Plant Biotechnology 22, 393–399 (2005)

Review

Regulation of lateral root formation by auxin signaling in *Arabidopsis*

Hidehiro Fukaki, Yoko Okushima, Masao Tasaka*

Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, 630-0101 Ikoma, Nara, Japan
* E-mail: m-tasaka@bs.naist.jp Tel: +81-743-72-5480 Fax: +81-743-72-5489

Received August 8, 2005; accepted August 16, 2005 (Edited by Y. Hotta)

Abstract In higher plants, lateral root (LR) formation is a major contributor to root architecture in the soil. Although it is well known that auxin promotes LR formation, the molecular mechanisms that regulate auxin-mediated LR formation are still largely unknown. Recent molecular genetic studies using *Arabidopsis* mutants have shown that normal auxin signaling mediated by two families of transcriptional regulators, Aux/IAAs (Auxin/Indole-3-Acetic Acid; repressors of auxin-responsive transcription) and ARFs (Auxin Response Factor; activators or repressors of auxin-responsive transcription), is necessary for LR formation. Auxin-responsive transcription mediated by SLR/IAA14 and ARF7/ARF19 in the root pericycle is particularly important for LR initiation. This review summarizes what is known about the auxin signaling that regulates LR formation in *Arabidopsis*, and discusses the role of Aux/IAAs and ARFs for LR initiation and subsequent primordial development.

Key words: ARFs, Aux/IAAs, auxin, lateral root formation, pericycle, SLR/IAA14.

The root systems of higher plants are developed through the production of many lateral and adventitious roots which enable the plant to absorb water and nutrients efficiently and to sustain the aerial shoots (Fitter 1996). It is well known that LR formation is dependent on auxin, but the molecular mechanisms that regulate auxin-mediated LR formation are still largely unknown. Recently, the Aux/IAA and ARF (Auxin Response Factor) families of *Arabidopsis* transcriptional regulators have been shown to be important for mediating auxin signaling in LR formation.

Several good reviews on LR development (Malamy and Benfey, 1997a; Casimiro et al. 2003) and on auxin signaling (Berleth et al. 2004; Dharmasiri and Estelle 2004; Woodward and Bartel 2005) have been published. This mini-review focuses on what is known about auxin signaling in the regulation of LR formation in *Arabidopsis*.

Lateral root development in *Arabidopsis*

The primary root (tap root) of higher plants is derived from embryonic root developed during embryogenesis. In contrast, LRs develop from the inner cell layer of the parental roots (primary roots, existing LRs, and adventitious roots) as a post-embryonic organ. In the case of *Arabidopsis*, LRs are initiated from the anticlinal cell divisions in the root pericycle adjacent to the two-protoxylem poles (protoxylem pericycle) (Laskowski et al. 1995; Malamy and Benfey 1997a; Beeckman et al. 2001; Casimiro et al. 2001; Himanen et al. 2002; Casimiro et al. 2003). These divided cells then undergo periclinal cell divisions to make daughter cells (outer layer) and inner pericycle cells, becoming a young LR primordium (Malamy and Benfey 1997a; Casimiro et al. 2001). The LR meristem is established after a series of cell divisions and cell differentiation in the primordium, which promotes subsequent LR growth. The cellular organization of the established LR meristem is structurally indistinguishable from that of the primary root meristem, suggesting the existence of a common machinery for root patterning. However, the developmental processes of LR initiation are different from those of embryonic root initiation, indicating that there are important differences in the molecular mechanisms between embryonic root and LR initiation. A recent study has shown that most of the cells in *Arabidopsis* LR primordia are derived from the central of the three protoxylem pericycle cell files adjacent to the xylem pole (Kurup et al. 2005). Although apparently genetically determined in *Arabidopsis*, it is unknown why LRs are initiated from the protoxylem pericycle, rather than from the protophloem pericycle.

Abbreviations: ARF, Auxin Response Factor; Aux/IAA, Auxin/Indole-3-Acetic Acid; IAA, indole-3-acetic acid; LR, lateral root; NAA, 1-naphthaleneacetic acid; NPA, N-1-naphthylphthalamic acid; PCIB, p-chlorophenoxyisobutyric acid; SLR, SOLITARY-ROOT; 2,4-D, 2,4-dichlorophenoxyacetic acid.

