Auxin receptors: a new role for F-box proteins
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The plant hormone auxin regulates transcription by promoting the degradation of a family of transcriptional repressors called Aux/IAA proteins. Genetic and biochemical studies have shown that this degradation is dependent on a ubiquitin protein ligase called SCFTIR1. In the presence of auxin, the F-box protein TIR1 binds to the Aux/IAA proteins, resulting in their ubiquitination and degradation. Recent attention has focused on the nature of the auxin receptor and upstream signaling events involved in this process. Now, two recent papers demonstrate that auxin binds directly to TIR1 and promotes the interaction with the Aux/IAA proteins. Furthermore, TIR1 functions together with at least three other related F-box protein/receptors to mediate the auxin response throughout plant growth and development.

Introduction
In 1880 Charles Darwin proposed that some plant growth responses were regulated by a “matter... which transmits its effects” from one part of the plant to another. In the ensuing 125 years, this “matter”, later shown to be the plant hormone auxin, has been the subject of intense investigation by numerous plant biologists. One of the most pressing questions concerning auxin action has been the mechanism of auxin perception. During the past year this puzzle has been solved through the discovery that the Arabidopsis F-box protein TIR1 is an auxin receptor [1**,2**]. Direct binding of auxin to TIR1 promotes the degradation of transcriptional repressors called Aux/IAA proteins. This is the first demonstration that an SCF E3 is regulated by direct binding of a small ligand.

In this review we describe our current understanding of auxin-regulated transcription and highlight the results of recent studies indicating that TIR1 and related F-box proteins function as auxin receptors.

The auxin response
The name auxin is derived from the Greek ‘aux-ein’ meaning ‘to grow’. This name is appropriate because auxin, a small molecule related to tryptophan, impacts a wide array of growth processes throughout plant development [3]. Genetic studies in Arabidopsis have led to the identification of a number of genes that function in auxin signaling.

Auxin response is regulated by two large protein families: the ARF (auxin response factor) proteins and the Aux/IAA proteins [4*,5*,6]. The 22 ARF proteins in Arabidopsis each contain a conserved DNA binding domain near their N terminus and most also have a dimerization domain near their C terminus. Although the best studied ARF proteins function as transcriptional activators, other members of the family may be repressors [7–9]. Genetic studies have implicated different ARFs in diverse growth processes including embryogenesis (ARF5/MONOPTEROS, ARF17), root (ARF7/NPH4, ARF10, ARF16, ARF19) and floral development (ARF1, ARF2, ARF3/ETTIN, ARF6, ARF8), and senescence (ARF2) [4*,10,11,12*,13*,14*,15]. Although many single arf mutants lack an obvious mutant phenotype, this is probably because of functional redundancy, as some double mutants combinations exhibit enhanced growth defects [4*,10,11,13,15]. The most dramatic arf mutant is arf5/monopteros. These plants have severe embryonic growth defects that result in seedling lethality [16].

The ARF proteins are negatively regulated by the Aux/IAA proteins [7,17]. There are 29 Aux/IAA proteins encoded by the Arabidopsis genome, most of which contain four conserved domains. Domain I is responsible for transcriptional repression while domains III and IV are required for homo- or heterodimerization [18,19]. Domain II contains a 13-amino-acid motif that is necessary for Aux/IAA degradation [20]. Each of the Aux/IAA genes is closely related to at least one other family member. So far 13 different aux/iaa loss-of-function mutants have been identified and characterized and none have an obvious mutant phenotype [5*]. Somewhat surprisingly an iaa5 iaa6 iaa19 triple mutant is also very similar to wild-type plants. Because these three genes reside in distinct subclades, this result suggests that genetic redundancy may extend to more distantly related members of the family [5*]. So far, much of what we know about the function of the Aux/IAAs comes from the recovery and characterization of gain-of-function mutants. Such mutants have been identified in 10 Aux/IAA genes and each is caused by a substitution within the 13 amino acid degron in domain II. These mutations act to stabilize the affected protein [21–26]. This in turn inhibits ARF function, resulting in...
decreased auxin response. The most severe aux/iaa phenotypes are the related bdl/iaa12 and iala13 mutants. These plants have an embryonic/seedling phenotype that is very similar to the arfl/imp mutant [27,28]. BDL and MP interact in a yeast two-hybrid assay [23] and subsequent genetic analysis showed that over-expression of MP can rescue bdl mutant embryos [11]. These results strongly suggest that BDL and MP function antagonistically during embryogenesis. Studies to date indicate that there are tissue-specific differences in the interactions between ARFs and Aux/IAAs [28]. It will be informative to fully characterize the in planta interactions between the members of these protein families.

**Protein degradation and auxin response**

Accumulation of the Aux/IAA proteins causes numerous auxin-related defects. This discovery conveniently dovetails with other studies demonstrating that genes involved in ubiquitin-mediated protein degradation play a role in the auxin response. Ubiquitination of proteins by ubiquitin ligases (E3s) targets them for degradation by the proteasome. One type of E3 ligase, called an SCF, comprises a cullin, SKP1 and an F-box protein (hence its name) and the RBX1 protein [29,30]. The cullin subunit of the complex serves as a scaffold and binds both SKP1 and RBX1. The F-box protein interacts with SKP1, via the ~40 amino acid F-box domain. In addition, the F-box protein interacts directly with the SCF substrates and therefore confers specificity to the complex.

