0.80	0.30	0.10	2.27
261746_at	At1g08380	expressed protein	-0.63	0.17	0.30	1.30
262168_at	At1g74730	expressed protein	-0.67	0.43	0.33	1.97
262399_at	At1g49350	expressed protein	-0.63	0.00	0.03	0.60
262826_at	At1g13080	cytochrome p450 family	-0.67	-0.03	-0.67	0.80
263034_at	At1g24020	Bet v I allergen family	-1.00	0.80	0.37	1.20
263841_at	At2g36870	xyloglucan endotransglycosylase, putative	-1.23	0.43	-0.07	1.33
264839_at	At2g17360	putative ribosomal protein S4	-0.77	0.33	0.27	1.53
264857_at	At2g17370	3-hydroxy3methylglutaryl-coenzyme A reductase	-0.67	0.17	-0.13	0.87
266636_at	At2g35370	glycine decarboxylase complex H-protein	-0.63	0.43	0.43	0.87
266790_at	At2g29020	expressed protein	-0.77	-0.03	-0.17	0.80
266899_at	At2g34620	hypothetical protein	-0.80	1.10	0.90	2.77
266979_at	At2g39430	disease resistance response protein-related	-0.70	0.37	0.40	1.83
267294_at	At2g23670	expressed protein	-0.87	0.43	0.30	1.87
267635_at	At2g42220	rhodanese-like domain protein	-0.70	0.43	0.47	1.53

1) The level of auxin-responsive gene expression was sorted by P-value criteria from base line chip data from RNA samples of WT etiolated seedlings. WTe_I, WT etiolated seedlings with auxin treatment; *Axr3-1*e, *Axr3-1* etiolated seedlings; *Axr3-1*e_I, *Axr3-1* etiolated seedlings with auxin treatment.
2) The level of light-response in gene expression was sorted by P-value criteria from base line chip data from RNA of WT green seedlings. Therefore, a positive value means that the expression level was repressed by light. All values represent average $\log_2$ ratio.

Figure 4-1. Phenotypes of Five Day-Old Seedlings.

A, Five day-old plants grown in 17 hr light:7 hr dark cycle
B, Five day-old etiolated seedlings

A

WT *Axr3-1* *Axr3-1R4* *IAA17K*

B

Figure 4-2. Scatter Plots of Hybridization Intensities of Various Samples.

Plots were generated from raw hybridization intensities (numbers) before global normalization. Red dots represent Present; Blue dots represent Marginal; Yellow dots represent Absent.

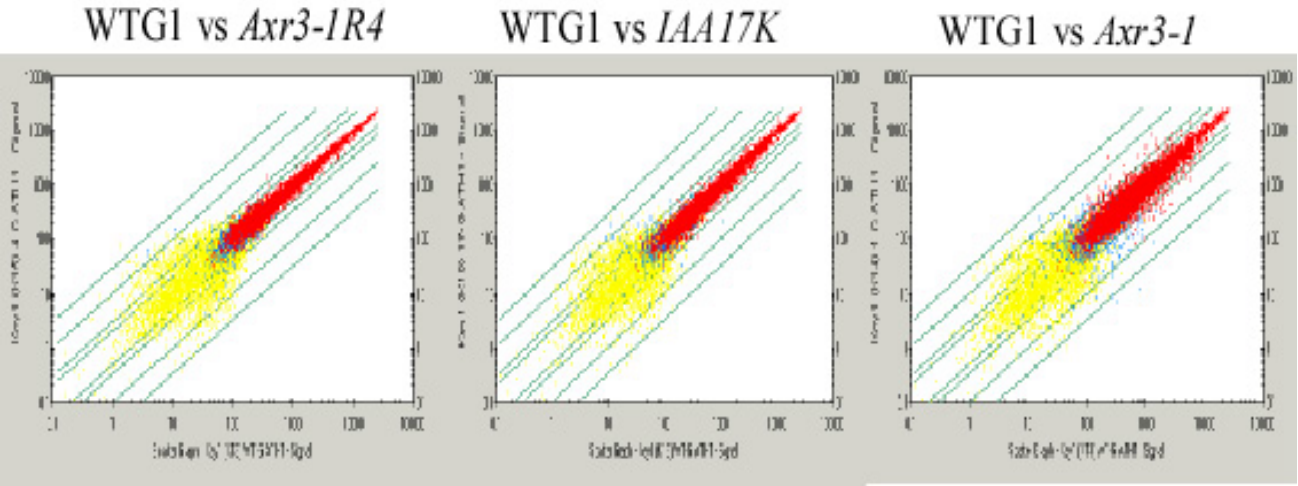
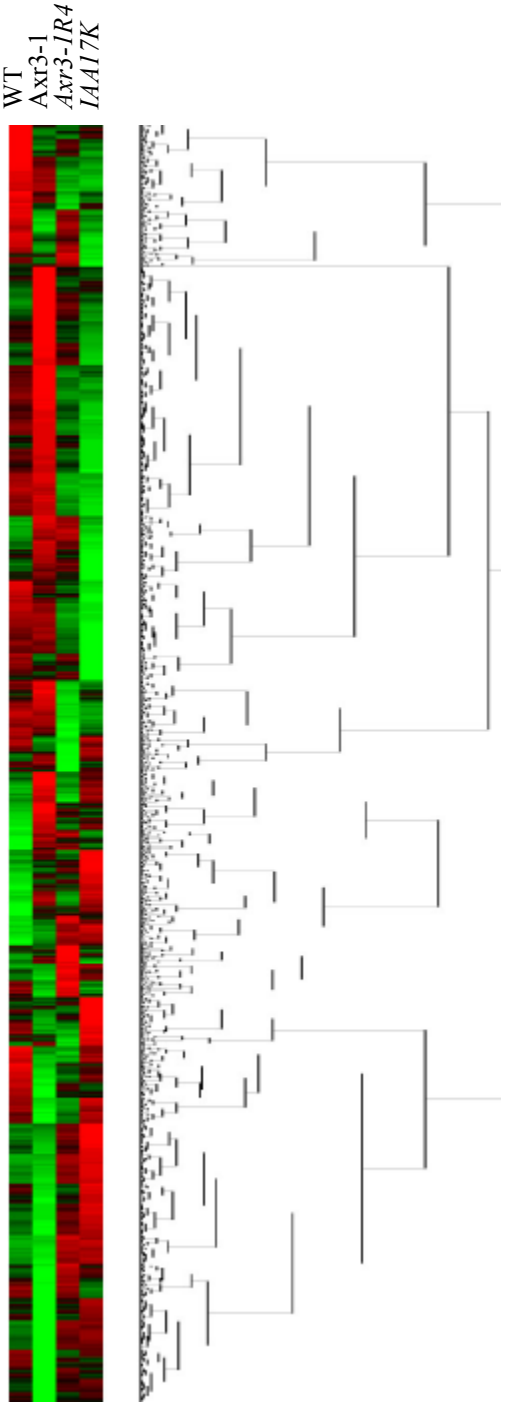

WTG1 vs *Axr3-1R4*
WTG1 vs *IAA17K*
WTG1 vs *Axr3-1*

Figure 4-3. Hierarchical (A) and Selected K-Means (B) Clustering of Global Transcriptional Profiles from WT, *Axr3-1*, *Axr3-1R4*, *IAA17K*

Hierarchical clustering was generated with uncentered distance with average linkage. K-Means clustering was generated with Euclidean distance. A total of 10,338 genes were used for clustering from the four different genetic backgrounds. Here, K-Means cluster is one subtree showing gene expression up-regulated in *axr3-1* and down-regulated in *IAA17K* compared to WT and *axr3-1R4*. Green color represents down-regulated gene expressions, and red color represents up-regulated gene expressions.

A.


WT
Axr3-1
Axr3-1R4
IAA17K

B.

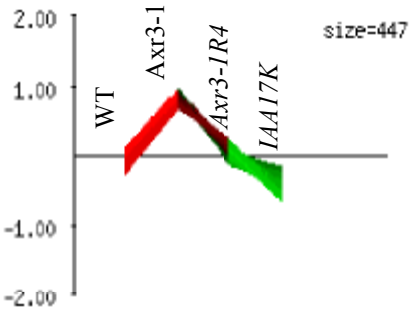

2.00
1.00
-1.00
-2.00
WT
Axr3-1
Axr3-1R4
IAA17K
size=447

Figure 4-4. Transcriptional Profiles of Auxin Up-Regulated Genes in *Axr3-1* Compared to Those of Etiolated WT.

Auxin up-regulated genes were sorted by P-value criteria in etiolated WT by auxin treatment, and average $\log_2$ ratio as a fold change were divided by average signal intensities compared to those of WT.

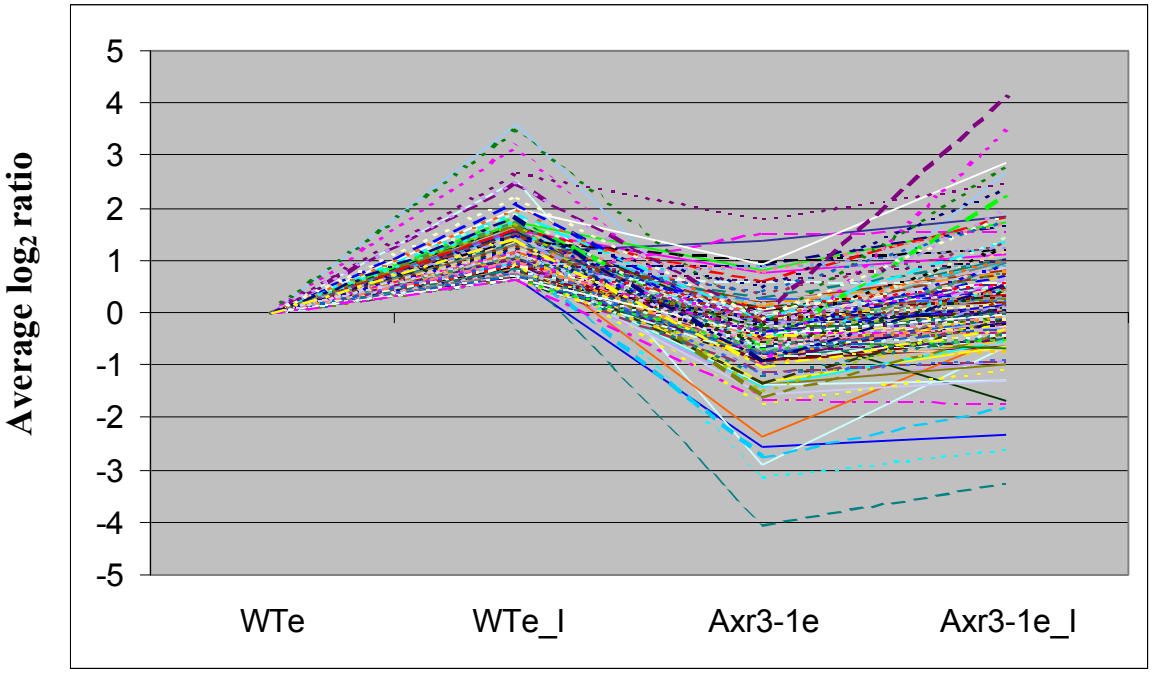

Average log2 ratio
5
4
3
2
1
0
-1
-2
-3
-4
-5
WTe
WTe_I
Axr3-1e
Axr3-1e_I

Figure 4-5. Transcriptional Profiles of Auxin Down-Regulated Genes in *Axr3-1* Compared to those of Etiolated WT.

Auxin down-regulated genes were sorted by P-value criteria in etiolated WT by auxin treatment, and Average $\log_2$ ratio as fold change were divided by average signal intensities compared to those of WT.

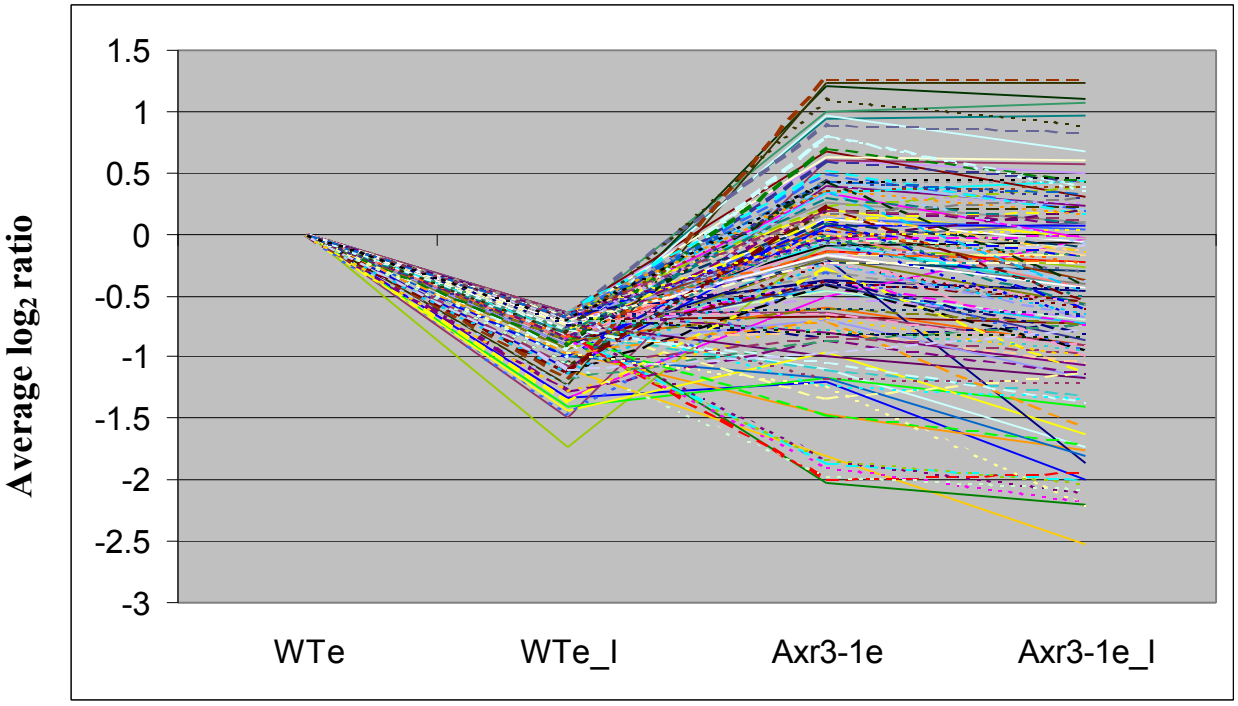
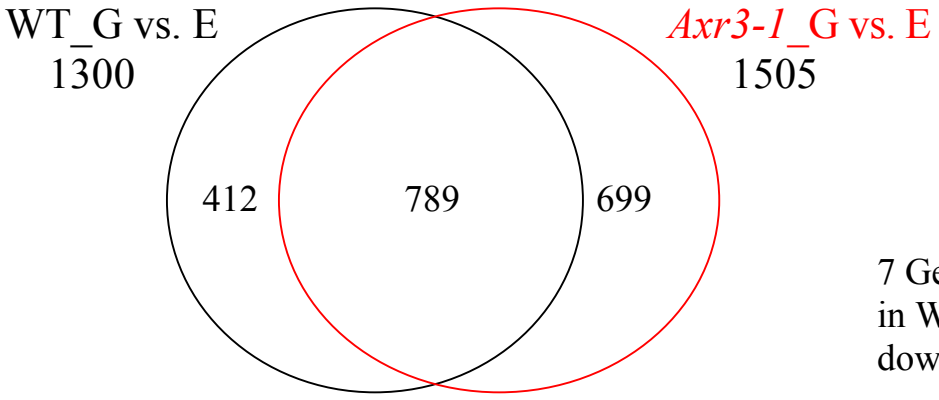

Average log2 ratio
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
WTe
WTe_I
Axr3-1e
Axr3-1e_I

Figure 4-6. Global Transcriptional Profiles of WT and *Axr3-1* for Light Response.

GeneChip expression data of light-grown and etiolated WT were pair-wise compared from three independent repeats and then sorted (P-value criteria), and then again sorted by average $\log_2$ ratio by 0.8 as a fold change (signal difference criteria). WT_G vs E means etiolated data was compared to light-grown data. For examples, WT_G vs E (1208) means 1283 genes were up-regulated in etiolated WT compared to light-grown WT. The mutant (*axr3-1*) data were generated the same way as WT.