This article can be found at http://www.jspcmb.jp/

Lateral root initiation is dependent on auxin

In *Arabidopsis*, exogenous auxin (eg. IAA, NAA, and 2,4-D) promotes LR initiation (Laskowski et al. 1995; Fukaki et al. 2002; Himanen et al. 2002), whereas auxin transport inhibitors (eg. NPA) and the anti-auxin PCIB block LR initiation (Reed et al. 1998; Casimiro et al. 2001; Oono et al. 2003), indicating that LR initiation is dependent on auxin. With the use of NPA and NAA, Himanen et al. (2002) established an inducible LR system. In this system, when *Arabidopsis* seedlings grown on NPA-containing medium for 72 hours after germination are transferred onto NAA-containing medium, the pericycle cells of these seedlings begin synchronous division. This allows changes in the expression of cell cycle-related genes during auxin-induced LR initiation to be monitored, and indicate that cell cycle regulation is one of the critical points for auxin-mediated LR initiation (Himanen et al. 2002, 2004).

Mutational analyses in *Arabidopsis* have also shown that LR initiation depends on auxin, as mutations affecting auxin biosynthesis and transport have been shown to affect the number of LRs. The *super root1*/*rooty*/*alf1* mutants that overproduce IAA have an increased number of LRs (Boerjan et al. 1995; Celenza et al. 1995; King et al. 1995). On the other hand, mutants defective in polar auxin transport, such as the *aux1*, *axr4* and *tir3*/*doc1*/*big*, have fewer LRs (Hobbie and Estelle 1995; Ruegger et al. 1997; Gil et al. 2001; Bhalerao et al. 2002; Marchant et al. 2002). These mutations change the level of endogenous free IAA in roots, affecting the number of LR initiations along the primary root. In addition, overexpression of the *DFL1*/*GH3-6* gene, which encodes an enzyme that decreases free IAA level by amino acid conjugation, also results in a reduction in LR initiation (Nakazawa et al. 2001; Staswick et al. 2005). These observations indicate that normal auxin biosynthesis and transport are necessary for LR initiation.

The role of auxin signaling in LR initiation

In addition to the mutations affecting auxin levels, studies on mutations affecting auxin sensitivity have shown the importance of auxin signaling in LR initiation. In plant cells, auxin signals are captured by TIR1 (TRANSPORT INHIBITOR RESISTANCE1), an F box protein that has been shown to function as an auxin receptor (Dharmasiri et al. 2005a; Kepinski and Leyser 2005). The *tir1* mutant has a decreased number of LRs compared to the wild type (Ruegger et al. 1998). Three additional TIR1-related F box proteins, called AFB1, AFB2, and AFB3, also function in auxin response (Dharmasiri et al. 2005b). Triple or quadruple mutants in *TIR1*/*AFBs* have pleiotropic auxin-related phenotypes, including the lack of LRs, indicating that *TIR1*/*AFBs* act as auxin receptors for LR formation in addition to the other auxin-mediated growth and developmental processes (Dharmasiri et al. 2005b). Auxin signals captured by *TIR1*/*AFBs* promote the ubiquitination of the Aux/IAA (Auxin/Indole-3-Acetic Acid) proteins, repressors of auxin-responsive transcription, through $SCF^{TIR1/AFB1/2/3}$ E3 ubiquitin-ligase complexes and the degradation of Aux/IAA proteins by the 26S proteasome (Gray et al. 2001; Zenser et al. 2001; reviewed by Dharmasiri and Estelle 2004; Dharmasiri et al. 2005b). This allows auxin-responsive transcription regulated by ARF (Auxin Response Factor) proteins that can act as a transcriptional activator or repressor. Mutations in the subunits of $SCF^{TIR1/AFB1/2/3}$ complexes (ASK1, ASK2, AtCulin1/AXR6 and RBX1) and in the components that regulate the activity of $SCF^{TIR1/AFB1/2/3}$ complexes through the RUB/Nedd8 conjugation pathway (AXR1, ECR1, RCE1, RUBs, SGT1b/ETA3, CAND1/ETA2 and COP9 signalosome) result in the accumulation of Aux/IAA proteins, thereby repressing the normal auxin response (reviewed by Dharmasiri and Estelle 2004; Woodward and Bartel 2005). Such defects in the $SCF^{TIR1/AFB1/2/3}$-dependent degradation of Aux/IAA proteins also decrease the number of LRs (Dharmasiri et al. 2003).

