Auxins, mainly indole-3-acetic acid (IAA), play a critical role in numerous plant growth processes, including embryo development, root and flower development, vascular differentiation, stem elongation, apical dominance, and tropic responses. Charles Darwin and his son Francis performed some of the earliest work relating to the effect of auxin on plant growth with their experiments on phototropism (bending of plants toward a light source) in oat (Avena sativa) and canary grass (Phalaris canariensis). Their experiments, reported in the monograph entitled “The Power of Movement in Plants,” led them to the conclusion that “some influence” was transmitted from the upper to the lower part of the seedlings, causing them to bend (Darwin, 1880). This “influence” was isolated in 1926 by Frits W. Went in his famous experiments with oat coleoptiles and later named auxin (Went, 1935). The diverse effects of auxin on plant growth are mainly the result of auxin’s effect on cell elongation, cell division, or cell differentiation and are dependent on the control of auxin biosynthesis and degradation and the polar transport to and the perception of auxin at the site of action. Although much has been discovered about the effects and regulation of auxins on plant growth since the Darwin experiments, there is much that remains unknown.

**Auxin/IAA Proteins**

The response to auxin includes a rapid initial cell growth response (within 15 to 20 min) that may involve auxin-induced changes in pH and calcium and a second phase that involves auxin-induced changes in gene expression. Auxin-responsive genes include SAUR (Small Auxin Upregulated), GH3, and Auxin(Aux)/IAA gene families, which have distinct conserved cis-acting sequences in their promoter regions, various glutathione S-transferase genes, and a gene for ACC synthase (Abel and Theologis, 1996). The functions of most of these genes are unknown. The Aux/IAA genes constitute a large family of genes that are induced specifically in response to auxin. They encode small (~20 to 35 kD) nuclear proteins that share four highly conserved domains (I, II, III, and IV) and likely function as transcription factors that regulate downstream auxin responses (Guilfoyle, 1998; Reed, 2001). Aux/IAA genes were first identified in screens for mRNA transcripts that are induced rapidly by auxin. Arabidopsis has at least 20 Aux/IAA family members, which show differences in tissue specificity and auxin induction kinetics.

Aux/IAA proteins likely function as homodimers and/or heterodimers. Domain III contains a putative βαβ DNA binding domain that is present in a prokaryotic family of transcriptional repressors (Arc proteins) and whose formation in this family requires protein dimerization. Aux/IAA proteins have been found to interact in homotypic and heterotypic associations in yeast two-hybrid experiments, and these interactions were dependent on the presence of domains III and IV (Kim et al., 1997; Rouse et al., 1998). Domains III and IV are shared with another family of proteins called auxin response factors, which bind to an early auxin-responsive element. Thus, it has been suggested that different combinations among the various members of the Aux/IAA and auxin response factor families regulate a variety of late auxin-responsive genes and function as autoregulators of early auxin-responsive genes (Kim et al., 1997; Rouse et al., 1998).

Many Aux/IAA proteins have extremely short half-lives (6 to 8 min), suggesting a primary role for protein degradation in the regulation of their activity. Guilfoyle et al. (1998) suggested that domain II was responsible for the rapid turnover of these proteins, because mutations in this domain (such as in a number of axr3 mutants) led to a semidominant gain-of-function phenotype. Worley et al. (2001) provided data that supported this hypothesis and further suggested that rapid degradation of Aux/IAA proteins is essential for a normal auxin response. Fusion proteins were constructed comprising firefly luciferase (LUC) and a portion of wild type or mutant Aux/IAA proteins. Single amino acid substitutions in domain II, equivalent to two alleles of axr3 mutants, resulted in an over 50-fold increase in fusion protein accumulation compared to the wild type, as measured by LUC activity in transient assays. It was also found that overexpression of the IAA17 protein in Arabidopsis resulted in plants with an axr3-like phenotype.

In this issue of The Plant Cell, Ramos et al. (pages 2349–2360) extended the work of Worley et al. (2000) to show that domain II of Aux/IAA proteins is required for the rapid proteasome-mediated degradation of Aux/IAA proteins in response to auxin. The group again made use of fusion proteins that constitute luciferase (LUC) fused to various portions of Aux/IAA proteins, including wild type and mutant Aux/IAA sequences. Protein abundance and half-life were measured by monitoring LUC activity in transient assays in tobacco protoplasts and in vivo in transgenic Arabidopsis seedlings expressing the fusion proteins. Sequences were analyzed from a number of conserved Aux/IAA proteins, including Arabidopsis IAA1 and
IAA17 and PSIAA6 from pea. A 13–amino acid consensus sequence in domain II was found to be sufficient to target fusion proteins for low accumulation. The single amino acid change in domain II equivalent to the axr3 mutation resulted in a fusion protein with an in vivo half-life that was 30-fold longer than the protein with a wild type sequence, although significantly shorter than LUC alone. Additional sequence within a region equivalent to the N-terminal 73 amino acids of PSIAA6 was required to confer the extremely short half-life of the wild-type protein (~8 min). Experiments using proteasome-specific and other protease inhibitors indicated that degradation of the Aux/IAA::LUC fusion proteins was accomplished via the proteasome pathway.