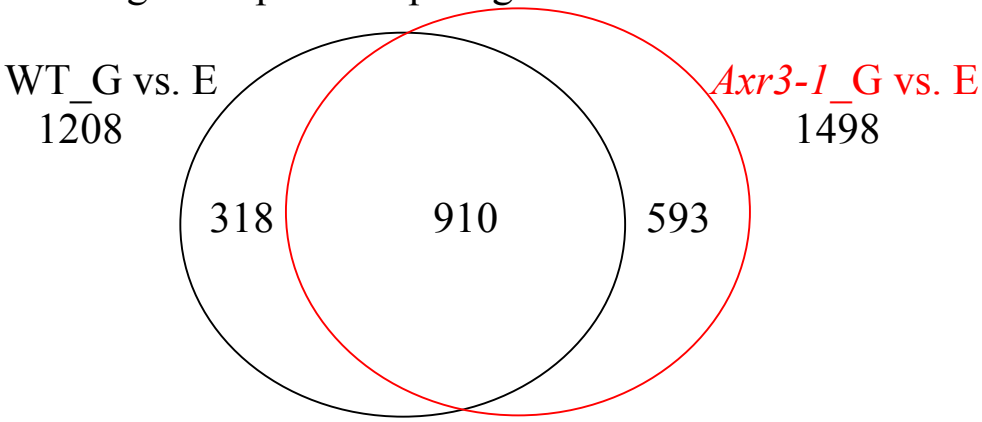
Light Response: Down-Regulated Genes



7 Genes were common in WT up and *axr3-1* down

2 Genes were common in WT down and *axr3-1* up

Light Response: Up-Regulated Genes



References

Abel S, Nguyen MD, Theologis A (1995). The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. J. Mol. Biol. 251, 533-549

Abel S, Theologis A (1996). Early genes and auxin action. Plant Physiol. **111**, 9-17

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997). Genes involved in organ separation in Arabidopsis: An analysis of the cup-shaped cotyledon mutant. Plant Cell **9:** 841–857

Barkan A, Goldschmidt-Clermont M (2000). Participation of nuclear genes in chloroplast gene expression. Biochemie **82**:559-572

Baulcombe D, Giorgini J, Key JL (1980). The effect of auxin on the polyadenylated RNA of soybean hypocotyls *In* Nato Advanced Studies Institute Published in Genome Organization and Expression in Plants edited by CJ Leaver Plenum Press, pp 175-185

Baulcombe DC, Key JL (1980). Polyadenylated RNA sequences which are reduced in concentration following auxin treatment of soybean hypocotyls. J. Biol. Chem. 255:8907-8913

Behringer FJ, Davies PJ (1992). Indole-3-acetic acid levels after phytochrome-mediated changes in the stem elongation rate of dark- and light-grown *Pisum* seedlings. Planta **188:** 85-92

Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN (2003). A gene expression map of the Arabidopsis root. Science **302**:1956-1960

Borevitz JO, Liang D, Plouffe D, Chang HS, Zhu T, Weigel D, Berry CC, Winzeler E, Chory J (2003). Large-scale identification of single-feature polymorphisms in complex genomes. Genome Research **13**: 513-23

Borevitz JO, Nordborg M (2003). The impact of genomics on the study of natural variation in arabidopsis. Plant Physiol. **132**: 718-25

Carson JA, Nettleton D, Reecy JM (2002). Differential gene expression in the rat soleus muscle during early work overload-induced hypertrophy. FASEB J. **16**: 207-209

Chen W, Provart NJ, Glazebrook J (2002). Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell **14**: 559-574

Chen JG, Ullah H, Young JC, Sussman MR, Jones AM (2001). ABP1 is required for organized cell elongation and division in Arabidopsis embryogenesis. Genes Dev. **15**: 902-911

Christensen S, Dagenais N, Chory J, Weigel D (2000). Regulation of auxin response by the protein kinase PINOID. Cell **100**: 469-478

Colon-Carmona A, Chen DL, Yeh KC, Abel S (2000). Aux/IAA proteins are phosphorylated by phytochrome in vitro. Plant Physiol. **124**:1728-38

Conner T, Goekjian V, LaFayette P, Key J (1990). Structure and expression of two auxin-inducible genes from *Arabidopsis*. Plant Mol. Bio. **15**:623-632

DeLong A, Mockaitis K, Christen S (2002). Protein phosphorylation in the delivery of and response to auxin signal. Plant Mol. Biol. **49** :285-303

Firn RD (1994). Phototropism. *In* Kendrick RE, Kronrnberg GHM, eds, Photomorphogenesis in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 659–681

Fukaki H, Tameda S, Masuda H, Tasaka M (2002). Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsi*s. Plant J. **29**:153-168

Gee MA, Hagen G, Guilfoyle TJ (1991). Tissue-specific and organ-specific expression of soybean auxin-responsive transcripts GH3 and SAURs. Plant Cell **3**:419-430

Gil P, Green PJ (1997). Regulatory activity exerted by the *SAUR-AC1* promoter region in transgenic plants. Plant Mol. Biol. **34**: 803–808

Girke T, Todd J, Ruuska S, White J, Benning C, Ohlrogge J (2000). Microarray analysis of developing Arabidopsis seeds. Plant Physiol. **124**: 1570-1581

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001). Auxin regulates SCF^{TIR1}-dependent degradation of Aux/IAA proteins. Nature **414:**271 -276

Greve K, LaCour T, Jensen MK, Poulsen FM, Skriver K (2003). Interactions between plant RING-H2 and plant-specific NAC (NAM/ATAF1/2/CUC2) proteins: RING-H2 molecular specificity and cellular localization. Biochem J. **371**:97-108

Guilfoyle T (1999). Auxin-regulated genes and promoters. *In* Biochemistry and Molecular Biology of Plant Hormones eds by Hooykaas P, Hall M, and Libbenga K. Elsevier Science B.V. pp 423-459

Hagen G, Kleinschmidt A, Guilfoyle T (1984). Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections. Planta **162** :147-1 53

Halliday KJ, Fankhauser C (2003). Phytochrome-hormonal signalling networks. New Phytologist **157**: 449-463

Hellmann H, Hobbie L, Chapman A, Dharmasiri S, Dharmasiri N, del Pozo C, Reinhardt D, Estelle M (2003). Arabidopsis AXR6 encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis. EMBO J. **22**:3314-3325

Hamann T, Benkova E, Baurle I, Kientz M, Jurgens G (2002). The *Arabidopsis* BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS –mediated embryo patterning. Genes Dev. **16:**1610 -1615

Hsieh HL, Okamoto H, Wang M, Ang LH, Matsui M, Goodman H, Deng XW (2000). FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development. Genes Dev. **14**:1958-1970

Jensen PJ, Hangarter RP, Estelle M (1998). Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown Arabidopsis. Plant Physiol. **116**: 455-4621

Jones AM, Cochran DS, Lamerson PM, Evans ML, Cohen JD (1991). Red light-regulated growth. I. Changes in the abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize

mesocotyl. Plant Physiol. **97:** 352-358

Kepinski S, Leyser O (2002). Ubiquitination and auxin signaling: a degrading story. Plant Cell **14:**S81 - S95

Kim BC, Soh MS, Kang BJ, Furuya M, Nam HG (1996). Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2*. Plant J. **9:**441-456

Kim J, Harter K, Theologis A (1997). Protein-protein interactions among the Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **94**:11786-11791

Leyser O, Pickett FB, Dharmasiri S, Estelle M (1996). Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. Plant J. **10**: 403-413

Liscum M, Reed J (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol. Biol. **49**:387-400

Ma L, Li J, Qu L, Hagar J, Chen Z, Zhao H, Deng XW (2001). Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. Plant Cell **13**: 2589-2607

Marrs KA (1996). The functions and regulation of glutathione S-transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47**:127–158

McClure B, Guilfoyle T (1987). Characterization of a class of small auxin-inducible soybean polyadenylated RNAs. Plant Mol. Biol. **6**:611-623

Monroe-Augustus M, Zolman BK, Bartel B (2003). IBR5, a dual-specificity phosphatase-like protein modulating auxin and abscisic acid responsiveness in Arabidopsis. Plant cell **15:** 2979-91

Mussig C, Shin GH, Altmann T (2003). Brassinosteroids promote root growth in Arabidopsis. Plant Physiol. **133**: 1261-71

Nagpal P, Walker LM, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW (2000). *AXR2* encodes a member of the Aux/IAA protein family. Plant Physiol. **123**:563-574

Nakazawa M, Yabe N, Ichikawa T, Yamamoto YY, Yoshizumi T, Hasunuma K, Matsui M (2001). DFL1, an auxin-responsive GH3 gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. Plant J. **25**:213-221

Nemhauser J, Chory J (2002). Photomorphogenesis, *In* The Arabidopsis Book, eds. C.R. Somerville and E.M. Meyerowitz, American Society of Plant Biologists, Rockville, MD, doi/10.1199/tab.0054, http://www.aspb.org/publications/arabidopsis/

op den Camp RGL, Przybyla D, Ochsenbein C, Laloi C, Kim C, Danon A, Wagner D, Hideg E, Gobel C, Feussner I, Nater M, Apel K (2003). Rapid induction of distinct stress responses after the release of singlet oxygen in Arabidopsis. Plant Cell **15**: 2320-2332

Ouellet F, Overvoorde PJ, Theologis A (2001). IAA17/AXR3: Biochemical insight into an auxin mutant phenotype. Plant Cell **13:**829-842

Pérez-Amador MA, Lidder P, Johnson MA, Landgraf J, Wisman E, Green PJ (2001). New molecular phenotypes in the dst mutants of Arabidopsis revealed by DNA microarray analysis. Plant Cell **13**: 2703-2717

Pufky J, Qiu Y, Rao MV, Hurban P, Jones AM (2003). The auxin-induced transcriptome for etiolated *Arabidopsis* seedlings using a structure/function approach. Funct Integr. Genomics **3**:135-43

Puthoff DP, Nettleton D, Rodermel SR, Baum TJ (2003). Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant J. **33**: 911-921

Ramos JA, Zenser N, Leyser O, Callis J (2001). Rapid degradation of Auxin/Indoleacetic Acid proteins requires conserved amino acids of Domain II and is proteasome dependent. Plant Cell **13:**2349 – 2360

Reed JW (2001). Roles and activities of Aux/IAA proteins in Arabidopsis. Trends Plant Sci. **6 :**420–425

Reymond P, Weber H, Damond M, Farmer EE (2000). Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell **12**: 707-719

Rhee SY, Osborne E, Poindexter PD, Somerville CR (2003). Microspore separation in the quartet 3 mutants of Arabidopsis is impaired by a defect in a developmentally regulated polygalacturonase required for pollen mother cell wall degradation. Plant Physiol. **133**:1170-80

Richards KD, Schott EJ, Sharma YK, Davis KR, Gardner RC (1998). Aluminum induces oxidative stress genes in Arabidopsis thaliana. Plant Physiol. **116**:409-18

Rizhsky L, Liang H, Mittler R (2003). The water-water cycle is essential for chloroplast protection in the absence of stress. J. Biol. Chem. **278**:38921-38925

Rogg LE, Lasswell J, Bartel B (2001). A gain-of-function mutation in *IAA28* suppresses lateral root development. Plant Cell **13**:465-480

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O (1998). Changes in auxin response from mutations in an *AUX/IAA* gene. Science **279**:1371-1373

Sablowski RWM, Meyerowitz EM (1998). A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*. Cell **92:**93–103

Sambrook J, Fritsch EF, Maniatis T (1992). Molecular cloning:a laboratory manual, second edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, Deshaies RJ, Gray WM, Estelle M, Deng XW (2001). Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIRI in mediating auxin response. Science **292**:1379-1382

Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Carninci P, Hayashizaki Y, Shinozaki K (2001). Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stress by using full-length cDNA microarray. Plant Cell **13**: 61-72

Sessions A, Nemhauser JL, McColl A, Roe JL, Feldmann KA, Zambryski PC (1997). *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. Development **124**: 4481-4491

Showalter AM (2001). Arabinogalactan-proteins: structure, expression and function. Cell. Mol. Life Sci. **58**:1399–1417

Shirano Y, Shimada H, Kanamaru K, Fujiwara M, Tanaka K, Takahashi H, Unno K, Sato S, Tabata S, Hayashi H, Miyake C, Yokota A, Shibata D (2000). Chloroplast development in Arabidopsis thaliana requires the nuclear-encoded transcription factor sigma B. FEBS Lett. **485**:178-182

Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I (1999). Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development **126**:4235-4245

Tanaka S, Mochizuki N, Nagatani A (2002). Expression of the AtGH3a gene, an Arabidopsis homologue of the soybean GH3 gene, is regulated by phytochrome B. Plant and Cell Physiol. **43**: 281-289

Tatematsu K, Watahiki K, Yamamoto K (1999). Evidences for a dominant mutation of IAA19 that disrupts hypocotyl growth curvature responses and alters auxin sensitivity. *In* 10th International Conference on Arabidopsis Research (Melbourne, Australia). Abstract No. 8-39

Tatematsu K, Kumagaia S, Mutob H, Satoa A, Watahikia MK, Harperc RM, Liscumc E, Yamamotoa KT (2004). *MASSUGU2* encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana*. Plant Cell 16:379-393

Theologis A, Huynh TV, Davis RW (1985). Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J. Mol. Biol. **183**:53-68

Thimann KV (1977). Hormone action *In* the Whole Life of Plants. Amherst, MA: University of Massachusetts Press

Tian Q, Reed JW (1999). Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. Development **126**:711-721

Tian Q, Uhlir JU, Reed JW (2002). Arabidopsis SHY2/IAA3 inhibits auxin-regulated gene expression. Plant Cell **14**: 301-319

Tiwari S, Wang WJ, Hagen G, Guilfoyle T (2001). AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell **13**:2809-2822

Tiwari S, Hagen G, Guilfoyle T (2003). The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell **15**:533-543

Tiwari SB, Hagen G, Guilfoyle TJ (2004). Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell **16**:533-543.

Ulm R, Baumann A, Oravecz A, Mate Z, Adam E, Oakeley EJ, Schafer E, Nagy F (2004). Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. Proc. Natl. Acad. Sci. USA **101**:1397-402

Ulmasov T, Hagen G, and Guilfoyle TJ (1997a). ARF1, a transcription factor that binds auxin response elements. Science **276**:1865-1868

Ulmasov T, Hagen G, Guilfoyle TJ (1999a). Activation and repression of transcription by auxin response factors. Proc. Natl. Acad. Sci. USA **96**:5844-5849

Ulmasov T, Hagen G, Guilfoyle TJ (1999b). Dimerization and DNA binding of auxin response factors. Plant J. **19**:309-319

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997b). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9**: 1963-1971

von Arnim AG (2003). On again - off again: COP9 signalosome turns the key on protein degradation. Current Opinion in Plant Biology **6**:520-529

Walker J, Key J (1982). Isolation of cloned cDNAs to auxin-responsive poly(A)$^+$RNAs of elongating soybean hypocotyls. Proc. Natl. Acad. Sci. USA **79**:7185-7189

Walker J, Legocka J, Edelman L, Key J (1985). An analysis of growth regulator interactions and gene expression during auxin-induced cell elongation using cloned complementary DNAs to auxin-responsive messenger RNAs. Plant Physiol. **77**:847-850

Wang R, Okamoto M, Xing X, Crawford NM (2003). Microarray analysis of the nitrate response in Arabidopsis roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. Plant Physiol. **132**:556-567

Xie Q, Guo HS, Dallman G, Fang S, Weissman AM, Chua NH (2002). SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. Nature **419:**167–170

Zenser N, Ellsmore A, Leasure C, Callis J (2001). Auxin modulates the degradation rate of Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **98 :** 11795–11800

Zenser N, Dreher KA, Edwards SR, Callis J (2003). Acceleration of Aux/IAA proteolysis is specific for auxin and independent of *AXR1*. Plant J. **35**: 285-294

Zhang Y, Brown G, Whetten R, Loopstra CA, Neale D, Kieliszewski MJ, Sederoff RR (2003). An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. Plant Mol Biol. **52**:91-102

Zurfluh LL, Guilfoyle TJ (1980). Auxin-induced changes in the patterns of protein synthesis in soybean hypocotyls. Proc. Natl. Acad. Sci. USA **77**:357-361

Zurfluh LL, Guilfoyle TJ (1982). Auxin-Induced Changes in the Population of Translatable Messenger RNA in Elongationg Sections of Soybean Hypocotyl. Plant Physiol. **69**:332-337

CHAPTER V

SUMMARY AND CONCLUSIONS

Auxin mediates multiple aspects of plant growth and development. Auxin up-regulated genes have been studied for their function and relationship with auxin in plant development. *Axr3-1* is the first characterized Aux/IAA mutant that has a mutation in Domain II of *IAA17*, an auxin up-regulated gene. All identified Aux/IAA mutants have mutations in Domain II centered within the GVWPP motif, and display various auxin-related pleiotropic phenotypes such as loss of tropic responses, small sized root and shoot, and strong apical dominance (Leyser et al., 1996; Tian and Reed, 1999; Nagpal et al., 2000; Fukaki et al., 2002; Rogg et al., 2001). An intragenic revertant of *axr3-1*, *axr3-1R4*, screened for suppression of *axr3-1* phenotype, has an additional mutation resulting in loss of the half of the conserved Domain IV; *axr3-1R4* plant displays WT-like phenotype (Rouse et al., 1998). To gain insight into how Domain IV of *axr3-1R4* overcomes the severe phenotypes caused by the Domain II mutations, the level of transcriptional expression of a large member of auxin responsive genes and protein-protein interactions among IAA17, axr3-1, and axr3-1R4 were examined by Northern analysis and yeast two-hybrid analyses, respectively. Four classes of early auxin-responsive genes were tested, with emphasis on Aux/IAA genes in the three genetic backgrounds, WT, *axr3-1*, and *axr3-1R4*. All auxin up-regulated genes in five day-old WT etiolated seedlings including SAUR, GST, and GH3 exhibited reduced message levels in the *axr3-1* background, but the message levels in *axr3-1R4* seedlings were essentially that of WT levels. However *IAA14, IAA16, IAA18, IAA28*, and *PAP2* were not auxin-responsive in WT and in revertant, and their message levels were not reduced in

axr3-1. Thus auxin responsiveness correlates with reduced message levels in the *axr3-1* and with recovery to near WT in the revertant.

Aux/IAAs interact with other Aux/IAAs and ARFs through Domains III and IV in the yeast two-hybrid system (Kim et al., 1997; O'Grady et al., unpublished). While not studied in detail, there appears to be substantial preferential selectivity in interactions leading to homo- and heterodimer formation and the relative strength of interactions between different pairs. The revertant protein, axr3-1R4, showed no protein-protein interaction with other Aux/IAAs or with ARFs, while the axr3-1 protein showed the same protein-protein interactions as that of the WT protein. The combined data of Northern analyses of mRNA levels of multiple auxin-responsive genes and protein-protein interaction analyses suggest that normal transcript levels of auxin up-regulated genes in *axr3-1R4* to WT levels compared to those of *axr3-1* seems to result from the loss of protein-protein interactions with other Aux/IAAs and ARFs. This probably accounts for the *axr3-1R4* plants having a WT-like phenotype. The Domain II mutation of IAA17 results in increased protein stability by 7- to 20-fold (Ramos et al., 2001; Ouellet et al., 2001; Gray et al., 2001).

Based on data presented here and on protein stability, a model is suggested that more stable Aux/IAAs resulting from the Domain II mutations interfere with the normal protein-protein interaction cycles with other Aux/IAAs, ARFs, and/or other proteins, resulting in down-regulation of most auxin up-regulated genes. This may lead to changes in the transcription of many genes including transcription factors, enzymes involved in metabolism, and other auxin-related genes resulting in abnormal auxin-related phenotypes and auxin responsiveness (i.e. show severe pleiotropic phenotypes and greatly reduced auxin sensitivity). However, the intragenic revertants (*axr3-1R4* and *shy2-22*) negate the effect of protein stability since the revertant

proteins do not interact with other Aux/IAAs, ARFs, and/or other proteins. This hypothesis may be implicated for other Domain II gain-of-function mutants that show various auxin-related pleiotropic phenotypes.

Since *axr3-1* is a gain-of-function mutant and showed reduced message levels of most auxin-responsive genes, it was not possible to study the specific function(s) of IAA17/AXR3 from *axr3-1* in auxin-related plant growth and development. *Axr3-1R4* and *IAA17K* were used in another approach to analyze the function of IAA17. Spatial and temporal expression patterns were studied using both Northern analysis and GUS expression of *pIAA17*::*GUS* in transgenic plants to obtain insight into the phenotypes of *axr3-1R4* and *IAA17K* in more detail. GUS expression was highest from the elongation zone to the root and hypocotyl junction. The staining patterns in the floral organs were very interesting in that GUS expression was confined to the base of flowers at early stages, and it remained in the base of young siliques. Expression subsequently extended throughout the entire silique at later stages of silique maturation. Based on GUS expression patterns, it is proposed that IAA17 is involved after organogenesis and may be involved in cell expansion and organ maturation in the case of flowers and siliques.