Aux/IAAs and ARFs: transcriptional regulators for auxin-mediated growth and development, including LR formation

The control of auxin-responsive transcription mediated by Aux/IAAs and ARFs also has an important role in auxin-mediated growth and development (reviewed by Hagen and Guilfoyle 2002; Liscum and Reed 2002; Berleth et al. 2004). There are 29 *Aux*/*IAA* and 23 *ARF* family members in the *Arabidopsis* genome (Hagen and Guilfoyle 2002; Liscum and Reed 2002; Remington et al. 2004). ARF proteins bind to the auxin-responsive elements (AuxREs) in the promoters of the many auxin-responsive genes, and activate or repress the transcription of their target genes (Guilfoyle et al. 1998; Ulmasov et al. 1997a, 1997b, 1999a, 1999b, Tiwari et al. 2003; Wang et al. 2005). The C-terminus domains (CTD) of ARFs are responsible for homodimerization and heterodimerization with other ARFs, and also for heterodimerization with Aux/IAA proteins (Figure 1; Kim et al. 1997; Ulmasov et al. 1999b; Hardtke et al. 2004; Tatematsu et al. 2004; Fukaki et al. 2005). Most Aux/IAA proteins have four highly-conserved domains (I–IV) (Figure 1; Abel et al. 1995; reviewed by Reed 2001; Liscum and Reed 2002). Domains III and IV are similar to the CTDs of ARFs, and are also responsible for heterodimerization with ARF proteins (Figure 1; Kim

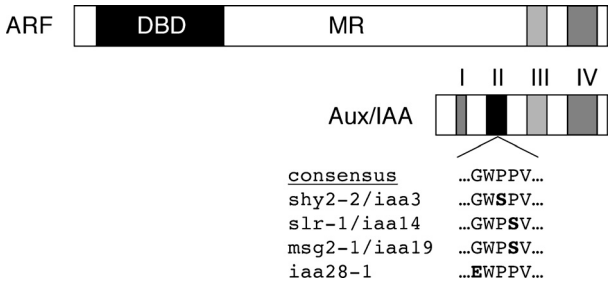

Figure 1. Structure of Aux/IAA and ARF proteins. Domain II of Aux/IAA protein is important for stability of the protein; an amino acid substitution in the highly-conserved five amino acids (GWPPV; Gly-Trp-Pro-Pro-Val, a consensus among IAA3, IAA14, IAA19, and IAA28 proteins) in this domain stabilizes the protein. The amino acid sequences in this domain are shown for the shy2-1/iaa3, slr-1/iaa14, msg2-1/iaa19, and iaa28-1 mutant proteins. Substituted amino acids are indicated in bold. The Aux/IAAs and ARFs interact through domains III and IV. DBD; DNA binding domain, MR; middle region.