THE AUXIN RESPONSE AND SCF\textsuperscript{TIR1}

The proteasome pathway performs the degradation of proteins conjugated to ubiquitin. The ubiquitination of a substrate protein requires the activity of three enzymes, a ubiquitin-activating enzyme (E1), which activates ubiquitin via the formation of a thiolester linkage between itself and ubiquitin, a ubiquitin-conjugating enzyme (E2), which binds E1-activated ubiquitin, and a ubiquitin-protein ligase (E3), which works together with E2 to transfer activated ubiquitin to a target protein. The repeated action of these enzymes links multiple ubiquitin molecules together to generate a poly-ubiquitin chain covalently linked to a substrate protein. The 26S proteasome complex recognizes and degrades poly-ubiquitinated proteins, cleaving and releasing free ubiquitin in the process. Whereas E1 and E2 proteins are encoded by families of related genes, E3 ligases are highly diverse, and this diversity is thought to allow for a wide range of target proteins—and, consequently, a wide range of developmental processes that may be controlled via proteasome-mediated degradation (Scheffner et al., 1995).

There are five main classes of E3s, all of which are present in plants; the HECT domain proteins, anaphase-promoting complex proteins, Ubr1-like E3s, monomeric RING–H2-type E3s, and the S-phase kinase–associated protein (Skp1)–cullin–F-box (SCF)-type E3s (Estelle, 2001). The F-box protein of SCF-type E3s is the receptor subunit of the complex, which recognizes and interacts with specific substrate proteins. The Aux/IAA proteins are considered good candidates for substrates of SCF\textsuperscript{TIR1}. The Arabidopsis TIR1 gene encodes an F-box protein, and tir1 mutants show an auxin-defective phenotype (Ruegger et al., 1998). Gray et al. (1999) showed that the SCF\textsuperscript{TIR1} complex, consisting of TIR1, the Skp1-like proteins ASK1 and ASK2, and the cullin AtCUL1, is required for the auxin response in Arabidopsis. Overexpression of TIR1 in transgenic plants resulted in enhanced auxin-induced gene expression, suggesting that principal targets of TIR1 are negative regulators of the auxin response.

Protein degradation also plays a critical role in photomorphogenesis, a process characterized by the inhibition of stem elongation (deetiolation), the activation of the shoot apical meristem, and the initiation of true leaf development in light-grown seedlings. Arabidopsis COP/DET/FUS genes are negative regulators of photomorphogenesis. Most of these genes encode subunits of the COP9 signalosome, which is a complex that is required for the proteasome-mediated degradation of positive regulators of photomorphogenesis, such as the HY5 transcription factor. Schwechheimer et al. (2001) showed that plants with reduced amounts of the COP9 signalosome had an auxin response defect similar to that of SCF\textsuperscript{TIR1} loss-of-function mutants. They further found that SCF\textsuperscript{TIR1} interacts with the COP9 signalosome in vivo and that the COP9 signalosome is required for the efficient degradation of the Aux/IAA protein PSIAA6. Thus, a hypothesis emerged in which SCF\textsuperscript{TIR1} interacts with Aux/IAA proteins, targeting them for interaction with the COP9 signalosome and subsequent proteasome-mediated degradation. Complicating matters, Zenser et al. (2001) have shown that Aux/IAA::LUC fusion proteins exhibit an equivalent auxin-induced increase in degradation rate in tir1 mutants as well as TIR1 functional plants, suggesting that TIR1 is not required for this response, but it is possible that other F-box proteins in this large family compensate for the loss of TIR1 function in the tir1 mutants. There are at least three close relatives of TIR1 in the Arabidopsis genome, designated Leucine-Rich Repeat F-box genes (Gray and Estelle, 2000).

THE AUX/IAA PARADOX

The auxin response is complicated by the fact that auxin induces Aux/IAA gene expression, yet many Aux/IAA proteins have extremely short half-lives. Furthermore, Zenser et al. (2001) showed that exogenous auxin promotes more rapid degradation of Aux/IAA::LUC fusion proteins in vivo, and they hypothesized that endogenous auxin enhances the degradation rates of at least some Aux/IAA proteins. How can this be explained?

Gray and Estelle (2000) postulated a regulatory loop wherein constitutive low-level expression of certain Aux/IAA proteins represses the auxin response pathway, and auxin acts to relieve this repression by promoting proteasome-mediated degradation of the repressors. There is evidence that some ARF proteins function as activators and others as repressors of transcription (Ulmasov et al., 1999). Auxin-upregulated proteins might include positive ARF regulators of downstream auxin-response genes as well as the repressor Aux/IAA proteins, which then act to restore the...
basal repression of auxin responses. There is new evidence that Aux/IAA proteins function exclusively as transcriptional repressors (Tiwari et al., 2001), and we are left with the apparent paradox that Aux/IAA proteins are induced by auxin and function as repressors of transcription, yet their rapid degradation is also enhanced by auxin (Zenser et al., 2001). It is evident that auxin regulation of development involves extremely tight control of the level of Aux/IAA proteins present, which is dependent on interactions of these proteins with the proteasome pathway.

The proteasome pathway is thus emerging as a powerful regulator of plant developmental processes. The proteasome complex may be a master integrator of diverse developmental pathways, providing a meeting point for a range of E3 ligases and target proteins (e.g., Aux/IAA and related proteins) and their myriad downstream effects. For example, all known target substrates of SCF E3s must be phosphorylated to trigger their association with the SCF complex (Gray and Estelle, 2000). Colón-Carmona et al. (2000) showed that Aux/IAA proteins are phosphorylated by phytochrome, providing a possible link for integrating auxin and light signals. Further investigations into the function of TIR1 and related proteins and their relationship to Aux/IAA proteins are needed to elucidate the complex interactions that converge on the proteasome. Watch this space for further developments.

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