Leyser et al. (1996) showed that *axr3-1* is 500-fold less sensitive (or more resistant) to auxin in terms of root growth inhibition. The *axr3-1* was only 100-fold less auxin sensitive than the WT to auxin in inhibition of root growth in the work reported here while a*xr3-1R4* and *IAA17K* basically showed similar auxin sensitivity as WT. Based on the spatial and temporal expression patterns of IAA17 and the shorter root lengths of *axr3-1R4* and *IAA17K*, root cell length of *axr3-1R4* and *IAA17K* were also studied by SEM and confocal microscopy. The general trends of root cell sizes of *axr3-1R4* and *IAA17K* were shorter than WT, but these differences in root cell size were not statistically significant based on variation within a relatively

small sample size. It is concluded, however, that IAA17 is involved in root growth, especially in enhancing root cell elongation. Because of the high conservation of the protein structure and similar/overlapping expression patterns of Aux/IAAs, IAA17 may play a role in root cell elongation in conjunction with other Aux/IAAs causing subtle phenotypic variation in roots as well as in shoot parts.

In order to search for more distinct phenotypes, double knockouts of *IAA17* and *IAA19* were made. Tissue-specific Northern analysis of IAA19 showed that the message levels were high in the stem, flower, and root. *IAA17K* and *IAA19K* did not show distinct phenotypes except at the early stage of root development where the root cell size was much shorter, and root hairs were longer and were more dense, suggesting redundancy in function among Aux/IAAs. The higher density of root hairs resulted from the much shorter root cell length in the epidermis. The double knockout of IAA17 and IAA19 showed a synergistic effect of the two genes in reducing root size. The root hair pattern of the double knockout mutant was very similar to that of *eto1-1*, an ethylene over-producing mutant (Pitts et al., 1998). Auxin is known to induce ethylene production by increasing *ACS* transcription as does CHX treatment (Abel and Theologis, 1996), indicating that transcription of the ACS gene is repressed by a short half-life protein such as an Aux/IAA. WT IAA19 and IAA17 may negatively regulate ACS gene transcription by interacting with ARFs. Therefore, an ACS gene in the absence of IAA17 and IAA19 may have high constitutive activity, leading to a higher ethylene level in cells (tissues). This may result in an *eto1-1*-like phenotype as was observed in the double knockout of IAA17 and IAA19 in root hair formation. Measurement of the message level of the ACS genes in the double mutant background would be informative.

In general, some aspects of auxin signaling are affected by levels of Aux/IAAA proteins, by auxin-dependent degradation of Aux/IAA proteins, and by protein-protein interactions through Domains III and IV. Data presented here identify additional factors that affect auxin signaling including the spatial and temporal specificity of Aux/IAA promoters and the potential synergism of various Aux/IAAs, ARFs, and/or other unknown proteins (?). There are additional levels of complexity of auxin regulation including "cross-talk" between auxin and ethylene in plant processes, auxin-light interactions, and auxin-cytokinin interactions to list a few of the more dominant and well studied phenomena (see Swarup et al., 2002), and it seems at this point based on mutant studies that Aux/IAAs and ARFs are centrally involved in these interactions.

Using Affymetrix ATH1 GeneChips, global transcriptional profiles of WT, *axr3-1*, *axr3-1R4*, and *IAA17K* were identified. A total of 231 genes were up-regulated and 293 genes were down-regulated in *axr3-1* compared to WT. Most of the genes, which showed altered levels of expression in *axr3-1* vs. WT, did not respond to auxin treatment in WT-etiolated seedlings even though *axr3-1* showed auxin-related severe phenotypes. Relatively fewer changes in gene expression occurred in *axr3-1R4* and *IAA17K* compared to *axr3-1*, correlating with the scatter plots and phenotypes of the revertant and the knockout. Gene expression changes in *axr3*-1 were much higher than in *axr3-1R4* and *IAA17K* in terms of fold ratio. The global transcriptional profiles among the four different genetic backgrounds of Arabidopsis seedlings appear to correlate well with phenotype differences.

In this research, the global transcriptional patterns of WT and *axr3-1* with respect to auxin and light treatments were examined to assess whether there is a correlation between auxin and light in effects on gene expression and growth responses to each. *Axr3-1* forms true leaves and floral organs in the dark, suggesting that this mutant overcomes dark-repressed

photomorphogenesis (e.g. de-etiolation process in the dark). By analyzing transcriptional profiles of WT and *axr3-1* with or without auxin and light treatment, gene expression changes that correlate in multiple parameters may be identified which cause or relate to the *axr3-1* phenotypes. A total of 169 genes were induced by auxin treatment in etiolated WT seedlings, and a total of 2508 genes responded to light treatment. *Axr3-1* seedlings generally exhibited reduced message levels of auxin up-regulated genes, but did respond to auxin treatment by enhanced expression of these genes compared to *axr3-1* control seedlings while remaining much lower than in auxin-treated WT seedlings. Results of our research show that auxin-induced changes in gene expression were generally repressed by light, suggesting a somewhat opposing mode of action between light and auxin.

Putative common or independent genes between light and auxin involved in photomorphogenesis were identified, and they seem to work down stream of protein degradation steps that are key regulatory steps in both light and auxin signaling. It would appear from the data presented above and the known strong interactions of auxin and light in the control of growth and development that further analysis of this interaction at the gene expression level would be in order along with further analysis of whether the COP9 Signalosome plays a central role in regulating the balance of, for example, phytochrome and AUX/IAAs as they relate to growth, development, and photomorphogenesis. Additional analysis of the microarray data might also provide some additional insights into the interactions of auxin and light at the level of gene expression. Additional experiments in this area would also be in order.

The possible combinations of protein-protein interactions among Aux/IAAs, ARFs, and Aux/IAA related proteins (do not have Domain I and II) in terms of regulatory signals by forming heterodimers with other groups (e.g. Aux/IAA with ARF or ARP, *vise versa*) are 3,174

(= 23 X 23 X 6); the possible combinations would be 2^n (where n is the number of family members; in this case n is 52) since they can also form homodimers. These combinations suggest the complexity of at least one area of auxin signaling in plant growth and development. This very large number would surely be reduced many fold based on insufficient interaction (or no interaction) and on tissue/organ-specific patterns of expression; other factors might also reduce the redundant number of interactions. However, there are certain redundancies among Aux/IAAs and ARFs in that knockouts of some Aux/IAAs and ARFs do not show distinct phenotypes (reviewed by Liscum and Reed, 2002; this study). Further analysis of spatial and temporal expression patterns of Aux/IAAs and ARFs is necessary in order to understand how these proteins are involved in auxin signaling in plant growth and development. Based on the expression patterns, knockouts of double and/or triple mutants can be screened, and/or RNAi silencing of Aux/IAAs and ARFs (since they share high sequence homologies) can be made. Analysis of their phenotypes should provide additional valuable insights into the role(s) of these proteins in auxin signaling and auxin-related plant growth and development.

References

Abel S, Theologis A (1996). Early genes and auxin action. Plant Physiol. **111**, 9-17

Fukaki H, Tameda S, Masuda H, Tasaka M (2002). Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. Plant J. **29**:153-168

Gray WM, Östin A, Sandberg G, Romano CP, Estelle M (1998). High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. Proc Natl Acad Sci USA **95**: 7197–7202

Kim J, Harter K, Theologis A (1997). Protein-protein interactions among the Aux/IAA proteins. Proc. Natl. Acad. Sci. USA **94**:11786-11791

Leyser O, Pickett FB, Dharmasiri S, Estelle M (1996). Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. Plant J. **10**: 403-413

Liscum M, Reed J (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol. Biol. **49**:387-400

Nagpal P, Walker L, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW (2000). AXR2 encodes a member of the Aux/IAA protein family. Plant Physiol. **123**:563-573

Ouellet F, Overvoorde P, Theologis A (2001). IAA17/AXR3: Biochemical Insight into an Auxin Mutant Phenotype. Plant Cell **13**: 829-842

Pitts RJ, Cernac A, Estelle M. (1998) Auxin and ethylene promote root hair elongation in *Arabidopsis.* The Plant Journal **16:**553 -560

Ramos JA, Zenser N, Leyser O, Callis J (2001). Rapid degradation of Auxin/Indoleacetic Acid proteins requires conserved amino acids of Domain II and is proteasome dependent. Plant Cell **13:**2349 – 2360

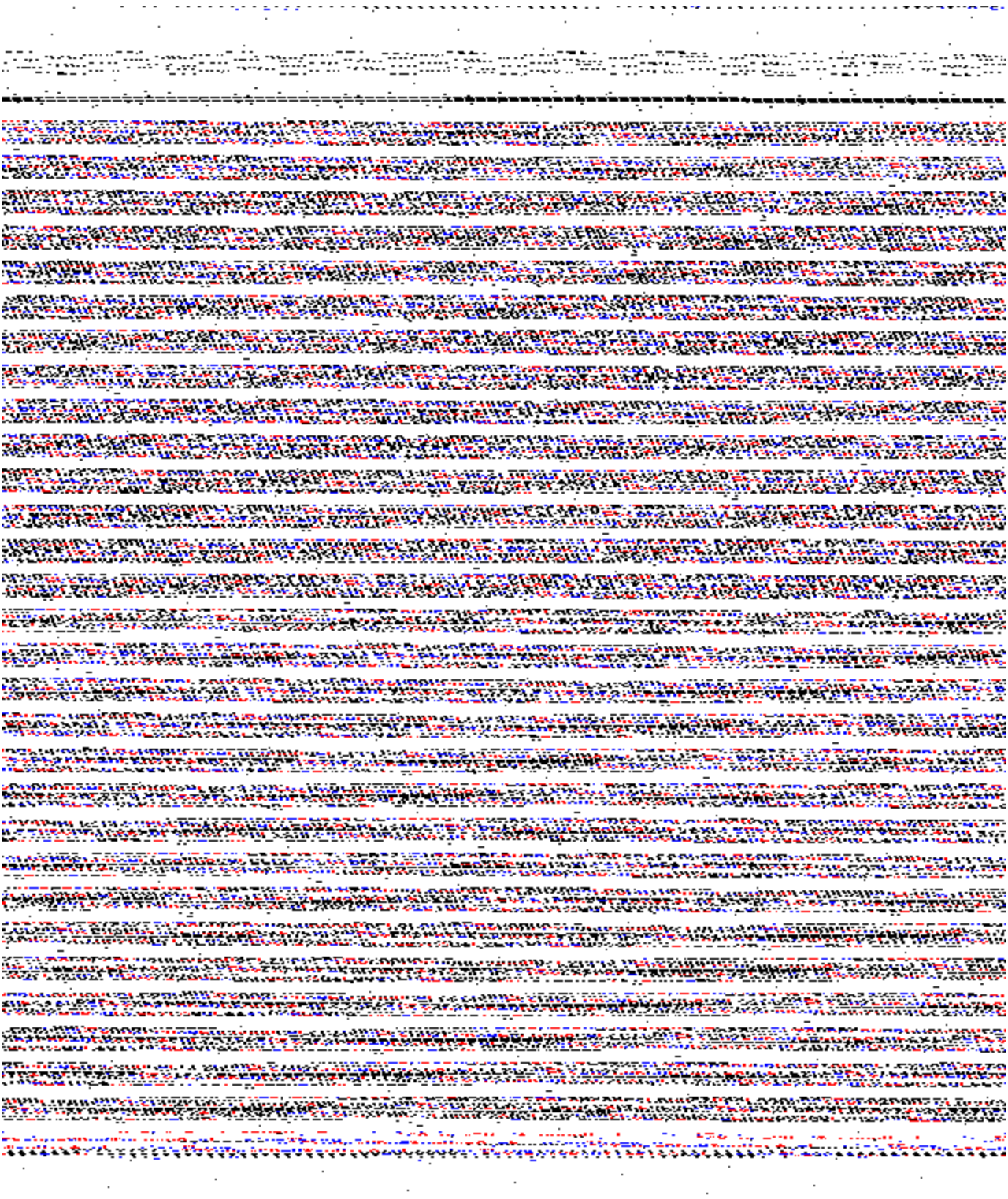
Rogg LE, Lasswell J, Bartel B (2001). A gain-of-function mutation in *IAA28* suppresses lateral root development. Plant Cell **13**:465-480

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O (1998). Changes in auxin response from mutations in an *AUX/IAA* gene. Science **279**:1371-1373

Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M (2001). Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. Genes Dev. **15**: 2648-2653

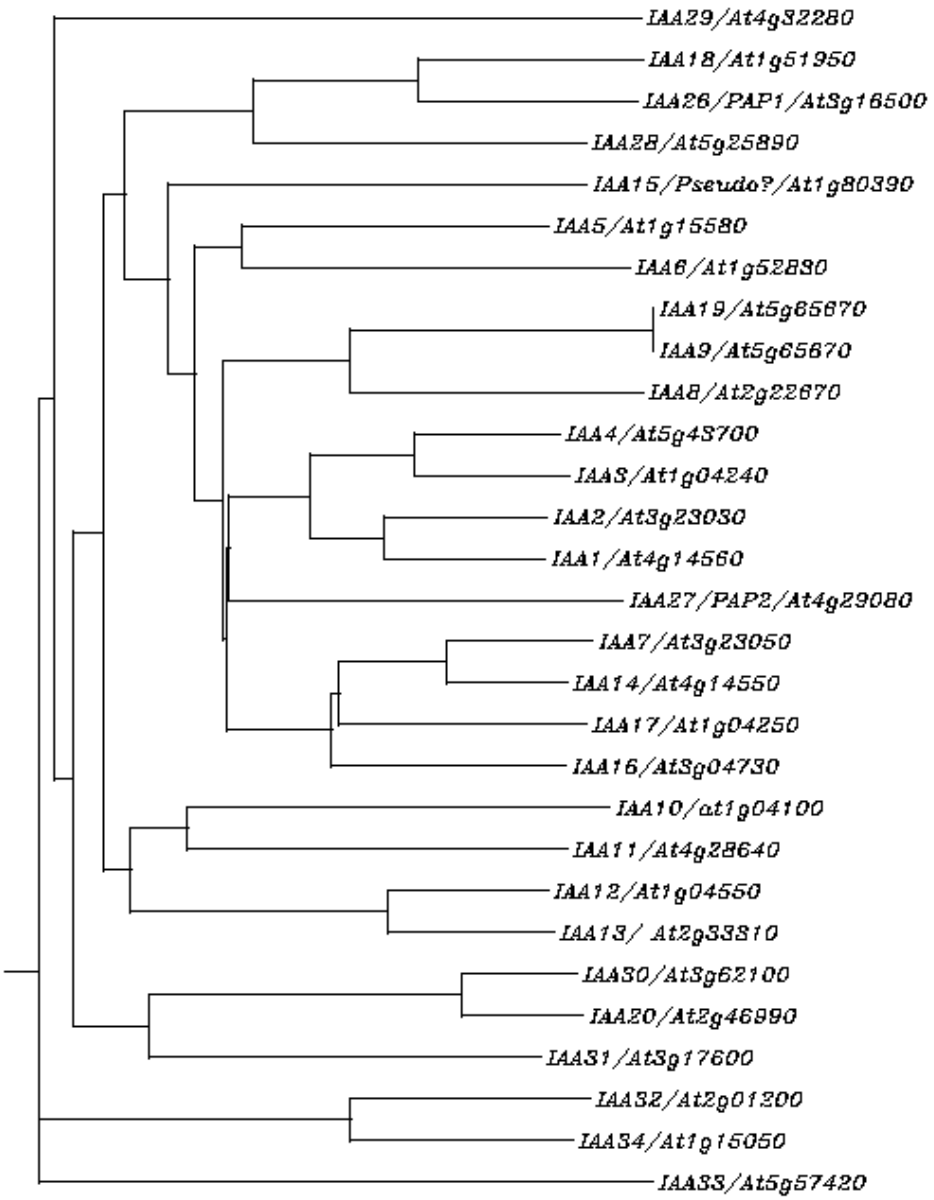
Tian Q, Reed JW (1999). Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. Development **126**:711-721

APPENDIX A. MULTIPLE SEQUNECE ALIGNMENT OF AUX/IAA PROTEINS[1]



[1] Multiple sequence alignment of Aux/IAA proteins was done by using MultiAlign (http://prodes.toulouse.inra.fr/multalin/multalin.html) with default parameters

APPENDIX B. PHYLOGENIC TREE OF AUX/IAA POTEINS[1]



[1] Phylogenic tree was generated by using Clustal W (http://clustalw.genome.ad.jp/) with parameter of "Nabhor-Joining with branch length"