et al. 1997; Ulmasov et al. 1999b; Ouellet et al. 2001; Hardtke et al. 2004; Tatematsu et al. 2004). Domain I can inactivate ARF function, thereby repressing auxin-responsive transcription (Tiwari et al. 2004). Domain II is important for instability of the protein and the interactions with $SCF^{TIR1/AFB1/2/3}$ complexes (Figure 1; Colón-Carmona et al. 2000; Worley et al. 2000; Ouellet et al. 2001; Ramos et al. 2001; Tiwari et al. 2001; Dharmasiri et al. 2005b). As noted above, auxin-dependent degradation of the Aux/IAA proteins through $SCF^{TIR1/AFB1/2/3}$ complexes allows the ARFs to function in auxin-responsive transcription (Dharmasiri et al. 2005b). On the other hand, gain-of-function mutations in domain II block interactions between Aux/IAA proteins and the $SCF^{TIR1/AFB1/2/3}$ complexes, thus increasing the stability of Aux/IAA proteins (Figure 1; Colón-Carmona et al. 2000; Worley et al. 2000; Gray et al. 2001; Ouellet et al. 2001; Dharmasiri et al. 2005b), resulting in constitutive inactivation of ARF functions (Tiwari et al. 2004). These gain-of-function mutations in domain II have been identified in several *Aux*/*IAA* genes (*iaa1*/*axr5*, *iaa3*/*shy2*, *iaa6*/*shy1*, *iaa7*/*axr2*, *iaa12*/*bdl*, *iaa14*/*slr*, *iaa17*/*axr3*, *iaa18*, *iaa19*/*msg2*, *iaa28*/*iar2*) (Rouse et al. 1998; Tian and Reed 1999; Nagpal et al. 2000; Reed et al. 2001; Rogg et al. 2001; Fukaki et al. 2002; Hamann et al. 2002; Tatematsu et al. 2004; Yang et al. 2004; Figure 1). Most of these *iaa* mutants have pleiotropic phenotypes in auxin-related growth and development, reduced sensitivity to exogenous auxin, and altered gene expression in response to auxin (reviewed by Reed et al. 2001; Liscum and Reed 2002).

Among the gain-of-function mutants, *shy2*/*iaa3*, *slr*/*iaa14*, *msg2*/*iaa19* and *iaa28-1* are severely defective in LR formation (Figure 2; Tian and Reed 1999; Rogg et al. 2001; Fukaki et al. 2002; Tatematsu et al. 2004). *Shy2*/*iaa3* and *msg2*/*iaa19* form few LRs in young seedlings until 10 days after germination, but these

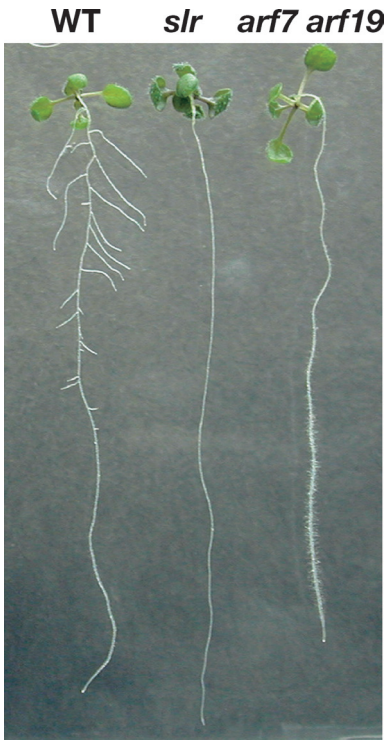

Figure 2. LR formation is blocked in the slr and *arf7 arf19* mutants in *Arabidopsis*. Ten-day-old wild type (WT; Columbia), *slr-1*/*iaa14* (*slr*; Fukaki et al. 2002) and *nph4-1 arf19-1* (*arf7 arf19*; Okushima et al. 2005) mutant plants are shown. After growth on standard MS medium (Fukaki et al. 2002), seedlings were transferred to fresh medium for the photograph.

mutants formed several LRs in mature plants 3 weeks after germination (Tian and Reed 1999; Tatematsu et al. 2004; Fukaki et al. unpublished results). The *iaa28-1* mutant also has few LRs in mature plants (Rogg et al. 2001). In contrast, the *slr-1*/*iaa14* mutant forms no LRs even after the inflorescence stems bolt (Fukaki et al. 2002), indicating that the stabilized *slr-1*/*iaa14* protein has the ability to constitutively block LR initiation. These observations suggest either that there are functional differences among these iaa proteins or that expression of *IAA* genes are regulated differently in roots.

Forward and reverse genetic analyses have identified the *ARF* genes required for LR formation (Tatematsu et al. 2004; Okushima et al. 2005; Wilmoth et al. 2005). The *arf7 arf19* double mutants also have few LRs, while single *arf7* or *arf19* mutants have a weak or subtle phenotype in LR formation, indicating that there are overlapping functions of *ARF7* and *ARF19* (Figure 2; Okushima et al. 2005; Wilmoth et al. 2005). Both ARF7 and ARF19 act as transcriptional activators (Tiwari et al. 2003; Wang et al. 2005), suggesting that ARF7 and ARF19 activate the target genes necessary for LR initiation. Since *ARF7* and *ARF19* are expressed in the stele tissues where *IAA14* is expressed (Fukaki et al. 2002; Okushima et al. 2005; Wilmoth et al. 2005), it is

thought that the stabilized slr-1/iaa14 protein in the *slr-1* mutant may repress ARF7/ARF19 functions through interactions between domains III and IV. Indeed, IAA14 was shown to interact with ARF7 and ARF19 in the yeast two-hybrid system (Fukaki et al. 2005), strongly suggesting that ARF7 and ARF19 are partners of SLR/IAA14 for LR initiation (Figure 3). Since the other IAAs, including SHY2/IAA3, MSG2/IAA19, and IAA28 also interact with ARF7 and ARF19 in the yeast two-hybrid assays (Tatematsu et al. 2004; Weijers et al. 2005; Okushima, Fukaki and Tasaka, unpublished results), these Aux/IAA proteins probably contribute to the inactivation of ARF7/ARF19 function, thereby blocking LR initiation with SLR/IAA14 (Figure 3). It will be necessary to confirm whether these Aux/IAAs interact with ARF7/ARF19 *in planta*, especially in the root pericycle. However, there remains the possibility that the other ARFs are also involved in LR initiation.

Auxin-responsive transcription mediated by SLR/IAA14 and ARF7/ARF19 in LR initiation

The *slr* mutation blocks auxin-induced pericycle cell divisions for LR initiation, indicating that auxin-responsive transcription mediated by SLR/IAA14 is important for LR initiation in *Arabidopsis* (Fukaki et al. 2002). These results strongly suggest that the stabilized slr-1 protein inactivates the ARFs (probably ARF7 and ARF19) that positively regulate LR initiation. Expression profiling with DNA microarray analysis has shown that the *slr* mutation alters expression of many kinds of genes (genes involved in cell cycle regulation, auxin-biosynthesis, -metabolism, -transport, and -signaling) (Vanneste et al. 2005). Target genes that trigger LR initiation, and which are regulated by ARFs interacting with SLR/IAA14 (probably ARF7 and ARF19; Figure 3) should be present in the *Arabidopsis* genome. Global expression analysis in the *arf7 arf19* double mutant using DNA microarrays has also shown that both ARF7 and ARF19 regulate transcription of many kinds of auxin-responsive genes containing AuxRE in their promoters (Okushima et al. 2005). Functional and expression analyses of these candidates will give us the necessary information to understand how ARF7/ARF19 promotes LR initiation.