APPENDIX C. LIST OF GENES UP-REGULATED IN *AXR3-1* COMPARED TO WT IN FIVE DAY-OLD GREEN SEEDLINGS

Affy ID	TAIR I.D.	Affy ID	TAIR I.D.	Affy ID	TAIR I.D.
244979_at	AtCg00750	249217_at	At5g42300	252965_at	At4g38860
244980_at	AtCg00760	249271_at	At5g41790	252970_at	At4g38850
244981_at	AtCg00770	249378_at	At5g40450	253005_at	At4g38100
244982_at	AtCg00780	249380_at	At5g40370	253103_at	At4g36110
244983_at	AtCg00790	249441_at	At5g39730	253635_at	At4g30620
244984_at	AtCg00800	249711_at	At5g35680	253660_at	At4g30150
244985_at	AtCg00810	249752_at	At5g24660	253794_at	At4g28720
244993_s_at	AtCg01000	249826_at	At5g23310	253917_at	At4g27240
245049_at	AtCg00050	249866_at	At5g23010	253996_at	At4g26110
245076_at	At2g23170	249867_at	At5g23020	254012_at	At4g26230
245096_at	At2g40880	249886_at	At5g22320	254234_at	At4g23680
245385_at	At4g14020	249894_at	At5g22580	254327_at	At4g22490
245392_at	At4g15680	249916_at	At5g22880	254654_at	At4g18040
245505_at	At4g15690	250304_at	At5g12110	254687_at	At4g13720
245575_at	At4g14760	250832_at	At5g04910	254705_at	At4g17870
245629_at	At1g56580	250863_at	At5g04750	254805_at	At4g12480
245861_at	At5g28300	250919_at	At5g03660	254818_at	At4g12470
246228_at	At4g36430	250936_at	At5g03120	254819_at	At4g12500
246265_at	At1g31860	251005_at	At5g02590	254828_at	At4g12650
246303_at	At3g51870	251017_at	At5g02760	254832_at	At4g12490
246476_at	At5g16730	251065_at	At5g01870	254889_at	At4g11650
246479_at	At5g16060	251109_at	At5g01600	254910_at	At4g11175
246932_at	At5g25190	251155_at	At3g63160	254955_at	At4g10920
247109_at	At5g65870	251195_at	At3g62930	255088_at	At4g09350
247252_at	At5g64770	251311_at	At3g61140	255284_at	At4g04610
247327_at	At5g64120	251438_s_at	At5g33355	255749_at	At1g32000
247331_at	At5g63530	251496_at	At3g59040	255791_at	At2g33430
247474_at	At5g62280	251690_at	At3g56510	256675_at	At3g52170
247549_at	At5g61420	251727_at	At3g56290	256796_at	At3g22210
247780_at	At5g58770	251785_at	At3g55130	256880_at	At3g26450
247942_at	At5g57120	251935_at	At3g54090	257008_at	At3g26920
248186_at	At5g53880	251951_s_at	At3g53600	257021_at	At3g19710
248279_at	At5g52910	252011_at	At3g52720	257054_at	At3g15353
248282_at	At5g52900	252034_at	At3g52040	257066_at	At3g18280
248386_at	At5g51940	252170_at	At3g50480	257206_at	At3g16530
248434_at	At5g51440	252206_at	At3g50360	257506_at	At1g29440
248657_at	At5g48570	252362_at	At3g48500	257730_at	At3g18420
248763_at	At5g47550	252414_at	At3g47420	257891_at	At3g17170
248801_at	At5g47370	252607_at	At3g44990	257937_at	At3g25450
249211_at	At5g42680	252612_at	At3g45160	258158_at	At3g17790

258245_at	At3g29075	261762_at	At1g15510	264698_at	At1g70200
258333_at	At3g16000	261824_at	At1g11430	264803_at	At1g08580
258419_at	At3g16670	261911_at	At1g80750	264899_at	At1g23130
258434_at	At3g16770	261951_at	At1g64490	265006_at	At1g61570
258453_at	At3g22320	262049_at	At1g80180	265116_at	At1g62560
258521_at	At3g06680	262119_s_at	At1g02930	265561_s_at	At2g05520
258623_at	At3g02790	262314_at	At1g70810	265588_at	At2g19970
258675_at	At3g08770	262373_at	At1g73130	265819_at	At2g17972
258736_at	At3g05900	262388_at	At1g49320	266074_at	At2g18700
258897_at	At3g05730	262657_at	At1g14210	266168_at	At2g28080
259311_at	At3g05060	262912_at	At1g59740	266265_at	At2g29340
259560_at	At1g21270	262913_at	At1g59960	266521_at	At2g24020
259640_at	At1g52400	262931_at	At1g65720	266537_at	At2g16860
259645_at	At1g69010	263034_at	At1g24020	266700_at	At2g19740
259706_at	At1g77540	263039_at	At1g23280	266746_s_at	At2g02930
259790_s_at	At1g29430	263046_at	At2g05380	266801_at	At2g22870
259813_at	At1g49860	263209_at	At1g10522	266822_at	At2g44860
259842_at	At1g73600	263216_s_at	At1g30720	266874_at	At2g44760
259871_at	At1g76800	263251_at	At2g31410	266916_at	At2g45860
260004_at	At1g67860	263318_at	At2g24762	266957_at	At2g34520
260025_at	At1g30070	263420_at	At2g17220	266965_at	At2g39480
260143_at	At1g71880	263467_at	At2g31730	267076_at	At2g41090
260157_at	At1g52930	263545_at	At2g21590	267127_at	At2g23610
260221_at	At1g74670	263597_at	At2g01870	267459_at	At2g33850
260343_at	At1g69200	264002_at	At2g22360	267553_s_at	At2g32650
260425_at	At1g72440	264016_at	At2g21150	267586_at	At2g41950
260556_at	At2g43620	264089_at	At2g31200		
260745_at	At1g78370	264121_at	At1g02280		
260824_at	At1g06720	264201_at	At1g22630		
261301_at	At1g48570	264346_at	At1g03370		
261406_at	At1g18800	264507_at	At1g09415		
261576_at	At1g01070	264527_at	At1g30760		

APPENDIX D. LIST OF GENES DOWN-REGULATED IN *AXR3-1* COMPARED TO WT IN FIVE DAY-OLD GREEN SEEDLINGS

Affy ID	TAIR I.D	Affy ID	TAIR I.D	Affy ID	TAIR I.D
245041_at	At2g26530	247094_at	At5g66280	249289_at	At5g41040
245113_at	At2g41660	247125_at	At5g66070	249337_at	At5g41080
245119_at	At2g41640	247170_at	At5g65530	249375_at	At5g40730
245151_at	At2g47550	247189_at	At5g65390	249469_at	At5g39320
245172_at	At2g47540	247280_at	At5g64260	249522_at	At5g38700
245181_at	At5g12420	247297_at	At5g64100	249626_at	At5g37540
245250_at	At4g17490	247337_at	At5g63660	249675_at	At5g35940
245305_at	At4g17215	247477_at	At5g62340	249719_at	At5g35735
245317_at	At4g15610	247543_at	At5g61500	249732_at	At5g24420
245336_at	At4g16515	247586_at	At5g60660	249767_at	At5g24090
245382_at	At4g17800	247604_at	At5g60950	249800_at	At5g23660
245399_at	At4g17340	247645_at	At5g60530	249848_at	At5g23220
245412_at	At4g17280	247655_at	At5g59820	249881_at	At5g23190
245593_at	At4g14550	247708_at	At5g59550	249882_at	At5g22890
245668_at	At1g28330	247727_at	At5g59490	249928_at	At5g22250
245711_at	At5g04340	247871_at	At5g57530	249934_at	At5g22410
245731_at	At1g73500	247914_at	At5g57540	249979_s_at	At5g18860
245777_at	At1g73540	247937_at	At5g57110	250059_at	At5g17820
245794_at	At1g32170	247947_at	At5g57090	250090_at	At5g17330
245885_at	At5g09440	247965_at	At5g56540	250098_at	At5g17350
245891_at	At5g09220	248040_at	At5g55970	250157_at	At5g15180
245904_at	At5g11110	248118_at	At5g55050	250165_at	At5g15290
245944_at	At5g19520	248138_at	At5g54960	250207_at	At5g14040
246099_at	At5g20230	248164_at	At5g54490	250214_at	At5g13870
246133_at	At5g20960	248178_at	At5g54370	250230_at	At5g13900
246229_at	At4g37160	248252_at	At5g53250	250239_at	At5g13580
246285_at	At4g36980	248337_at	At5g52310	250277_at	At5g12940
246289_at	At3g56880	248448_at	At5g51190	250339_at	At5g11670
246374_at	At1g51840	248486_at	At5g51060	250437_at	At5g10430
246375_at	At1g51830	248528_at	At5g50760	250469_at	At5g10130
246390_at	At1g77330	248636_at	At5g49050	250500_at	At5g09530
246403_at	At1g57590	248750_at	At5g47530	250541_at	At5g08580
246584_at	At5g14730	248761_at	At5g47635	250670_at	At5g06860
246633_at	At1g29720	248790_at	At5g47450	250676_at	At5g06320
246652_at	At5g35190	248799_at	At5g47230	250682_x_at	At5g06630
246825_at	At5g26260	248844_s_at	At5g46900	250683_x_at	At5g06640
246897_at	At5g25560	248964_at	At5g45700	250746_at	At5g05880
246913_at	At5g25830	249061_at	At5g44550	250778_at	At5g05500
246991_at	At5g67400	249167_at	At5g42860	250801_at	At5g04960
247047_at	At5g66650	249187_at	At5g43060	250916_at	At5g03630
247091_at	At5g66370	249227_at	At5g42180	250935_at	At5g03240

251010_at	At5g02550	254075_at	At4g25470	257481_at	At1g08430
251174_at	At3g63200	254120_at	At4g24780	257644_at	At3g25730
251226_at	At3g62680	254248_at	At4g23270	257654_at	At3g13310
251336_at	At3g61190	254385_s_at	At4g21830	257690_at	At3g12830
251668_at	At3g57010	254606_at	At4g19030	257824_at	At3g25290
251745_at	At3g56010	254718_at	At4g13570	257925_at	At3g23170
251824_at	At3g55090	254820_s_at	At4g12520	257946_at	At3g21710
251840_at	At3g54950	254893_at	At4g11830	258008_at	At3g19430
251843_x_at	At3g54590	254915_s_at	At4g11290	258121_s_at	At3g14530
251857_at	At3g54770	254926_at	At4g11280	258145_at	At3g18200
251942_at	At3g53480	255259_at	At4g05020	258207_at	At3g14050
252009_at	At3g52800	255310_at	At4g04955	258436_at	At3g16720
252045_at	At3g52450	255516_at	At4g02270	258498_at	At3g02480
252131_at	At3g50820	255568_at	At4g01250	258735_at	At3g05880
252209_at	At3g50400	255695_at	At4g00080	258745_at	At3g05920
252238_at	At3g49960	255733_at	At1g25400	258751_at	At3g05890
252278_at	At3g49530	255751_at	At1g31960	258792_at	At3g04640
252368_at	At3g48520	255814_at	At1g19900	258912_at	At3g06460
252474_at	At3g46830	255895_at	At1g17990	258941_at	At3g09940
252511_at	At3g46280	255934_at	At1g12750	258957_at	At3g01420
252536_at	At3g45700	256017_at	At1g19180	259276_at	At3g05330
252679_at	At3g44260	256217_at	At1g56320	259291_at	At3g11520
252833_at	At4g40090	256252_at	At3g11340	259328_at	At3g16440
253024_at	At4g38080	256302_at	At1g69526	259443_at	At1g02310
253061_at	At4g37610	256308_s_at	At1g30410	259445_at	At1g02370
253073_at	At4g37410	256349_at	At1g54890	259478_at	At1g18980
253177_s_at	At4g35150	256352_at	At1g54970	259511_at	At1g12520
253259_at	At4g34410	256356_s_at	At1g66500	259573_at	At1g20390
253268_s_at	At4g34135	256442_at	At3g10930	259596_at	At1g28130
253582_at	At4g30670	256527_at	At1g66100	259680_at	At1g77690
253628_at	At4g30280	256589_at	At3g28740	259875_s_at	At1g76660
253643_at	At4g29780	256617_at	At3g22240	259979_at	At1g76600
253667_at	At4g30170	256633_at	At3g28340	260130_s_at	At1g66280
253684_at	At4g29690	256933_at	At3g22600	260227_at	At1g74470
253786_at	At4g28650	256937_at	At3g22620	260230_at	At1g74370
253829_at	At4g28040	256994_s_at	At3g25830	260234_at	At1g74550
253830_at	At4g27652	257041_at	At3g19220	260243_at	At1g63720
253832_at	At4g27654	257053_at	At3g15210	260302_at	At1g80290
253872_at	At4g27440	257080_at	At3g15240	260527_at	At2g47270
253915_at	At4g27400	257154_at	At3g27210	260553_at	At2g41800
253957_at	At4g26320	257162_s_at	At3g24290	260557_at	At2g43610
253971_at	At4g26530	257175_s_at	At3g23470	260558_at	At2g43600
253998_at	At4g26010	257197_at	At3g23800	260560_at	At2g43590
254025_at	At4g25790	257217_at	At3g14940	260744_at	At1g15010
254044_at	At4g25820	257244_at	At3g24240	260758_at	At1g48930
254074_at	At4g25490	257280_at	At3g14440	260803_at	At1g78340

260950_s_at	At1g06120	263552_x_at	At2g24980	265680_at	At2g32150
261099_at	At1g62980	263613_at	At2g16440	265737_at	At2g01180
261157_at	At1g34510	263656_at	At1g04500	265902_at	At2g25590
261193_at	At1g32920	263664_at	At1g04430	265974_at	At2g11260
261405_at	At1g18740	263797_at	At2g24570	266125_at	At2g45050
261470_at	At1g28370	263904_at	At2g36380	266165_at	At2g28130
261562_at	At1g01750	263931_at	At2g36310	266191_at	At2g39040
261606_at	At1g49570	263935_at	At2g35930	266196_at	At2g39110
261648_at	At1g27730	263998_at	At2g22520	266356_at	At2g32300
261691_at	At1g50060	264000_at	At2g22370	266368_at	At2g41380
261713_at	At1g32640	264005_at	At2g22470	266514_at	At2g47890
261892_at	At1g80840	264026_at	At2g21050	266545_at	At2g35290
261930_at	At1g22460	264157_at	At1g65310	266581_at	At2g46140
261956_at	At1g64590	264213_at	At1g65400	266711_at	At2g46740
261985_at	At1g33750	264217_at	At1g60190	266752_at	At2g47020
261999_at	At1g33800	264289_at	At1g61890	266791_at	At2g28950
262045_at	At1g80240	264318_at	At1g70330	266834_s_at	At2g30030
262105_at	At1g02810	264338_at	At1g70300	266838_at	At2g25980
262131_at	At1g02900	264415_at	At1g43160	266920_at	At2g45750
262133_at	At1g78000	264497_at	At1g30840	266941_at	At2g18980
262317_at	At2g48140	264567_s_at	At1g05250	266967_at	At2g39520
262349_at	At2g48160	264577_at	At1g05260	266977_at	At2g39410
262427_s_at	At1g47600	264580_at	At1g05340	266978_at	At2g39420
262575_at	At1g15210	264758_at	At1g61340	267028_at	At2g38470
262797_at	At1g20840	264787_at	At2g17920	267121_at	At2g23540
262813_at	At1g11670	264809_at	At1g08830	267240_at	At2g02680
262832_s_at	At1g14870	264998_at	At1g67330	267287_at	At2g23630
262838_at	At1g14960	265031_at	At1g61610	267293_at	At2g23810
262978_at	At1g75780	265048_at	At1g52050	267307_at	At2g30210
263073_at	At2g17500	265049_at	At1g52060	267337_at	At2g19310
263227_at	At1g30750	265050_at	At1g52070	267355_at	At2g39900
263250_at	At2g31390	265102_at	At1g31010	267393_at	At2g44500
263284_at	At2g36100	265119_at	At1g62450	267456_at	At2g33770
263379_at	At2g40140	265169_x_at	At1g23720	267457_at	At2g33790
263406_at	At2g04160	265184_at	At1g23710		
263437_at	At2g28670	265355_at	At2g16760		
263478_at	At2g31880	265480_at	At2g15970		
263496_at	At2g42570	265539_at	At2g15830		
263548_at	At2g21680	265645_at	At2g27370		

APPENDIX E. AUXIN UP-REGULATED GENES BY AUXIN TREATMENT IN FIVE DAY-OLD ETIOLATED SEEDLINGS

Affy ID	TAIR I.D.	Affy ID	TAIR I.D.	Affy ID	TAIR I.D
245076_at	At2g23170	250062_at	At5g17760	253779_at	At4g28480
245108_at	At2g41510	250182_at	At5g14470	253791_at	At4g28640
245140_at	At2g45420	250201_at	At5g14230	253872_at	At4g27440
245233_at	At4g25580	250252_at	At5g13750	253908_at	At4g27260
245251_at	At4g17615	250327_at	At5g12050	253994_at	At4g26080
245277_at	At4g15550	250443_at	At5g10520	254300_at	At4g22780
245397_at	At4g14560	250493_at	At5g09800	254318_at	At4g22500
245416_at	At4g17350	250509_at	At5g09970	254409_at	At4g21400
245528_at	At4g15530	250662_at	At5g07010	254630_at	At4g18360
245821_at	At1g26270	250670_at	At5g06860	254665_at	At4g18340
245947_at	At5g19530	250803_at	At5g04980	254685_at	At4g13850
246485_at	At5g16080	250820_at	At5g05100	254759_at	At4g13180
247023_at	At5g67060	250907_at	At5g03670	254761_at	At4g13195
247148_at	At5g65670	251013_at	At5g02540	254784_at	At4g12720
247151_at	At5g65640	251017_at	At5g02760	254805_at	At4g12480
247283_at	At5g64250	251144_at	At5g01210	254860_at	At4g12110
247351_at	At5g63790	251178_at	At3g63440	255028_at	At4g09890
247474_at	At5g62280	251246_at	At3g62100	255177_at	At4g08040
248028_at	At5g55630	251261_at	At3g62130	255543_at	At4g01990
248104_at	At5g55250	251372_at	At3g60520	255695_at	At4g00080
248162_at	At5g54500	251436_at	At3g59900	255742_at	At1g25410
248163_at	At5g54510	251565_at	At3g58160	255788_at	At2g33310
248164_at	At5g54490	251643_at	At3g57520	255802_s_at	At4g10150
248213_at	At5g53660	251770_at	At3g55970	255905_at	At1g17810
248253_at	At5g53290	251839_at	At3g54940	255959_at	At1g21980
248282_at	At5g52900	251946_at	At3g53540	256024_at	At1g58340
248381_at	At5g51830	252103_at	At3g51410	256097_at	At1g13670
248509_at	At5g50335	252204_at	At3g50340	256426_at	At1g33560
248563_at	At5g49690	252970_at	At4g38850	256877_at	At3g26470
248564_at	At5g49700	253011_at	At4g37890	256981_at	At3g13380
248713_at	At5g48180	253047_at	At4g37295	257643_at	At3g25710
248801_at	At5g47370	253054_at	At4g37470	257766_at	At3g23030
248870_at	At5g46710	253062_at	At4g37590	257858_at	At3g12920
249065_at	At5g44260	253065_at	At4g37740	257900_at	At3g28420
249087_at	At5g44210	253066_at	At4g37770	257975_at	At3g20820
249109_at	At5g43700	253103_at	At4g36110	258075_at	At3g25900
249467_at	At5g39610	253155_at	At4g35720	258253_at	At3g26760
249951_at	At5g18930	253423_at	At4g32280	258367_at	At3g14370
249983_at	At5g18470	253483_at	At4g31910	258399_at	At3g15540
249992_at	At5g18560	253500_at	At4g31920	258516_at	At3g06490
250007_at	At5g18670	253722_at	At4g29190	258878_at	At3g03170