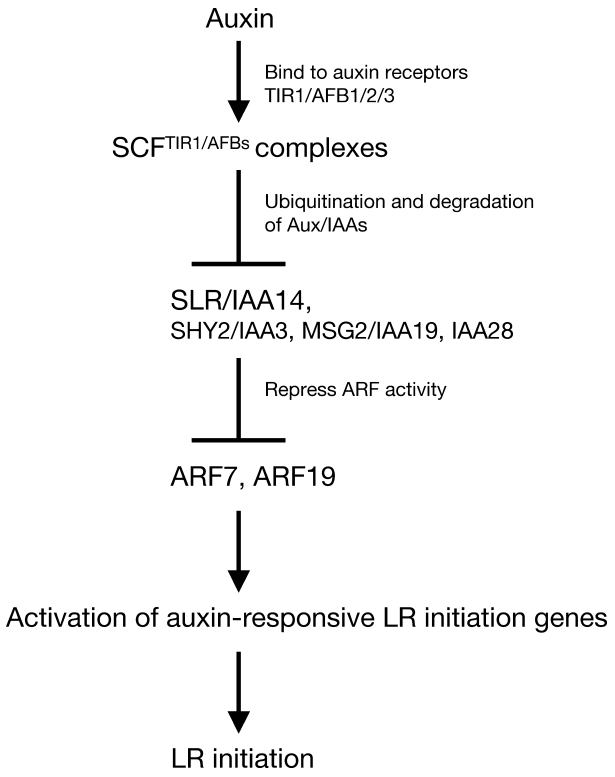

Figure 3. Auxin signaling pathway model for LR initiation. Auxin is captured by TIR1/AFB1/2/3 auxin receptors. Auxin signals promote the ubiquitination of Aux/IAAs through the $SCF^{TIR1/AFB1/2/3}$ E3 ubiquitin ligase complexes and degradation of Aux/IAAs by the 26S proteasome. Degradation of Aux/IAAs (SLR/IAA14, SHY3/IAA3, MSG2/IAA19, and IAA28), resulting in inactivation of ARF7/ARF19 function, allows ARF7/ARF19 to activate the target genes required for LR initiation.

The role of tissue-specific auxin signaling in LR development

Although LRs are initiated from the protoxylem pericycle in *Arabidopsis*, it has not been shown which tissues are important for auxin signaling in LR initiation, nor where the individual signal events take place. To understand the role of tissue-specific auxin signaling for LR formation, transgenic *Arabidopsis* plants expressing the stabilized slr-1/iaa14-GR (glucocorticoid receptor hormone binding domain) fusion protein under the control of tissue-specific promoters were analyzed in detail (Fukaki et al. 2005). This study has shown that expression of stabilized slr-1/iaa14-GR either in the stele or specifically limited to the protoxylem pericycle blocks LR initiation in a dexamethazone-dependent manner. These results indicate that normal auxin signaling mediated by ARFs and Aux/IAAs in the root stele, especially in the protoxylem pericycle, is necessary for LR initiation. In addition, expression of slr-1/iaa14-GR under the control of either the *ARF7* or *ARF19* promoters also blocked LR initiation (Fukaki et al. 2005). These results support the hypothesis that mIAA14 inactivates ARF7/ARF19 functions in the *slr* mutant, thereby blocking LR initiation. On the other hand, ectopic expression of stabilized slr-1/iaa14-GR during LR primordium formation under the control of the *SCARECROW* promoter, which is expressed in the LR primordium (Di Laurenzio et al. 1996; Malamy and Benfey 1997a, 1997b), blocked normal LR primordial formation during early stages, or caused aberrant LR development with disorganized LR meristems (Fukaki et al. 2005). These results indicate that normal auxin

signaling mediated by ARFs and Aux/IAAs in LR primordia is also important for correct LR primordial development (Fukaki et al. 2005). This conclusion posed a new question as to how auxin signaling regulates LR primordial development. It has been shown that auxin signaling mediated by ARF5/MP and BDL/IAA12 regulate embryonic root formation during embryogenesis (Hardtke and Berleth 1998; Hamman et al. 2002; Weijers et al. 2005). Although it has not been determined whether a similar auxin signaling pathway functions during LR primordial development, further study with the tissue-specific expression system of the stabilized IAA protein will be helpful to understand the role of auxin signaling in LR primordial development.

Perspectives

Auxin biosynthesis, metabolism, transport, and signaling are important for LR formation. Of particular note has been the discovery that LR initiation in *Arabidopsis* is dependent on auxin signaling mediated by $SCF^{TIR1/AFB1/2/3}$ auxin receptor complexes and the transcriptional regulators ARF7/ARF19 and several Aux/IAAs (SLR/IAA14, SHY3/IAA3, MSG2/IAA19, and IAA28) (Figure 3). However, there are still unanswered questions as to the details of LR development in *Arabidopsis*: 1) what determines the LR initiation sites along the root apical-basal axis, 2) why are LRs initiated only from the protoxylem pericycle, and not from the protophloem pericycle, 3) what kinds of ARF7/ARF19-regulated genes contribute to initiate LRs, and 4) how does auxin signaling control LR primordial development? In order to identify the factors involved in LR initiation, the suppressor mutants of *slr-1* that initiate LRs in the *slr-1* background have been isolated (Fukaki and Tasaka, unpublished results). Mutational analyses in combination with global expression analyses should be able to allow dissection of the regulatory pathways of auxin-mediated LR formation.

Lateral root development in *Arabidopsis* is a good model system to study the important issues in plant biology, such as meristem and organ formation, plant hormone signaling, cell fate determination, and cell cycle regulation. Understanding the molecular basis of *Arabidopsis* LR development will be invaluable for understanding LR formation in other plant species, and to provide the tools for modification of plant root systems for efficient shoot growth and increasing crop yield, particularly in drought, high salinity or other high-stress environmental conditions.

Acknowledgements

We wish to thank Athanasios Theologis for the seeds of *nph4-1 arf19-1* double mutant. The work of our group was supported in part by a grant to M.T. from the 'Research for the Future' program of the Japan Society for the Promotion of Science, and a Grant-in-Aid to H.F. for Scientific Research on Priority Areas and a Grant-in-Aid to H.F. for Scientific Research for Young Scientists from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

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

Beeckman T, Burssens S. and Inze D. (2001) The peri-*cell*-cycle in *Arabidopsis*. *J Exp Bot* 52: 403–411

Berleth T, Krogan NT, Scarpella E (2004) Auxin signals—turning genes on and turning cells around. *Curr Opin in Plant Biol* 7: 553–563

Bhalerao RP, Eklof J, Ljung K, Marchant A, Bennett M, Sandberg G (2002) Shoot-derived auxin is essential for early lateral root emergence in *Arabidopsis* seedlings. *Plant J* 29: 325–332

Boerjan W, Cervera M-T, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, van Onckelen H, van Montagu M, Inze D (1995) *superroot*, a recessive mutation in *Arabidopsis*, confers auxin overproduction. *Plant Cell* 7: 1405–1419

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inze D, Sandberg G, Casero PJ, Bennett M (2001) Auxin transport promotes *Arabidopsis* lateral root development. *Plant Cell* 13: 843–852

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: 165–171

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

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

Dharmasiri S, Dharmasiri N, Hellmann H, Estelle M (2003) The RUB/Nedd8 conjugation pathway is required for early development in *Arabidopsis*. *EMBO J* 22: 1762–1770

Dharmasiri N, Estelle M (2004) Auxin signaling and regulated protein degradation. *Trends Plant Sci* 9: 302–308

Dharmasiri N, Dharmasiri S, Estelle M (2005a) The F-box protein TIR1 is an auxin receptor. *Nature* 435: 4441–445

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jürgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F box proteins. *Dev Cell* 9: 109–119

Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN (1996) The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* 86: 423–433

Fitter A (1996) Characteristics and functions of root systems. In: Waisel Y, Eshel A, Kfkafi U (eds) Plant Roots: *The Hidden Half, 2nd edn*. New York: Marcel Dekker, pp 1–20

Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA1*4 gene of *Arabidopsis. Plant J* 29: 153–168

Fukaki H, Nakao Y, Okushima Y, Theologis A, Tasaka M (2005) Tissue-specific expression of stabilized SOLITARY-ROOT/

IAA14 alters lateral root development in *Arabidopsis*. *Plant J* (in press)

Gil P, Dewey E, Friml J, Zhao Y, Snowden KC, Putterill J, Palme K, Estelle M, Chory J (2001) BIG: a calossin-like protein required for polar auxin transport in *Arabidopsis.* *Genes Dev* 15: 1985–1997

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 (1998) How does auxin turn on genes? *Plant Physiol* 118: 341–347

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

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

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

Hardtke CS, Ckurshumova W, Vidaurre DP, Singh SA, Stamatiou G, Tiwari SB, Hagen G, Guilfoyle TJ, Berleth T (2004) Overlapping and non-redundant functions of the *Arabidopsis* auxin response factors *MONOPTEROS* and *NONPHOTOTROPIC HYPOCOTYL 4*. *Development* 131: 1089–1100

Himanen K, Boucheron E, Vanneste S, de Almeida Engler J, Inzé D, Beeckman T (2002) Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell* 14: 2339–2351

Himanen K, Vuylsteke M, Vanneste S, Vercruysse S, Boucheron E, Alard P, Chriqui D, Van Montagu M, Inzé D, Beeckman T (2004) Transcript profiling of early lateral root initiation. *Proc Natl Acad Sci USA* 101: 5146–5151

Hobbie L, Estelle M (1995) The *axr4* auxin-resistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation. *Plant J* 7: 211–220.