258907_at	At3g06370	262643_at	At1g62770	266507_at	At2g47860
258935_at	At3g10140	262844_at	At1g14687	266611_at	At2g14960
259297_at	At3g05360	262912_at	At1g59740	266663_at	At2g25790
259507_at	At1g43910	262933_at	At1g65840	266761_at	At2g47130
259735_at	At1g64405	262971_at	At1g75640	266800_at	At2g22880
259773_at	At1g29480	263436_at	At2g28690	266908_at	At2g34650
259845_at	At1g73590	263653_at	At1g04330	266974_at	At2g39620
260058_at	At1g78100	263931_at	At2g36310	267008_at	At2g39350
260363_at	At1g70530	264025_at	At2g21180	267083_at	At2g41100
260900_s_at	At1g21400	264479_at	At1g77280	267134_at	At2g23450
261114_at	At1g75390	264537_at	At1g55660	267230_at	At2g44080
261327_at	At1g44830	264777_at	At1g08630	267300_at	At2g30140
261467_at	At1g28520	264788_at	At2g17840	267337_at	At2g19310
261766_at	At1g15580	264929_at	At1g60730	267614_at	At2g26710
262001_at	At1g33790	265084_at	At1g03830		
262045_at	At1g80240	265144_at	At1g51170		
262099_s_at	At1g59500	265856_at	At2g42430		
262229_at	At1g68620	266017_at	At2g18690		
262381_at	At1g72900	266364_at	At2g41230		
262525_at	At1g17060	266368_at	At2g41380		

APPENDIX F. AUXIN DOWN-REGULATED GENES BY AUXIN TREATMENT IN FIVE DAY-OLD ETIOLATED SEEDLINGS

Affy ID	TAIR I.D.	Affy ID	TAIR I.D.	Affy ID	TAIR I.D.
245176_at	At2g47440	252057_at	At3g52480	256321_at	At1g55020
245196_at	At1g67750	252130_at	At3g50890	256626_at	At3g20015
245242_at	At1g44446	252168_at	At3g50440	256796_at	At3g22210
245304_at	At4g15630	252272_at	At3g49670	257066_at	At3g18280
245306_at	At4g14690	252363_at	At3g48460	257204_at	At3g23805
245637_at	At1g25230	252534_at	At3g46130	257673_at	At3g20290
245736_at	At1g73330	252536_at	At3g45700	257867_at	At3g17780
245924_at	At5g28750	252659_at	At3g44430	258003_at	At3g29030
246002_at	At5g20740	252711_at	At3g43720	258181_at	At3g21670
246011_at	At5g08330	253024_at	At4g38080	258239_at	At3g27690
246142_at	At5g19970	253050_at	At4g37450	258321_at	At3g22840
246481_s_at	At5g15960	253362_s_at	At4g33110	258468_at	At3g06070
246825_at	At5g26260	253667_at	At4g30170	258497_at	At3g02380
247246_at	At5g64620	253684_at	At4g29690	258589_at	At3g04290
247406_at	At5g62920	253738_at	At4g28750	258742_at	At3g05800
247899_at	At5g57345	253790_at	At4g28660	259276_at	At3g05330
247946_at	At5g57180	253794_at	At4g28720	259391_s_at	At1g06340
248140_at	At5g54980	253814_at	At4g28290	259660_at	At1g55260
248186_at	At5g53880	254024_at	At4g25780	259840_at	At1g52230
248572_at	At5g49790	254056_at	At4g25250	259892_at	At1g72610
248683_at	At5g48490	254119_at	At4g24640	260081_at	At1g78170
248727_at	At5g47980	254193_at	At4g23850	260097_at	At1g73220
248844_s_at	At5g46900	254573_at	At4g19420	260266_at	At1g68520
248921_at	At5g45950	254606_at	At4g19030	260453_s_at	At1g72510
249472_at	At5g39210	254644_at	At4g18510	260806_at	At1g78260
249750_at	At5g24570	254820_s_at	At4g12520	260877_at	At1g21500
249876_at	At5g23060	254954_at	At4g10910	261488_at	At1g14345
250207_at	At5g14040	255127_at	At4g08300	261727_at	At1g76090
250500_at	At5g09530	255248_at	At4g05180	261746_at	At1g08380
250582_at	At5g07580	255298_at	At4g04840	261768_at	At1g15550
250892_at	At5g03760	255302_at	At4g04830	261769_at	At1g76100
250936_at	At5g03120	255433_at	At4g03210	261942_at	At1g22590
251028_at	At5g02230	255506_at	At4g02130	261949_at	At1g64670
251031_at	At5g02120	255608_at	At4g01140	261975_at	At1g64640
251714_at	At3g56140	255732_at	At1g25450	262029_at	At1g35680
251762_at	At3g55800	255942_at	At1g20350	262128_at	At1g52690
251814_at	At3g54890	255962_at	At1g22335	262168_at	At1g74730
251857_at	At3g54770	255969_at	At1g22330	262236_at	At1g48330
251885_at	At3g54150	256066_at	At1g06980	262315_at	At1g70990
251928_at	At3g53980	256275_at	At3g12110	262376_at	At1g73100
251977_at	At3g53250	256309_at	At1g30380	262399_at	At1g49350

262516_at	At1g17190	264839_at	At2g17360	266790_at	At2g29020
262608_at	At1g14120	264857_at	At2g17370	266873_at	At2g44740
262733_s_at	At1g28670	264884_at	At1g61170	266899_at	At2g34620
262736_at	At1g28570	265067_at	At1g03850	266979_at	At2g39430
262826_at	At1g13080	265149_at	At1g51400	267209_at	At2g30930
262830_at	At1g14700	265296_at	At2g14060	267294_at	At2g23670
263034_at	At1g24020	265443_at	At2g20750	267635_at	At2g42220
263098_at	At2g16070	265716_at	At2g03350		
263595_at	At2g01890	265819_at	At2g17972		
263765_at	At2g21540	266001_at	At2g24150		
263841_at	At2g36870	266636_at	At2g35370		
264501_at	At1g09390	266703_at	At2g19880		

APPENDIX G. LIGHT UP-REGULATED GENES IN FIVE DAY-OLD WT GREEN SEEDLINGS COMPARED TO ETIOLATED SEEDLINGS

Affy ID	TAIR I.D.	Affy ID	TAIR I.D	Affy ID	TAIR I.D
244961_at	AtCg01040	245593_at	At4g14550	246268_at	At1g31800
244966_at	AtCg00600	245616_at	At4g14480	246272_at	At4g37150
244972_at	AtCg00680	245635_at	At1g25250	246276_at	At4g37270
244977_at	AtCg00730	245690_at	At5g04230	246330_at	At3g43600
244995_at	AtCg00150	245701_at	At5g04140	246374_at	At1g51840
245010_at	AtCg00420	245716_at	At5g08740	246375_at	At1g51830
245011_at	AtCg00430	245736_at	At1g73330	246411_at	At1g57770
245016_at	AtCg00500	245744_at	At1g51110	246445_at	At5g17630
245024_at	AtCg00120	245745_at	At1g51115	246449_at	At5g16810
245026_at	AtCg00140	245748_at	At1g51140	246454_at	At5g16710
245041_at	At2g26530	245757_at	At1g35140	246468_at	At5g17050
245047_at	AtCg00020	245765_at	At1g33600	246486_at	At5g15910
245061_at	At2g39730	245768_at	At1g33590	246487_at	At5g16030
245075_at	At2g23180	245790_at	At1g32200	246490_at	At5g15950
245130_at	At2g45340	245793_at	At1g32220	246491_at	At5g16100
245150_at	At2g47590	245806_at	At1g45474	246502_at	At5g16240
245152_at	At2g47490	245852_at	At5g13510	246540_at	At5g15600
245155_at	At5g12470	245866_s_at	At1g57990	246547_at	At5g14970
245172_at	At2g47540	245867_at	At1g58080	246554_at	At5g15450
245181_at	At5g12420	245877_at	At1g26220	246597_at	At5g14760
245195_at	At1g67740	245889_at	At5g09480	246633_at	At1g29720
245201_at	At1g67840	245891_at	At5g09220	246651_at	At5g35170
245213_at	At1g44575	245904_at	At5g11110	246652_at	At5g35190
245228_at	At3g29810	245936_at	At5g19850	246736_at	At5g27560
245242_at	At1g44446	245943_at	At5g19500	246783_at	At5g27360
245287_at	At4g14910	245985_at	At5g13120	246792_at	At5g27290
245296_at	At4g16370	246007_at	At5g08410	246860_at	At5g25840
245318_at	At4g16985	246011_at	At5g08330	246880_s_at	At5g25980
245346_at	At4g17090	246021_at	At5g21100	246947_at	At5g25120
245347_at	At4g14890	246027_at	At5g21060	246966_at	At5g24850
245362_at	At4g17460	246069_at	At5g20220	246998_at	At5g67370
245388_at	At4g16410	246110_at	At5g20140	247025_at	At5g67030
245448_at	At4g16860	246122_at	At5g20380	247040_at	At5g67150
245450_at	At4g16880	246154_at	At5g19940	247073_at	At5g66570
245451_at	At4g16890	246158_at	At5g19855	247101_at	At5g66520
245456_at	At4g16950	246194_at	At4g37000	247118_at	At5g65890
245460_at	At4g16990	246199_at	At4g36530	247131_at	At5g66190
245501_at	At4g15620	246203_at	At4g36610	247162_at	At5g65730
245502_at	At4g15640	246226_at	At4g37200	247166_at	At5g65840
245558_at	At4g15430	246229_at	At4g37160	247214_at	At5g64850

247218_at	At5g65010	248105_at	At5g55280	248851_s_at	At5g46490
247222_at	At5g64840	248128_at	At5g54770	248873_at	At5g46450
247232_at	At5g64940	248178_at	At5g54370	248875_at	At5g46470
247255_at	At5g64780	248190_at	At5g54120	248886_at	At5g46110
247261_at	At5g64460	248191_at	At5g54130	248890_at	At5g46270
247268_at	At5g64080	248238_at	At5g53900	248910_at	At5g45820
247282_at	At5g64240	248246_at	At5g53200	248921_at	At5g45950
247289_at	At5g64290	248263_at	At5g53370	248950_at	At5g45390
247304_at	At5g63850	248270_at	At5g53470	248959_at	At5g45630
247320_at	At5g64050	248273_at	At5g53500	248962_at	At5g45650
247337_at	At5g63660	248282_at	At5g52900	248986_at	At5g45170
247347_at	At5g63780	248287_at	At5g52970	249002_at	At5g44520
247357_at	At5g63710	248311_at	At5g52550	249008_at	At5g44680
247400_at	At5g62840	248336_at	At5g52420	249010_at	At5g44580
247415_at	At5g63060	248377_at	At5g51720	249029_at	At5g44870
247417_at	At5g63040	248392_at	At5g52050	249035_at	At5g44190
247452_at	At5g62430	248404_at	At5g51410	249037_at	At5g44130
247467_at	At5g62130	248419_at	At5g51660	249046_at	At5g44400
247470_at	At5g62220	248437_at	At5g51230	249061_at	At5g44550
247486_at	At5g62140	248449_at	At5g51110	249063_at	At5g44110
247523_at	At5g61410	248459_at	At5g51020	249071_at	At5g44050
247544_at	At5g61670	248491_at	At5g51010	249075_at	At5g44000
247549_at	At5g61420	248537_at	At5g50140	249094_at	At5g43890
247563_at	At5g61130	248540_at	At5g50130	249118_at	At5g43870
247637_at	At5g60600	248560_at	At5g49970	249120_at	At5g43750
247693_at	At5g59730	248562_at	At5g49680	249122_at	At5g43850
247694_at	At5g59750	248566_s_at	At5g49730	249134_at	At5g43150
247709_at	At5g59250	248576_at	At5g49840	249137_at	At5g43140
247714_at	At5g59340	248582_at	At5g49910	249191_at	At5g42760
247760_at	At5g59130	248607_at	At5g49480	249193_at	At5g42480
247766_at	At5g58870	248613_at	At5g49555	249208_at	At5g42650
247780_at	At5g58770	248624_at	At5g48790	249214_at	At5g42720
247813_at	At5g58330	248636_at	At5g49050	249218_at	At5g42120
247816_at	At5g58260	248640_at	At5g48930	249230_at	At5g42070
247848_at	At5g58120	248683_at	At5g48490	249235_at	At5g42100
247853_at	At5g58140	248684_at	At5g48485	249244_at	At5g42270
247875_at	At5g57720	248686_at	At5g48540	249247_at	At5g42310
247899_at	At5g57345	248687_at	At5g48300	249288_at	At5g41050
247902_at	At5g57350	248748_at	At5g47840	249289_at	At5g41040
247914_at	At5g57540	248750_at	At5g47530	249331_at	At5g40950
247916_at	At5g57590	248756_at	At5g47560	249355_at	At5g40500
247925_at	At5g57560	248759_at	At5g47610	249366_at	At5g40610
247931_at	At5g57040	248772_at	At5g47800	249434_at	At5g39960
247977_at	At5g56850	248838_at	At5g46800	249477_s_at	At5g38930
248028_at	At5g55630	248839_at	At5g46690	249480_s_at	At5g38990
248080_at	At5g55380	248845_at	At5g46480	249482_at	At5g38980

249510_at	At5g38480	250149_at	At5g14700	250985_at	At5g02830
249519_at	At5g38660	250200_at	At5g14130	251006_at	At5g02600
249524_at	At5g38520	250207_at	At5g14040	251024_at	At5g02180
249610_at	At5g37360	250230_at	At5g13900	251031_at	At5g02120
249622_at	At5g37550	250239_at	At5g13580	251056_at	At5g01770
249645_at	At5g36910	250243_at	At5g13630	251068_at	At5g01920
249658_s_at	At5g36700	250247_at	At5g13720	251072_at	At5g01740
249677_at	At5g35970	250256_at	At5g13650	251082_at	At5g02070
249685_at	At5g36120	250278_at	At5g12860	251091_at	At5g01410
249694_at	At5g35790	250291_at	At5g13280	251098_at	At5g01650
249710_at	At5g35630	250304_at	At5g12110	251118_at	At3g63410
249732_at	At5g24420	250305_at	At5g12150	251142_at	At5g01015
249759_at	At5g24380	250327_at	At5g12050	251143_at	At5g01220
249769_at	At5g24120	250337_at	At5g11790	251157_at	At3g63140
249773_at	At5g24140	250429_at	At5g10470	251181_at	At3g62640
249774_at	At5g24150	250435_at	At5g10380	251183_at	At3g62600
249775_at	At5g24160	250469_at	At5g10130	251218_at	At3g62410
249777_at	At5g24320	250471_at	At5g10170	251226_at	At3g62680
249785_at	At5g24280	250496_at	At5g09650	251241_s_at	At3g62530
249798_at	At5g23730	250498_at	At5g09660	251243_at	At3g61870
249810_at	At5g23910	250500_at	At5g09530	251305_at	At3g62030
249813_at	At5g23940	250505_at	At5g09870	251309_at	At3g61220
249834_at	At5g23440	250531_at	At5g08630	251330_at	At3g61550
249866_at	At5g23010	250533_at	At5g08540	251353_at	At3g61080
249867_at	At5g23020	250541_at	At5g08580	251461_at	At3g59770
249875_at	At5g23120	250563_at	At5g08050	251497_at	At3g59060
249876_at	At5g23060	250576_at	At5g08250	251519_at	At3g59400
249881_at	At5g23190	250613_at	At5g07240	251524_at	At3g58990
249899_at	At5g22620	250665_at	At5g06980	251536_at	At3g58510
249918_at	At5g19240	250668_at	At5g07020	251539_at	At3g58470
249920_at	At5g19260	250682_x_at	At5g06630	251599_at	At3g57610
249932_at	At5g22390	250683_x_at	At5g06640	251639_at	At3g57490
249934_at	At5g22410	250690_at	At5g06530	251658_at	At3g57020
249945_at	At5g22440	250733_at	At5g06290	251679_at	At3g57030
250006_at	At5g18660	250763_at	At5g06060	251701_at	At3g56650
250043_at	At5g18420	250778_at	At5g05500	251713_at	At3g56080
250058_at	At5g17870	250794_at	At5g05270	251714_at	At3g56140
250073_at	At5g17170	250801_at	At5g04960	251720_at	At3g56160
250074_at	At5g17310	250824_at	At5g05180	251727_at	At3g56290
250075_at	At5g17670	250832_at	At5g04910	251740_at	At3g56170
250095_at	At5g17230	250846_at	At5g04590	251741_at	At3g56070
250098_at	At5g17350	250867_at	At5g03880	251745_at	At3g56010
250099_at	At5g17300	250892_at	At5g03760	251746_at	At3g55980
250128_at	At5g16540	250906_at	At5g03650	251762_at	At3g55800
250133_at	At5g16400	250926_at	At5g03555	251784_at	At3g55330
250146_at	At5g14660	250967_at	At5g02790	251814_at	At3g54890