Kepinski S, Leyser O (2005) The *Arabidopsis* TIR1 protein is an auxin receptor. *Nature* 435: 446–451

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

King JJ, Stimart DP, Fisher RH, Bleecker AB (1995) A mutation altering auxin homeostasis and plant morphology in *Arabidopsis*. *Plant Cell* 7: 2023–2037

Kurup S, Runions J, Köhler U, Laplaze L, Hodge S, Haseloff J (2005) Marking cell lineages in living tissues. *Plant J* 42: 444–453

Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a two-stage process. *Development* 121: 3303–3310

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

Malamy JE, Benfey PN (1997a) Down and out in *Arabidopsis*: the formation of lateral roots. *Trends Plant Sci* 2: 390–396

Malamy JE, Benfey PN (1997b) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* 124: 33–44

Marchant A, Bhalerao R, Casimiro I, Eklof 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

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

Okushima Y, Overvoorde PJ, Arima K, Alonso JM, Chan A, Chang C, Ecker JR, Hughes B, Lui A, Nguyen D, Onodera C, Quach H, Smith A, Yu G, Theologis A (2005) Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: unique and overlapping functions of *ARF7* and *ARF19*. *Plant Cell* 17: 444–463

Oono Y, Ooura C, Rahman A, Aspuria ET, Hayashi K, Tanaka A, Uchimiya H (2003) p-Chlorophenoxyisobutyric acid impairs auxin response in *Arabidopsis* root. *Plant Physiol* 133: 1135–1147

Ouellet F, Overvoorde PJ, 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 RC, Brady SR, Muday GR (1998) Inhibition of auxin movement from the shoot into the root inhibits lateral root development in *Arabidopsis*. *Plant Physiol* 118: 1369–1378

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

Remington DL, Vision TJ, Guilfoyle TJ, Reed JW (2004) Contrasting modes of diversification in the *Aux*/*IAA* and *ARF* gene families. *Plant Physiol* 135: 1738–1752

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 WM, 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

Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Suza W (2005) Characterization of an *Arabidopsis* enzyme family that conjugates amino acids to indole-3-acetic acid. *Plant Cell* 17: 616–627

Tatematsu K, Kumagai S, Muto H, Sato A, Watahiki MK, Harper RM, Liscum E, Yamamoto 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 LRs in *Arabidopsis thaliana*. *Plant Cell* 16: 379–393

Tian Q, Reed JW (1999) Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA*3 gene.

Development 126: 711–721

Tiwari SB, Wang XJ, 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 (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

Vanneste S Rybel BD, Beemster GTS, Ljung K, Smet ID, Van Isterdael G, Naudts M, Iida R, Gruissem W, Tasaka M, Inzé M, Fukaki H, Beeckman T (2005) Cell cycle progression in the pericycle is not sufficient for SOLITARY-ROOT/IAA14-mediated lateral root initiation in Arabidopsis. *Plant Cell* (in press)

Wang S, Tiwari SB, Hagen G, Guilfoyle TJ (2005) AUXIN RESPONSE FACTOR7 restores the expression of auxin-responsive genes in mutant *Arabidopsis* leaf mesophyll protoplasts. *Plant Cell* 17: 1979–1993

Weijers D, Benkova E, Jäger KE, Schlereth A, Hamann T, Kientz M, Wilmoth JC, Reed JW, Jürgens G (2005) Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcription regulators. *EMBO J* 24: 1874–1885

Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, Guilfoyle TJ, Alonso JM, Ecker JR, Reed J (2005) NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *Plant J* 43: 118–130

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. *Annals of Bot* 95: 707–735

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

Yang X, Lee S, So JH, Dharmasiri S, Dharmasiri N, Ge L, Jensen C, Hangarter R, Hobbie L, Estelle M (2004) The IAA1 protein is encoded by *AXR5* and is a substrate of SCF^{TIR1}. *Plant J* 40: 772–782

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