251815_at	At3g54900	252463_at	At3g47070	253228_at	At4g34630
251820_at	At3g55040	252481_at	At3g46630	253233_at	At4g34290
251824_at	At3g55090	252485_at	At3g46530	253235_at	At4g34350
251826_at	At3g55110	252502_at	At3g46900	253237_at	At4g34240
251827_at	At3g55120	252529_at	At3g46490	253238_at	At4g34480
251843_x_at	At3g54590	252618_at	At3g44735	253245_at	At4g34590
251860_at	At3g54660	252619_at	At3g45140	253247_at	At4g34610
251869_at	At3g54500	252625_at	At3g44750	253256_at	At4g34360
251885_at	At3g54150	252629_at	At3g44970	253272_at	At4g34190
251906_at	At3g53720	252648_at	At3g44630	253283_at	At4g34090
251916_at	At3g53960	252652_at	At3g44720	253284_at	At4g34150
251920_at	At3g53900	252661_at	At3g44450	253302_at	At4g33720
251929_at	At3g53920	252688_at	At3g44020	253305_at	At4g33640
251941_at	At3g53470	252724_at	At3g43540	253308_at	At4g33670
251982_at	At3g53080	252831_at	At4g39980	253310_at	At4g33790
251984_at	At3g53210	252853_at	At4g39710	253335_at	At4g33500
251993_at	At3g52960	252858_at	At4g39770	253342_at	At4g33520
252001_at	At3g52750	252863_at	At4g39800	253387_at	At4g33010
252011_at	At3g52720	252870_at	At4g39940	253391_at	At4g32590
252123_at	At3g51160	252876_at	At4g39970	253394_at	At4g32770
252130_at	At3g50890	252878_at	At4g39460	253397_at	At4g32710
252132_at	At3g50930	252886_at	At4g39350	253412_at	At4g33000
252160_at	At3g50570	252896_at	At4g39480	253420_at	At4g32260
252167_at	At3g50560	252911_at	At4g39510	253429_at	At4g32420
252178_at	At3g50730	252929_at	At4g38970	253440_at	At4g31875
252181_at	At3g50630	252965_at	At4g38860	253480_at	At4g31840
252193_at	At3g50060	252972_at	At4g38840	253496_at	At4g31870
252199_at	At3g50270	252973_s_at	At4g38740	253547_at	At4g30950
252202_at	At3g50300	252983_at	At4g37980	253548_at	At4g30993
252209_at	At3g50400	252989_at	At4g38420	253579_at	At4g30610
252238_at	At3g49960	252995_at	At4g38370	253598_at	At4g30800
252274_at	At3g49680	253002_at	At4g38530	253688_at	At4g29590
252280_at	At3g49260	253009_at	At4g37930	253736_at	At4g28780
252317_at	At3g48720	253024_at	At4g38080	253738_at	At4g28750
252325_at	At3g48560	253039_at	At4g37760	253740_at	At4g28706
252333_at	At3g48830	253040_at	At4g37800	253751_at	At4g29070
252343_at	At3g48610	253048_at	At4g37560	253753_at	At4g28910
252353_at	At3g48200	253053_at	At4g37310	253790_at	At4g28660
252366_at	At3g48420	253061_at	At4g37610	253815_at	At4g28250
252377_at	At3g47960	253073_at	At4g37410	253823_at	At4g28030
252411_at	At3g47650	253124_at	At4g36030	253825_at	At4g28025
252412_at	At3g47295	253165_at	At4g35320	253835_at	At4g27820
252429_at	At3g47500	253174_at	At4g35090	253849_at	At4g28080
252430_at	At3g47470	253197_at	At4g35250	253851_at	At4g28110
252441_at	At3g46780	253200_at	At4g34720	253856_at	At4g28100
252462_at	At3g47250	253227_at	At4g35030	253860_at	At4g27700

253875_at	At4g27450	254642_at	At4g18810	255457_at	At4g02770
253911_at	At4g26900	254649_at	At4g18570	255488_at	At4g02630
253934_at	At4g26830	254686_at	At4g13790	255502_at	At4g02410
253943_at	At4g27030	254687_at	At4g13720	255503_at	At4g02420
253951_at	At4g26860	254691_at	At4g17840	255504_at	At4g02200
253971_at	At4g26530	254697_at	At4g17970	255513_at	At4g02060
253998_at	At4g26010	254710_at	At4g18050	255516_at	At4g02270
254016_at	At4g26150	254737_at	At4g13840	255558_at	At4g01940
254044_at	At4g25820	254746_at	At4g12980	255567_at	At4g01150
254053_s_at	At4g25300	254770_at	At4g13340	255572_at	At4g01050
254075_at	At4g25470	254783_at	At4g12830	255622_at	At4g01070
254080_at	At4g25630	254790_at	At4g12800	255626_at	At4g00780
254098_at	At4g25100	254798_at	At4g13050	255645_at	At4g00895
254099_at	At4g25130	254804_at	At4g13010	255674_at	At4g00430
254102_at	At4g25050	254831_at	At4g12600	255676_at	At4g00490
254109_at	At4g25240	254835_s_at	At4g12310	255685_s_at	At4g00600
254118_at	At4g24790	254862_at	At4g12030	255694_at	At4g00050
254119_at	At4g24640	254874_at	At4g11570	255710_at	At4g00030
254120_at	At4g24780	254931_at	At4g11460	255718_at	At1g32070
254125_at	At4g24670	254980_at	At4g10450	255719_at	At1g32080
254134_at	At4g24830	254995_at	At4g10370	255720_at	At1g32060
254137_at	At4g24930	255016_at	At4g10120	255764_at	At1g16720
254145_at	At4g24700	255025_at	At4g09900	255773_at	At1g18590
254174_at	At4g24120	255026_at	At4g09910	255785_at	At1g19860
254187_at	At4g23890	255046_at	At4g09650	255787_at	At1g19670
254201_at	At4g24130	255051_at	At4g09710	255798_at	At2g33255
254202_at	At4g24140	255065_s_at	At4g08870	255809_at	At4g10300
254250_at	At4g23290	255078_at	At4g09010	255814_at	At1g19900
254298_at	At4g22890	255088_at	At4g09350	255842_at	At2g33530
254305_at	At4g22200	255131_at	At4g08280	255852_at	At1g66970
254331_s_at	At4g22710	255140_x_at	At4g08410	255856_at	At1g66940
254370_at	At4g21750	255248_at	At4g05180	255877_at	At2g40460
254388_at	At4g21860	255284_at	At4g04610	255886_at	At1g20340
254398_at	At4g21690	255290_at	At4g04640	255913_at	At1g66980
254460_at	At4g21210	255298_at	At4g04840	255982_at	At1g34000
254474_at	At4g20390	255304_at	At4g04850	256000_at	At1g29780
254485_at	At4g20760	255305_at	At4g04770	256007_at	At1g34065
254505_at	At4g19985	255344_s_at	At4g04540	256015_at	At1g19150
254535_at	At4g19710	255364_s_at	At4g04020	256020_at	At1g58290
254544_at	At4g19820	255379_at	At4g03520	256024_at	At1g58340
254545_at	At4g19830	255411_at	At4g03110	256049_at	At1g07010
254553_at	At4g19140	255435_at	At4g03280	256057_at	At1g07180
254564_at	At4g19170	255440_at	At4g02530	256076_at	At1g18060
254577_at	At4g19450	255450_at	At4g02850	256093_at	At1g20823
254623_at	At4g18480	255455_at	At4g02930	256096_at	At1g13650
254638_at	At4g18740	255456_at	At4g02920	256125_at	At1g18250

256140_at	At1g48650	257033_at	At3g19170	258049_at	At3g16220
256145_at	At1g48750	257044_at	At3g19700	258055_at	At3g16250
256160_at	At1g30120	257045_at	At3g19730	258087_at	At3g26060
256168_at	At1g51805	257100_at	At3g25010	258106_at	At3g23580
256185_at	At1g51700	257129_at	At3g20100	258155_at	At3g18130
256215_at	At1g50900	257148_at	At3g27240	258159_at	At3g17840
256217_at	At1g56320	257149_at	At3g27280	258181_at	At3g21670
256228_at	At1g56290	257168_at	At3g24430	258188_at	At3g17800
256266_at	At3g12320	257183_at	At3g13220	258200_at	At3g13900
256275_at	At3g12110	257204_at	At3g23805	258239_at	At3g27690
256299_at	At1g69530	257222_at	At3g27925	258250_at	At3g15850
256304_at	At1g69523	257247_at	At3g24140	258285_at	At3g16140
256309_at	At1g30380	257253_at	At3g24170	258295_at	At3g23400
256349_at	At1g54890	257262_at	At3g21890	258299_at	At3g23410
256352_at	At1g54970	257264_at	At3g22060	258342_at	At3g22800
256386_at	At1g66540	257311_at	At3g26570	258349_at	At3g17610
256427_at	At1g33420	257451_at	At1g05690	258350_at	At3g17510
256441_at	At3g10940	257519_at	At3g01210	258359_s_at	At3g14415
256446_at	At3g11110	257533_at	At3g10840	258365_s_at	At3g14390
256454_at	At1g75280	257543_at	At3g28960	258386_at	At3g15520
256497_at	At1g31520	257547_at	At3g13000	258398_at	At3g15360
256514_at	At1g31480	257556_at	At3g28090	258418_at	At3g16660
256526_at	At1g66090	257635_at	At3g26280	258419_at	At3g16670
256527_at	At1g66100	257673_at	At3g20290	258456_at	At3g22420
256548_at	At3g14770	257698_at	At3g12730	258460_at	At3g17330
256577_at	At3g28220	257699_at	At3g12780	258495_at	At3g02690
256585_at	At3g28750	257722_at	At3g18490	258497_at	At3g02380
256603_at	At3g28270	257723_at	At3g18500	258505_at	At3g06530
256613_at	At3g29290	257746_at	At3g29200	258512_at	At3g06510
256655_at	At3g18890	257771_at	At3g23000	258535_at	At3g06750
256661_at	At3g11964	257793_at	At3g26960	258566_at	At3g04110
256674_at	At3g52360	257794_at	At3g27050	258607_at	At3g02730
256680_at	At3g52230	257801_at	At3g18750	258609_at	At3g02910
256819_at	At3g21390	257860_at	At3g13062	258613_at	At3g02870
256835_at	At3g22890	257903_at	At3g28460	258621_at	At3g02830
256856_at	At3g15110	257916_at	At3g23210	258622_at	At3g02720
256860_at	At3g23840	257954_at	At3g21760	258675_at	At3g08770
256872_at	At3g26490	257964_at	At3g19840	258676_at	At3g08600
256892_at	At3g19000	257966_at	At3g19900	258724_at	At3g09610
256907_at	At3g24030	257999_at	At3g27540	258729_at	At3g11900
256933_at	At3g22600	258003_at	At3g29030	258755_at	At3g11950
256937_at	At3g22620	258008_at	At3g19430	258837_at	At3g07110
256979_at	At3g21055	258021_at	At3g19380	258860_at	At3g02050
256994_s_at	At3g25830	258023_at	At3g19450	258884_at	At3g10050
257008_at	At3g26920	258025_at	At3g19480	258897_at	At3g05730
257021_at	At3g19710	258033_at	At3g21250	258925_at	At3g10420

258929_at	At3g10060	259658_at	At1g55370	260419_at	At1g69730
258956_at	At3g01440	259671_at	At1g52290	260427_at	At1g72430
258957_at	At3g01420	259681_at	At1g77760	260466_at	At1g10900
258962_at	At3g10570	259707_at	At1g77490	260481_at	At1g10940
258977_s_at	At3g02020	259761_at	At1g77590	260515_at	At1g51460
258997_at	At3g01810	259775_at	At1g29520	260542_at	At2g43560
259001_at	At3g01960	259786_at	At1g29660	260565_at	At2g43800
259012_at	At3g07360	259790_s_at	At1g29430	260570_at	At2g43710
259033_at	At3g09410	259791_at	At1g29700	260601_at	At1g55910
259036_at	At3g09220	259838_at	At1g52220	260603_at	At1g55960
259092_at	At3g04870	259840_at	At1g52230	260615_at	At1g53240
259096_at	At3g04840	259858_at	At1g68400	260653_at	At1g32440
259098_at	At3g04790	259860_at	At1g80640	260685_at	At1g17650
259138_s_at	At3g10270	259892_at	At1g72610	260696_at	At1g32520
259140_at	At3g10230	259896_at	At1g71500	260704_at	At1g32470
259161_at	At3g01500	259922_at	At1g72770	260709_at	At1g32500
259185_at	At3g01550	259927_at	At1g75100	260714_at	At1g14980
259188_at	At3g01510	259943_at	At1g71480	260727_at	At1g48100
259193_at	At3g01480	259945_at	At1g71460	260730_at	At1g48030
259222_at	At3g03540	259965_at	At1g53670	260745_at	At1g78370
259226_at	At3g07700	259970_at	At1g76570	260758_at	At1g48930
259237_at	At3g11630	260014_at	At1g68010	260769_at	At1g49010
259275_at	At3g01060	260035_at	At1g68850	260790_at	At1g06240
259277_at	At3g01190	260036_at	At1g68830	260821_at	At1g06840
259279_at	At3g01160	260045_at	At1g73670	260831_at	At1g06830
259282_at	At3g01140	260056_at	At1g78140	260837_at	At1g43670
259298_at	At3g05370	260070_at	At1g73830	260856_at	At1g21920
259325_at	At3g05320	260072_at	At1g73650	260872_at	At1g21350
259342_at	At3g03890	260076_at	At1g73630	260877_at	At1g21500
259347_at	At3g03920	260077_at	At1g73620	260916_at	At1g02475
259348_at	At3g03770	260137_at	At1g66330	260968_at	At1g12220
259432_at	At1g01520	260155_at	At1g52870	260969_at	At1g12230
259460_at	At1g44000	260207_at	At1g70730	260982_at	At1g53520
259475_at	At1g19140	260215_at	At1g74530	261012_at	At1g26600
259478_at	At1g18980	260227_at	At1g74470	261016_at	At1g26560
259491_at	At1g15820	260230_at	At1g74370	261033_at	At1g17360
259514_at	At1g12480	260232_at	At1g74410	261068_at	At1g07450
259537_at	At1g12370	260234_at	At1g74550	261075_at	At1g07280
259546_at	At1g35350	260294_at	At1g63680	261080_at	At1g07370
259549_at	At1g35290	260308_at	At1g70610	261108_at	At1g62960
259603_at	At1g56500	260309_at	At1g70580	261118_at	At1g75460
259625_at	At1g42970	260324_at	At1g63970	261122_at	At1g75330
259629_at	At1g56510	260331_at	At1g80270	261132_at	At1g19800
259632_at	At1g56430	260368_at	At1g69700	261139_at	At1g19700
259633_at	At1g56505	260380_at	At1g73870	261141_at	At1g19740
259640_at	At1g52400	260388_at	At1g74070	261175_at	At1g04800

261190_at	At1g32990	262160_at	At1g52590	262882_at	At1g64900
261191_at	At1g32900	262162_at	At1g78020	262883_at	At1g64780
261197_at	At1g12900	262168_at	At1g74730	262926_s_at	At1g65730
261218_at	At1g20020	262176_at	At1g74960	262945_at	At1g79510
261308_at	At1g48480	262194_at	At1g77930	262954_at	At1g54500
261338_at	At1g44920	262202_at	At2g01110	262963_at	At1g54220
261346_at	At1g79720	262231_at	At1g68740	262970_at	At1g75690
261351_at	At1g79790	262281_at	At1g68570	262986_at	At1g23390
261353_at	At1g79600	262287_at	At1g68660	262988_at	At1g23310
261368_at	At1g53070	262288_at	At1g70760	263000_at	At1g54350
261417_at	At1g07700	262290_at	At1g70985	263031_at	At1g24070
261439_at	At1g07600	262317_at	At2g48140	263034_at	At1g24020
261470_at	At1g28370	262349_at	At2g48160	263097_at	At2g16060
261480_at	At1g14280	262368_at	At1g73060	263111_s_at	At1g65220
261488_at	At1g14345	262374_s_at	At1g73120	263115_at	At1g03130
261519_at	At1g71810	262380_at	At1g73020	263119_at	At1g03110
261536_at	At1g01790	262382_at	At1g72920	263120_at	At1g78490
261562_at	At1g01750	262383_at	At1g72940	263121_at	At1g78530
261569_at	At1g01060	262418_at	At1g50320	263136_at	At1g78580
261597_at	At1g49780	262451_at	At1g11140	263142_at	At1g65230
261598_at	At1g49750	262473_at	At1g50250	263241_at	At2g16500
261618_at	At1g33110	262479_at	At1g11130	263252_at	At2g31380
261635_at	At1g49970	262483_at	At1g17220	263287_at	At2g36145
261640_at	At1g49960	262506_at	At1g21640	263298_at	At2g15290
261648_at	At1g27730	262526_at	At1g17050	263350_at	At2g13360
261667_at	At1g18460	262536_at	At1g17000	263380_at	At2g40200
261691_at	At1g50060	262566_at	At1g34310	263406_at	At2g04160
261746_at	At1g08380	262577_at	At1g15290	263410_at	At2g04039
261751_at	At1g76080	262598_at	At1g15260	263429_at	At2g22250
261776_at	At1g76190	262604_at	At1g15060	263432_at	At2g22230
261782_at	At1g76110	262609_at	At1g13930	263433_at	At2g22240
261788_at	At1g15980	262612_at	At1g14150	263449_at	At2g31670
261793_at	At1g16080	262626_at	At1g06430	263452_at	At2g22190
261804_at	At1g30530	262634_at	At1g06690	263477_at	At2g31790
261819_at	At1g11410	262645_at	At1g62750	263483_at	At2g04030
261834_at	At1g10670	262721_at	At1g43560	263491_at	At2g42600
261914_at	At1g65870	262745_at	At1g29020	263533_at	At5g37350
261933_at	At1g22420	262748_at	At1g28960	263541_at	At2g24780
261948_at	At1g64680	262783_at	At1g10850	263552_x_at	At2g24980
261956_at	At1g64590	262809_at	At1g11720	263558_at	At2g16380
261958_at	At1g64500	262811_at	At1g11700	263598_at	At2g01850
261981_at	At1g33811	262823_at	At1g11750	263606_at	At2g16480
262059_at	At1g80030	262825_at	At1g11790	263607_at	At2g16280
262097_at	At1g55990	262826_at	At1g13080	263632_at	At2g04795
262113_at	At1g02820	262875_at	At1g64700	263668_at	At1g04350
262151_at	At1g52510	262878_at	At1g64770	263676_at	At1g09340

263677_at	At1g04520	264372_at	At1g11840	265394_at	At2g20725
263705_at	At1g31190	264383_at	At2g25080	265415_at	At2g20890
263715_at	At2g20570	264394_at	At1g11860	265475_at	At2g15690
263726_at	At2g13610	264435_at	At1g10360	265569_at	At2g05620
263739_at	At2g21320	264436_at	At1g10370	265611_at	At2g25510
263755_at	At2g21340	264567_s_at	At1g05250	265628_at	At2g27290
263760_at	At2g21280	264584_at	At1g05140	265634_at	At2g25530
263761_at	At2g21330	264613_at	At1g04640	265665_at	At2g24420
263777_at	At2g46450	264636_at	At1g65490	265722_at	At2g40100
263779_at	At2g46340	264641_at	At1g09130	265724_at	At2g32100
263841_at	At2g36870	264692_at	At1g70000	265742_at	At2g01290
263845_at	At2g37040	264694_at	At1g70250	265768_at	At2g48020
263873_at	At2g21860	264716_at	At1g70170	265773_at	At2g48070
263875_at	At2g21970	264728_at	At1g22985	265846_at	At2g35770
263906_at	At2g36250	264738_at	At1g62200	265869_at	At2g01760
263935_at	At2g35930	264745_at	At1g62180	265892_at	At2g15020
263942_at	At2g35860	264839_at	At2g17360	265895_at	At2g15000
263951_at	At2g35960	264840_at	At1g03630	265939_at	At2g19650
263952_s_at	At2g35810	264843_at	At1g03700	265959_at	At2g37240
263954_at	At2g35840	264845_at	At1g03400	265962_at	At2g37460
263980_at	At2g42770	264850_at	At2g17350	265990_at	At2g24280
263985_at	At2g42750	264857_at	At2g17370	265993_at	At2g24160
263987_at	At2g42690	264872_at	At1g24260	266015_at	At2g24190
263995_at	At2g12900	264885_s_at	At1g61180	266038_at	At2g07680
263998_at	At2g22520	264898_at	At1g23205	266104_at	At2g45150
264012_at	At2g21090	264899_at	At1g23130	266184_s_at	At2g38940
264014_at	At2g21070	264909_at	At2g17300	266191_at	At2g39040
264022_at	At2g21200	264931_at	At1g60590	266196_at	At2g39110
264037_at	At2g03810	264959_at	At1g77090	266207_at	At2g27680
264052_at	At2g22330	264978_at	At1g27120	266209_at	At2g27550
264054_at	At2g22550	265049_at	At1g52060	266265_at	At2g29340
264078_at	At2g28470	265050_at	At1g52070	266275_at	At2g29370
264114_at	At2g31270	265073_at	At1g55580	266277_at	At2g29310
264153_at	At1g65390	265102_at	At1g31010	266278_at	At2g29300
264164_at	At1g65295	265109_s_at	At1g62610	266279_at	At2g29290
264182_at	At1g65360	265111_at	At1g62570	266286_at	At2g29180
264185_at	At1g54780	265117_at	At4g09455	266291_at	At2g29320
264195_at	At1g22690	265149_at	At1g51400	266293_at	At2g29360
264201_at	At1g22630	265169_x_at	At1g23720	266319_s_at	At3g10280
264207_at	At1g22750	265175_at	At1g23480	266329_at	At2g01590
264213_at	At1g65400	265182_at	At1g23740	266353_at	At2g01520
264240_at	At1g54820	265203_at	At2g36630	266363_at	At2g41250
264279_s_at	At1g78820	265248_at	At2g43010	266391_at	At2g41290
264319_at	At1g70310	265339_at	At2g18230	266395_at	At2g43100
264343_at	At1g11850	265340_at	At2g18330	266402_at	At2g38780
264360_at	At1g03310	265374_at	At2g06520	266413_at	At2g38740

266421_at	At2g38540	266920_at	At2g45750	267260_at	At2g23130
266460_at	At2g47970	266946_at	At2g07720	267287_at	At2g23630
266465_at	At2g47750	266963_at	At2g39440	267294_at	At2g23670
266481_at	At2g31070	266979_at	At2g39430	267344_at	At2g44230
266570_at	At2g24080	266990_at	At2g39190	267367_at	At2g44210
266572_at	At2g23830	267002_s_at	At2g34430	267402_at	At2g26180
266599_at	At2g46100	267005_at	At2g34460	267425_at	At2g34810
266625_at	At2g35380	267028_at	At2g38470	267430_at	At2g34860
266636_at	At2g35370	267029_at	At2g38460	267440_at	At2g19070
266638_at	At2g35490	267038_at	At2g38480	267457_at	At2g33790
266647_at	At2g25860	267053_s_at	At2g38390	267459_at	At2g33850
266649_at	At2g25810	267057_at	At2g32500	267471_at	At2g30390
266658_at	At2g25910	267061_at	At2g32480	267495_at	At2g30420
266672_at	At2g29650	267063_at	At2g41120	267516_at	At2g30520
266673_at	At2g29630	267066_at	At2g41040	267517_at	At2g30510
266687_at	At2g19670	267076_at	At2g41090	267526_at	At2g30570
266704_at	At2g19940	267078_at	At2g40960	267545_at	At2g32690
266716_at	At2g46820	267089_at	At2g38300	267549_at	At2g32640
266717_at	At2g46735	267115_s_at	At2g32540	267569_at	At2g30790
266719_at	At2g46830	267121_at	At2g23540	267635_at	At2g42220
266720_s_at	At2g46790	267126_s_at	At2g23590	267644_s_at	At2g32880
266790_at	At2g29020	267132_at	At2g23420	267645_at	At2g32860
266805_at	At2g30010	267169_at	At2g37540		
266813_at	At2g44920	267188_at	At2g44050		
266882_at	At2g44670	267196_at	At2g30950		
266892_at	At2g26080	267220_at	At2g02500		
266899_at	At2g34620	267247_at	At2g30170		

APPENDIX H LIGHT DOWN-REGULATED GENES IN FIVE DAY-OLD WT GREEN SEEDLINGS COMPARED TO ETIOLATED SEEDLINGS

Affy ID	TAIR I.D.	Affy ID	TAIR I.D.	Affy ID	TAIR I.D.
244985_at	AtCg00810	245629_at	At1g56580	246288_at	At1g31850
245046_at	At2g26510	245637_at	At1g25230	246304_at	At3g51840
245096_at	At2g40880	245642_at	At1g25275	246311_at	At3g51880
245098_at	At2g40940	245644_at	At1g25320	246319_at	At3g56680
245127_at	At2g47600	245668_at	At1g28330	246343_at	At3g56720
245134_s_at	At2g45250	245684_at	At5g22000	246371_at	At1g51940
245136_at	At2g45210	245694_at	At5g04170	246389_at	At1g77380
245193_at	At1g67810	245775_at	At1g30270	246390_at	At1g77330
245196_at	At1g67750	245781_at	At1g45976	246396_at	At1g58180
245233_at	At4g25580	245787_at	At1g32130	246421_at	At5g16880
245249_at	At4g16760	245789_at	At1g32090	246432_at	At5g17490
245258_at	At4g15340	245794_at	At1g32170	246476_at	At5g16730
245266_at	At4g17070	245821_at	At1g26270	246483_at	At5g16000
245270_at	At4g14960	245861_at	At5g28300	246506_at	At5g16110
245276_at	At4g16780	245868_at	At1g58032	246524_at	At5g15860
245277_at	At4g15550	245885_at	At5g09440	246550_at	At5g14920
245278_at	At4g17730	245887_at	At5g09390	246562_at	At5g15580
245302_at	At4g17695	245947_at	At5g19530	246580_at	At1g31770
245306_at	At4g14690	245970_at	At5g20710	246584_at	At5g14730
245317_at	At4g15610	246004_at	At5g20630	246595_at	At5g14780
245319_at	At4g16146	246018_at	At5g10695	246653_at	At5g35200
245323_at	At4g16500	246028_at	At5g21170	246702_at	At5g28050
245325_at	At4g14130	246062_at	At5g19330	246755_at	At5g27920
245336_at	At4g16515	246090_at	At5g20520	246779_at	At5g27520
245338_at	At4g16442	246149_at	At5g19890	246822_at	At5g26960
245349_at	At4g16690	246171_at	At5g32440	246825_at	At5g26260
245353_at	At4g16000	246178_s_at	At5g28430	246843_at	At5g26734
245359_at	At4g14430	246197_at	At4g37010	246862_at	At5g25760
245386_at	At4g14010	246205_at	At4g36970	246909_at	At5g25770
245398_at	At4g14900	246211_at	At4g36730	246933_at	At5g25160
245404_at	At4g17610	246212_at	At4g36930	246935_at	At5g25350
245410_at	At4g17220	246219_at	At4g36760	246951_at	At5g04880
245422_at	At4g17470	246222_at	At4g36900	246953_at	At5g04850
245431_at	At4g17080	246238_at	At4g36670	246975_at	At5g24890
245439_at	At4g16670	246244_at	At4g37250	247000_at	At5g67380
245528_at	At4g15530	246250_at	At4g36880	247002_at	At5g67320
245543_at	At4g15260	246251_at	At4g37220	247005_at	At5g67520
245550_at	At4g15330	246252_s_at	At4g37070	247009_at	At5g67600
245572_at	At4g14720	246267_at	At1g31812	247013_at	At5g67480
245602_at	At4g14270	246284_at	At4g36780	247023_at	At5g67060

247043_at	At5g66880	247924_at	At5g57655	248895_at	At5g46330
247095_at	At5g66400	247937_at	At5g57110	248905_at	At5g46250
247107_at	At5g66040	247942_at	At5g57120	248932_at	At5g46050
247113_at	At5g65960	247943_at	At5g57170	248941_s_at	At5g45400
247116_at	At5g65970	247946_at	At5g57180	248944_at	At5g45500
247130_at	At5g66180	247951_at	At5g57240	248968_at	At5g45280
247136_at	At5g66170	247954_at	At5g56870	248988_at	At5g45190
247137_at	At5g66210	247979_at	At5g56750	249009_at	At5g44610
247140_at	At5g66250	247992_at	At5g56520	249027_at	At5g44785
247156_at	At5g65760	248025_at	At5g55840	249033_at	At5g44930
247176_at	At5g65110	248050_at	At5g56100	249045_at	At5g44380
247189_at	At5g65390	248058_at	At5g55530	249053_at	At5g44440
247191_at	At5g65310	248100_at	At5g55180	249064_at	At5g44250
247199_at	At5g65210	248146_at	At5g54940	249065_at	At5g44260
247210_at	At5g65020	248162_at	At5g54500	249079_at	At5g43930
247246_at	At5g64620	248193_at	At5g54080	249097_at	At5g43520
247266_at	At5g64570	248197_at	At5g54190	249101_at	At5g43580
247273_at	At5g64300	248208_at	At5g53980	249109_at	At5g43700
247275_at	At5g64370	248213_at	At5g53660	249136_at	At5g43180
247284_at	At5g64410	248228_at	At5g53800	249174_at	At5g42900
247295_at	At5g64180	248236_at	At5g53870	249178_at	At5g42890
247328_at	At5g64130	248245_at	At5g53190	249187_at	At5g43060
247333_at	At5g63600	248252_at	At5g53250	249199_at	At5g42520
247348_at	At5g63810	248302_at	At5g53160	249217_at	At5g42300
247352_at	At5g63650	248337_at	At5g52310	249239_at	At5g41990
247353_at	At5g63620	248344_at	At5g52280	249293_at	At5g41260
247374_at	At5g63190	248386_at	At5g51940	249316_s_at	At5g41220
247418_at	At5g63030	248410_at	At5g51570	249346_at	At5g40780
247474_at	At5g62280	248435_at	At5g51210	249375_at	At5g40730
247524_at	At5g61440	248563_at	At5g49690	249378_at	At5g40450
247525_at	At5g61380	248564_at	At5g49700	249384_at	At5g39890
247541_at	At5g61590	248581_at	At5g49900	249454_at	At5g39520
247582_at	At5g60760	248606_at	At5g49450	249456_at	At5g39410
247586_at	At5g60660	248697_at	At5g48370	249459_at	At5g39580
247632_at	At5g60460	248698_at	At5g48380	249467_at	At5g39610
247677_at	At5g59420	248713_at	At5g48180	249493_at	At5g39080
247691_at	At5g59720	248727_at	At5g47980	249494_at	At5g39050
247696_at	At5g59780	248779_at	At5g47720	249527_at	At5g38560
247721_at	At5g59140	248790_at	At5g47450	249575_at	At5g37660
247731_at	At5g59590	248793_at	At5g47240	249581_at	At5g37600
247743_at	At5g59010	248796_at	At5g47180	249582_at	At5g37780
247800_at	At5g58570	248801_at	At5g47370	249606_at	At5g37260
247851_at	At5g58070	248813_at	At5g46860	249612_at	At5g37290
247863_at	At5g57900	248869_at	At5g46840	249614_at	At5g37300
247878_at	At5g57760	248879_at	At5g46180	249729_at	At5g24410
247912_at	At5g57480	248881_at	At5g46020	249750_at	At5g24570

249800_at	At5g23660	250385_at	At5g11520	251039_at	At5g02020
249823_s_at	At5g23350	250409_at	At5g10860	251050_at	At5g02430
249824_at	At5g23380	250412_at	At5g11150	251061_at	At5g01830
249838_at	At5g23460	250420_at	At5g11260	251074_at	At5g01800
249847_at	At5g23210	250421_at	At5g11270	251094_at	At5g01350
249848_at	At5g23220	250437_at	At5g10430	251109_at	At5g01600
249861_at	At5g22875	250438_at	At5g10580	251112_s_at	At5g01320
249863_at	At5g22950	250472_at	At5g10210	251144_at	At5g01210
249869_at	At5g23050	250476_at	At5g10140	251189_at	At3g62650
249894_at	At5g22580	250516_at	At5g09620	251199_at	At3g62980
249895_at	At5g22500	250524_at	At5g08510	251221_at	At3g62550
249996_at	At5g18600	250548_at	At5g08100	251227_at	At3g62700
249999_at	At5g18640	250556_at	At5g07950	251235_at	At3g62860
250000_at	At5g18650	250580_at	At5g07440	251274_at	At3g61700
250028_at	At5g18130	250609_at	At5g07470	251310_at	At3g61150
250032_at	At5g18170	250649_at	At5g06690	251317_at	At5g28350
250033_at	At5g18110	250661_at	At5g07030	251338_at	At3g60600
250053_at	At5g17830	250662_at	At5g07010	251356_at	At3g61060
250054_at	At5g17850	250666_at	At5g07100	251358_at	At3g61160
250079_at	At5g16650	250680_at	At5g06570	251373_at	At3g60530
250103_at	At5g16600	250737_at	At5g06370	251403_at	At3g60290
250109_at	At5g15230	250748_at	At5g05710	251406_at	At3g60330
250114_s_at	At5g16340	250753_at	At5g05860	251411_at	At3g60250
250119_at	At5g16470	250755_at	At5g05750	251427_at	At3g60130
250165_at	At5g15290	250781_at	At5g05410	251428_at	At3g60140
250175_at	At5g14390	250811_at	At5g05110	251436_at	At3g59900
250179_at	At5g14440	250819_at	At5g04930	251438_s_at	At5g33355
250182_at	At5g14470	250830_at	At5g05250	251450_at	At3g60030
250184_at	At5g14240	250844_at	At5g04470	251453_at	At3g60070
250191_at	At5g14270	250858_at	At5g04760	251478_at	At3g59690
250199_at	At5g14180	250860_at	At5g04770	251494_at	At3g59350
250201_at	At5g14230	250863_at	At5g04750	251517_at	At3g59370
250208_at	At5g13930	250868_at	At5g03860	251551_at	At3g58680
250273_at	At5g13010	250877_at	At5g04040	251600_at	At3g57840
250277_at	At5g12940	250881_at	At5g04080	251643_at	At3g57520
250287_at	At5g13330	250884_at	At5g03940	251758_at	At3g55770
250293_s_at	At5g13360	250891_at	At5g04530	251772_at	At3g55920
250296_at	At5g12020	250901_at	At5g03530	251789_at	At3g55450
250301_at	At5g11970	250909_at	At5g03700	251799_at	At3g55520
250302_at	At5g11920	250911_at	At5g03730	251927_at	At3g53990
250313_at	At5g12210	250921_at	At5g03460	251945_at	At3g53520
250316_at	At5g12140	250928_at	At5g03280	251954_at	At3g53660
250317_at	At5g12250	250935_at	At5g03240	251956_at	At3g53460
250339_at	At5g11670	250968_at	At5g02890	251974_at	At3g53200
250346_at	At5g11950	250992_at	At5g02260	252034_at	At3g52040
250351_at	At5g12030	251012_at	At5g02580	252067_at	At3g51370

252081_at	At3g51670	252993_at	At4g38540	253720_at	At4g29270
252091_at	At3g51390	252997_at	At4g38400	253722_at	At4g29190
252102_at	At3g50970	253011_at	At4g37890	253732_at	At4g29140
252133_at	At3g50790	253038_at	At4g37790	253762_at	At4g28820
252166_at	At3g50500	253041_at	At4g37870	253806_at	At4g28270
252188_at	At3g50860	253049_at	At4g37300	253822_at	At4g28410
252206_at	At3g50360	253054_at	At4g37470	253829_at	At4g28040
252213_at	At3g50210	253056_at	At4g37630	253864_at	At4g27460
252224_at	At3g49860	253065_at	At4g37740	253868_at	At4g27490
252234_at	At3g49780	253103_at	At4g36110	253871_at	At4g27530
252250_at	At3g49790	253104_at	At4g36010	253874_at	At4g27540
252282_at	At3g49360	253120_at	At4g35790	253887_at	At4g27730
252291_s_at	At3g49120	253140_at	At4g35480	253897_at	At4g27190
252296_at	At3g48970	253147_at	At4g35600	253900_at	At4g27080
252303_at	At3g49210	253155_at	At4g35720	253908_at	At4g27260
252310_at	At3g49350	253159_at	At4g35570	253981_at	At4g26670
252315_at	At3g48690	253163_at	At4g35750	254011_at	At4g26370
252323_at	At3g48530	253184_at	At4g35230	254043_at	At4g25990
252329_at	At3g48760	253217_at	At4g34970	254050_s_at	At4g25670
252331_s_at	At3g48790	253268_s_at	At4g34135	254057_at	At4g25170
252415_at	At3g47340	253273_at	At4g34180	254073_at	At4g25500
252419_at	At3g47510	253277_at	At4g34230	254110_at	At4g25260
252422_at	At3g47550	253279_at	At4g34030	254144_at	At4g24690
252464_at	At3g47160	253281_at	At4g34138	254157_at	At4g24220
252475_s_at	At3g46610	253285_at	At4g34250	254162_at	At4g24440
252488_at	At3g46700	253304_at	At4g33780	254193_at	At4g23850
252515_at	At3g46230	253322_at	At4g33980	254206_at	At4g24180
252565_at	At3g46000	253373_at	At4g33150	254215_at	At4g23700
252570_at	At3g45300	253400_at	At4g32860	254216_at	At4g23710
252591_at	At3g45330	253401_at	At4g32870	254225_at	At4g23670
252607_at	At3g44990	253418_at	At4g32760	254226_at	At4g23690
252622_at	At3g45270	253421_at	At4g32340	254234_at	At4g23680
252698_at	At3g43670	253422_at	At4g32240	254258_at	At4g23410
252738_at	At3g43240	253437_at	At4g32470	254269_at	At4g23050
252740_at	At3g43270	253462_at	At4g32150	254276_at	At4g22820
252751_at	At3g43430	253510_at	At4g31730	254292_at	At4g23030
252764_at	At3g42790	253519_at	At4g31240	254293_at	At4g23060
252789_s_at	At3g42150	253526_at	At4g31420	254306_at	At4g22330
252832_at	At4g39910	253556_at	At4g31100	254361_at	At4g22340
252855_at	At4g39660	253597_at	At4g30690	254385_s_at	At4g21830
252882_at	At4g39675	253608_at	At4g30290	254387_at	At4g21850
252885_at	At4g39260	253614_at	At4g30350	254392_at	At4g21600
252924_at	At4g39070	253617_at	At4g30410	254393_at	At4g21580
252927_at	At4g39090	253629_at	At4g30450	254399_at	At4g21280
252956_at	At4g38630	253697_at	At4g29700	254409_at	At4g21400
252991_at	At4g38470	253702_at	At4g29900	254422_at	At4g21560

254425_at	At4g21450	255430_at	At4g03320	256589_at	At3g28740
254432_at	At4g20830	255474_at	At4g02470	256601_s_at	At3g28290
254446_at	At4g20930	255517_at	At4g02290	256626_at	At3g20015
254477_at	At4g20380	255521_at	At4g02280	256627_at	At3g19970
254490_at	At4g20320	255543_at	At4g01990	256671_at	At3g52290
254492_at	At4g20260	255575_at	At4g01430	256697_at	At3g20650
254500_at	At4g20110	255590_at	At4g01610	256712_at	At2g34020
254506_at	At4g20140	255625_at	At4g01120	256725_at	At2g34070
254530_at	At4g19640	255671_at	At4g00335	256743_at	At3g29370
254559_at	At4g19200	255723_at	At3g29575	256747_at	At3g29180
254563_at	At4g19120	255788_at	At2g33310	256765_at	At3g22200
254580_at	At4g19390	255794_at	At2g33480	256799_at	At3g18560
254609_at	At4g18970	255795_at	At2g33380	256804_at	At3g20920
254644_at	At4g18510	255805_at	At4g10240	256848_at	At3g27960
254656_at	At4g18070	255811_at	At4g10250	256863_at	At3g24070
254665_at	At4g18340	255872_at	At2g30360	256870_at	At3g26300
254667_at	At4g18280	255923_at	At1g22080	256914_at	At3g23880
254682_at	At4g13640	255942_at	At1g20350	256948_at	At3g18920
254685_at	At4g13850	255994_at	At1g29760	256965_at	At3g13450
254702_at	At4g17940	256001_at	At1g29860	256970_at	At3g21090
254726_at	At4g13660	256030_at	At1g34110	257038_at	At3g19260
254767_s_at	At4g13290	256061_at	At1g07040	257052_at	At3g15290
254794_at	At4g12970	256091_at	At1g20693	257066_at	At3g18280
254797_at	At4g13030	256113_at	At1g16920	257122_at	At3g20250
254853_at	At4g12080	256114_at	At1g16810	257162_s_at	At3g24290
254861_at	At4g12040	256193_at	At1g30200	257171_at	At3g23760
254876_at	At4g11610	256221_at	At1g56300	257192_at	At3g13200
254889_at	At4g11650	256225_at	At1g56220	257293_at	At3g15580
254907_at	At4g11190	256232_at	At3g12630	257295_at	At3g17420
254915_s_at	At4g11290	256235_at	At3g12490	257323_at	AtMg01200
254919_at	At4g11360	256237_at	At3g12610	257502_at	At1g78110
254951_at	At4g10810	256240_at	At3g12600	257504_at	At1g52250
254954_at	At4g10910	256243_at	At3g12500	257516_at	At1g69040
254990_at	At4g10610	256252_at	At3g11340	257580_at	At1g50760
254999_at	At4g09830	256300_at	At1g69490	257621_at	At3g20410
255028_at	At4g09890	256301_at	At1g69510	257643_at	At3g25710
255064_at	At4g08950	256302_at	At1g69526	257670_at	At3g20340
255093_s_at	At5g17900	256311_at	At1g35890	257679_at	At3g20420
255127_at	At4g08300	256326_at	At3g02340	257689_at	At3g12810
255160_at	At4g07810	256340_at	At1g72070	257701_at	At3g12710
255216_s_at	At4g07670	256413_at	At3g11100	257743_at	At3g27390
255221_at	At4g05150	256422_at	At1g33520	257766_at	At3g23030
255230_at	At4g05390	256433_at	At3g10990	257769_at	At3g23050
255261_s_at	At4g05110	256452_at	At1g75240	257805_at	At3g18830
255283_at	At4g04620	256455_at	At1g75190	257830_at	At3g26690
255381_at	At4g03510	256583_at	At3g28790	257832_at	At3g26740

257852_at	At3g12950	258745_at	At3g05920	259598_at	At1g27980
257879_at	At3g17160	258749_at	At3g05760	259617_at	At1g47970
257891_at	At3g17170	258805_at	At3g04760	259642_at	At1g69030
257892_at	At3g17020	258878_at	At3g03170	259680_at	At1g77690
257894_at	At3g17100	258880_at	At3g06420	259694_at	At1g63180
257896_at	At3g16920	258901_at	At3g05640	259724_at	At1g60940
257926_at	At3g23280	258923_at	At3g10450	259736_at	At1g64390
257939_at	At3g19820	258935_at	At3g10140	259739_at	At1g64350
257947_at	At3g21720	258939_at	At3g10020	259756_at	At1g71080
257952_at	At3g21770	258954_at	At3g01400	259769_at	At1g29400
257967_at	At3g19800	258955_s_at	At3g01450	259788_at	At1g29670
257981_at	At3g20770	259047_at	At3g03390	259793_at	At1g64380
257985_at	At3g20810	259058_at	At3g03470	259794_at	At1g64330
257990_at	At3g19860	259118_at	At3g01310	259801_at	At1g72230
258005_at	At3g19390	259137_at	At3g10300	259803_at	At1g72150
258052_at	At3g16190	259143_at	At3g10190	259831_at	At1g69600
258061_at	At3g25910	259154_at	At3g10260	259839_at	At1g52190
258075_at	At3g25900	259162_at	At3g01640	259841_at	At1g52200
258091_at	At3g14560	259164_at	At3g01770	259865_at	At1g72710
258109_at	At3g23640	259165_at	At3g01470	259878_at	At1g76790
258114_at	At3g14660	259173_at	At3g03640	259911_at	At1g72680
258133_at	At3g24500	259181_at	At3g01690	259932_at	At1g34370
258141_at	At3g18035	259184_at	At3g01520	259976_at	At1g76560
258158_at	At3g17790	259230_at	At3g07780	259977_at	At1g76590
258206_at	At3g14010	259244_at	At3g07790	259982_at	At1g76410
258207_at	At3g14050	259257_at	At3g07760	259984_at	At1g76460
258225_at	At3g15630	259264_at	At3g01260	259996_at	At1g67910
258253_at	At3g26760	259302_at	At3g05120	260005_at	At1g67920
258272_at	At3g15610	259351_at	At3g05150	260020_at	At1g29990
258332_at	At3g16180	259364_at	At1g13260	260026_at	At1g29970
258362_at	At3g14280	259366_at	At1g13280	260030_at	At1g68880
258366_at	At3g14230	259381_s_at	At3g16390	260055_at	At1g78150
258367_at	At3g14370	259391_s_at	At1g06340	260083_at	At1g63220
258399_at	At3g15540	259395_at	At1g06400	260097_at	At1g73220
258402_at	At3g15450	259415_at	At1g02330	260101_at	At1g73260
258434_at	At3g16770	259416_at	At1g02305	260129_at	At1g36380
258455_at	At3g22440	259417_at	At1g02340	260138_at	At1g66410
258487_at	At3g02550	259426_at	At1g01470	260143_at	At1g71880
258491_at	At3g02700	259429_at	At1g01600	260179_at	At1g70690
258498_at	At3g02480	259479_at	At1g19020	260181_at	At1g70710
258507_at	At3g06500	259502_at	At1g15670	260226_at	At1g74660
258527_at	At3g06850	259516_at	At1g20450	260248_at	At1g74310
258555_at	At3g06860	259544_at	At1g20620	260264_at	At1g68500
258565_at	At3g04350	259570_at	At1g20440	260287_at	At1g70490
258617_at	At3g03000	259586_at	At1g28100	260301_at	At1g80340
258641_at	At3g08030	259595_at	At1g28050	260367_at	At1g69760

260371_at	At1g69690	261252_at	At1g05810	262114_at	At1g02860
260418_s_at	At1g66590	261253_at	At1g05840	262119_s_at	At1g02930
260431_at	At1g68190	261272_at	At1g26665	262128_at	At1g52690
260436_at	At1g68140	261318_at	At1g53035	262159_at	At1g52720
260442_at	At1g68220	261335_at	At1g44800	262166_at	At1g74840
260444_at	At1g68300	261395_at	At1g79700	262173_at	At1g74920
260484_at	At1g11000	261459_at	At1g21100	262204_at	At2g01100
260502_at	At1g47210	261484_at	At1g14400	262215_at	At1g74790
260527_at	At2g47270	261506_at	At1g71697	262220_at	At1g74740
260536_at	At2g43400	261534_at	At1g01820	262225_at	At1g53840
260549_at	At2g43535	261558_at	At1g01770	262226_at	At1g53885
260567_at	At2g43820	261559_at	At1g01780	262228_at	At1g68690
260588_at	At1g53320	261561_at	At1g01730	262236_at	At1g48330
260589_at	At1g53400	261567_at	At1g33055	262295_at	At1g27650
260610_at	At2g43680	261572_at	At1g01170	262312_at	At1g70830
260639_at	At1g53180	261586_at	At1g01640	262347_at	At1g63980
260662_at	At1g19540	261606_at	At1g49570	262373_at	At1g73130
260668_at	At1g19530	261607_at	At1g49660	262407_at	At1g34630
260697_at	At1g32530	261608_at	At1g49650	262412_at	At1g34760
260728_at	At1g48210	261615_at	At1g33050	262434_at	At1g47670
260741_at	At1g15045	261639_at	At1g49975	262456_at	At1g11260
260799_at	At1g78270	261644_s_at	At1g27840	262488_at	At1g21820
260818_at	At1g06900	261674_at	At1g18270	262495_at	At1g21780
260835_at	At1g06700	261711_at	At1g32700	262499_at	At1g21770
260855_at	At1g21970	261745_at	At1g08500	262503_at	At1g21670
260882_at	At1g29280	261749_at	At1g76180	262505_at	At1g21680
260887_at	At1g29160	261771_at	At1g76150	262516_at	At1g17190
260889_at	At1g29130	261823_at	At1g11400	262525_at	At1g17060
260900_s_at	At1g21400	261825_at	At1g11545	262570_at	At1g15200
260914_at	At1g02640	261827_at	At1g11480	262592_at	At1g15400
260944_at	At1g45145	261828_at	At1g11360	262599_at	At1g15350
260957_at	At1g06040	261838_at	At1g16030	262602_at	At1g15270
260964_at	At1g44990	261872_s_at	At1g11520	262603_at	At1g15380
260978_at	At1g53540	261927_at	At1g22490	262607_at	At1g13990
260989_at	At1g53450	261951_at	At1g64490	262621_at	At1g06530
261032_at	At1g17410	261957_at	At1g64660	262635_at	At1g06570
261046_at	At1g01390	262001_at	At1g33790	262640_at	At1g62763
261055_at	At1g01300	262038_at	At1g35580	262643_at	At1g62770
261109_at	At1g75450	262044_s_at	At1g80210	262657_at	At1g14210
261129_at	At1g04820	262047_at	At1g80160	262661_s_at	At1g14250
261140_at	At1g19680	262049_at	At1g80180	262682_at	At1g75900
261211_at	At1g12780	262053_at	At1g79940	262694_at	At1g62790
261224_at	At1g20160	262061_at	At1g80110	262709_at	At1g16240
261226_at	At1g20190	262072_at	At1g59590	262725_at	At1g43580
261230_at	At1g20010	262095_at	At1g56090	262727_at	At1g75800
261250_at	At1g05890	262110_at	At1g02840	262746_at	At1g28680

262749_at	At1g28540	263844_at	At2g36930	264577_at	At1g05260
262756_at	At1g16370	263856_at	At2g04410	264580_at	At1g05340
262844_at	At1g14687	263881_at	At2g21960	264588_at	At2g17730
262845_at	At1g14890	263894_at	At2g21910	264590_at	At2g17710
262908_at	At1g59900	263898_at	At2g21950	264633_at	At1g65660
262911_s_at	At1g59860	263901_at	At2g36320	264654_s_at	At1g08900
262958_at	At1g54410	263920_at	At2g36410	264663_at	At1g09970
262975_at	At1g75540	263921_at	At2g36460	264709_at	At1g09740
262978_at	At1g75780	263957_at	At2g35880	264756_at	At1g61370
263065_at	At2g18170	263963_at	At2g36080	264777_at	At1g08630
263073_at	At2g17500	263978_at	At2g42680	264787_at	At2g17920
263096_at	At2g16040	263981_at	At2g42870	264788_at	At2g17840
263098_at	At2g16070	263983_at	At2g42780	264851_at	At2g17340
263116_s_at	At1g03140	263986_at	At2g42790	264883_s_at	At1g61250
263117_at	At1g03040	264026_at	At2g21050	264900_at	At1g23080
263118_at	At1g03090	264045_at	At2g22450	264901_at	At1g23090
263137_at	At1g78660	264121_at	At1g02280	264908_at	At2g17440
263157_at	At1g54100	264124_at	At1g79360	264923_s_at	At1g60740
263184_at	At1g05570	264146_at	At1g02205	264938_at	At1g61130
263206_at	At1g10590	264147_at	At1g02200	264953_at	At1g77120
263207_at	At1g10550	264157_at	At1g65310	264985_at	At1g27150
263216_s_at	At1g30720	264209_at	At1g22740	264987_at	At1g27030
263231_at	At1g05680	264211_at	At1g22770	265023_at	At1g24440
263265_at	At2g38820	264229_at	At1g67480	265031_at	At1g61610
263275_at	At2g14170	264246_at	At1g60450	265035_at	At1g61660
263296_at	At2g38800	264247_at	At1g60140	265043_at	At1g03900
263319_at	At2g47160	264300_at	At1g78860	265044_at	At1g03950
263325_at	At2g04240	264338_at	At1g70300	265067_at	At1g03850
263333_at	At2g03890	264342_at	At1g12080	265070_at	At1g55545
263352_at	At2g22080	264346_at	At1g03370	265084_at	At1g03830
263403_at	At2g04040	264422_at	At1g43130	265107_s_at	At1g63340
263413_at	At2g21240	264447_at	At1g27300	265116_at	At1g62560
263437_at	At2g28670	264458_at	At1g10410	265131_at	At1g23760
263443_at	At2g28630	264460_at	At1g10170	265188_at	At1g23800
263509_s_at	AtMg00730	264462_at	At1g10200	265245_at	At2g43060
263513_at	At2g12400	264467_at	At1g10140	265246_at	At2g43050
263517_at	At2g21620	264486_at	At1g77180	265266_at	At2g42890
263548_at	At2g21680	264504_at	At1g09430	265296_at	At2g14060
263647_at	At2g04690	264507_at	At1g09415	265321_at	At2g18280
263653_at	At1g04330	264508_at	At1g09570	265330_at	At2g18440
263656_at	At1g04500	264510_at	At1g09530	265344_at	At2g22660
263711_at	At2g20630	264517_at	At1g10090	265345_at	At2g22680
263737_at	At1g60010	264524_at	At1g10070	265352_at	At2g16600
263765_at	At2g21540	264527_at	At1g30760	265358_at	At2g16710
263799_at	At2g24550	264551_at	At1g09460	265375_at	At2g06530
263839_at	At2g36900	264561_at	At1g55810	265427_at	At2g20730

265457_at	At2g46550	266294_at	At2g29500	267158_at	At2g37640
265460_at	At2g46600	266296_at	At2g29420	267161_at	At2g37680
265467_at	At2g37050	266322_at	At2g46690	267178_at	At2g37580
265471_at	At2g46480	266330_at	At2g01530	267181_at	At2g37760
265478_at	At2g15890	266348_at	At2g01450	267209_at	At2g30930
265481_at	At2g15960	266358_at	At2g32280	267226_at	At2g44010
265510_at	At2g05630	266360_at	At2g32250	267230_at	At2g44080
265539_at	At2g15830	266364_at	At2g41230	267238_at	At2g44130
265561_s_at	At2g05520	266415_at	At2g38530	267254_at	At2g23030
265627_at	At2g27285	266418_at	At2g38750	267261_at	At2g23120
265663_at	At2g24500	266423_at	At2g41340	267268_at	At2g02570
265670_s_at	At2g32210	266428_at	At2g07170	267278_at	At2g19350
265672_at	At2g31980	266489_at	At2g35190	267337_at	At2g19310
265679_at	At2g32240	266503_at	At2g47780	267340_at	At2g39870
265718_at	At2g03340	266507_at	At2g47860	267357_at	At2g40000
265720_at	At2g40110	266518_at	At2g35170	267361_at	At2g39920
265740_at	At2g01150	266580_at	At2g46260	267374_at	At2g26230
265790_at	At2g01170	266590_at	At2g46240	267376_at	At2g26330
265807_at	At2g17990	266613_at	At2g14900	267383_at	At2g44360
265817_at	At2g18050	266643_s_at	At2g29730	267461_at	At2g33830
265909_at	At2g25720	266644_at	At2g29660	267485_at	At2g02820
265911_at	At2g25670	266688_at	At2g19660	267488_at	At2g19110
265940_at	At2g19480	266695_at	At2g19810	267496_at	At2g30550
265952_at	At2g37480	266743_at	At2g02990	267500_s_at	At2g45510
265953_at	At2g37490	266752_at	At2g47020	267513_at	At2g45620
265968_at	At2g37410	266753_at	At2g47000	267536_at	At2g42010
265987_at	At2g24240	266783_at	At2g29120	267590_at	At2g39700
265989_at	At2g24260	266799_at	At2g22860	267591_at	At2g39705
266017_at	At2g18690	266825_at	At2g22890	267624_at	At2g39660
266019_at	At2g18750	266839_at	At2g25930	267637_at	At2g42190
266044_s_at	AtMg00210	266872_at	At2g44730		
266072_at	At2g18660	266884_at	At2g44790		
266090_at	At2g38050	266897_at	At2g45820		
266119_at	At2g02100	266906_at	At2g34585		
266121_at	At2g02160	266964_at	At2g39450		
266125_at	At2g45050	266974_at	At2g39620		
266150_s_at	At2g12290	266988_at	At2g39310		
266156_at	At2g28110	266993_at	At2g39210		
266181_at	At2g02390	266995_at	At2g34500		
266203_at	At2g02230	267008_at	At2g39350		
266222_at	At2g28780	267035_at	At2g38400		
266225_at	At2g28900	267040_at	At2g34300		
266228_at	At2g28910	267059_at	At2g32520		
266229_at	At2g28840	267060_at	At2g32580		
266259_at	At2g27830	267080_at	At2g41190		
266290_at	At2g29490	267157_at	At2g37630		