The involvement of the protein degradation machinery, and more specifically the SCF, in the auxin response was demonstrated through the identification and characterization of several auxin-resistant mutants. One of the affected genes, called TIR1 (TRANSPORT RESPONSE 1), encodes an F-box protein indicating that an SCF, in this case SCFTIR1, is required for auxin response [31]. Subsequent studies showed that mutations in other SCF subunits such as CUL1, or in genes that regulate SCF function, result in auxin response defects [32–36].

Once TIR1 was shown to be an F-box protein, the next task was to identify its substrates [37]. The Aux/IAA proteins were good candidates because earlier studies had shown that stable versions inhibited auxin response. Furthermore, several lines of evidence indicated that auxin promotes their degradation [20,38,39]. Sure enough, biochemical experiments showed that recombinant Aux/IAA protein, in this case IAA7, interacts with TIR1 in cell extracts [38]. Furthermore, a 30-amino-acid peptide, encompassing domain II of IAA7, was able to bind TIR1 in the same assay [40]. These results confirm that the Aux/IAA proteins are substrates for SCFTIR1 and highlight the importance of domain II to the interaction.

At this stage it was unclear what factors facilitated the interaction between TIR1 and the Aux/IAA proteins or how these factors might relate to auxin perception. Importantly, it was discovered that TIR1 can interact with Aux/IAA proteins in a membrane-free cell extract, suggesting that the receptor and other signaling proteins are soluble proteins [41]. In animal and fungal systems, substrate modification, typically phosphorylation, is required for recognition by the SCF [29]. In the case of SCFTIR1, however, genetic and pharmacological studies indicated that phosphorylation is not required for Aux/IAA recognition [20,40,41]. In addition, mass-spectrometric analysis of domain II peptide did not reveal an auxin-dependent modification, suggesting that auxin does not alter Aux/IAA proteins in order to facilitate their interaction with TIR1 [40].

**TIR1 is an auxin receptor**

The next obvious question was what additional proteins are required to promote the auxin-dependent of the Aux/IAA proteins by SCFTIR1. The surprising answer was none. When SCFTIR1 was recovered from protein extracts, either by immunoprecipitation or by GST pull-down, the preparation bound substrate in an auxin-dependent manner [1,2]. Furthermore, by performing pull-down experiments in the presence of [3H] IAA, it was possible to demonstrate specific and saturable binding of IAA to SCFTIR1 or a tightly associated protein [1,2]. Importantly, [3H] did not bind specifically to IAA7 [1]. Increasing the amount of TIR1 in cell extracts proportionally increased the recovery of [3H] IAA, indicating that it was binding to TIR1 or a closely associated protein [2].

Although compelling, these results do not distinguish between binding of IAA to TIR1 or to another tightly bound protein. To resolve this issue, TIR1 was synthesized in two heterologous systems: Xenopus laevis embryos and insect cells. TIR1 synthesized in either cell type was able to bind IAA7 or domain II peptide in an auxin-dependent manner, strongly suggesting that TIR1 is an auxin receptor [1,2].

So far there is little information on which regions of TIR1 are involved in auxin or Aux/IAA binding. However, there are some hints that the F-box motif may have a direct role in binding. Mutant forms of TIR1 that either lack the F-box or are disrupted at conserved residues within the F-box do not interact with IAA7 [1,2]. One possible explanation for these results is that TIR1 first must interact with SKP1 (or ASK1 in plants) in order to bind hormone and substrate. TIR1 does not appear to interact with frog SKP1, however, suggesting that TIR1 can bind substrate alone [2]. Further studies with purified proteins will be required to resolve this issue.

**The TIR1/AFB family of auxin receptors**

The tir1 mutants display moderate auxin resistance but are otherwise indistinguishable from wild-type plants
This is in marked contrast to the phenotype of gain-of-function mutations in the Aux/IAA genes such as bdl/iaa12. This paradox can be explained by the presence of three closely related proteins in Arabidopsis. Like TIR1, these AFB (auxin-signaling F-box protein) proteins contain 16 leucine-rich repeats and share a high level of amino acid identity [42**]. Also like TIR1, the AFB proteins interact with IAA7 in an auxin-dependent way, suggesting that they are also auxin receptors [42**]. Consistent with this hypothesis, saturable binding of [3H]IAA to a GST-IAA7 pulldown is greatly reduced in quadruple tir1 afb1 afb2 afb3 mutants [1**].

The characterization of single afb mutants as well as various higher order mutant combinations indicates that members of the family function in a redundant and additive fashion [42**]. The level of auxin resistance in the roots of seedlings is dependent on the number of functional AFB genes. With respect to morphology, striking defects are not observed until at least three members of the family are mutated. However, both triple tir1 afb2 afb3 and quadruple tir1 afb1 afb2 afb3 display a wide range of auxin-related defects throughout the life cycle of the plant [42**]. The phenotype of these plants is quite variable but a high proportion of triple and quadruple mutant embryos have severe patterning defects that result in early seedling lethality. This phenotype is very similar to that of the iaa12/bdl or arf5/mp mutants (Figure 1). Consistent with this hypothesis, BDL/IAA12 protein levels are elevated in tir1 afb2 afb3 plants [42**]. On the basis of these results, it is clear that the TIR1/AFB auxin receptors mediate auxin signaling during embryogenesis and throughout plant development.

Of course this does not imply that the TIR1/AFBs are the only auxin receptors in the plant cell. Some auxin responses appear to be too fast to be mediated by changes in transcription. These responses, primarily rapid changes in ion transport across the plasma membrane, may be mediated by a membrane-associated receptor. The best candidate for such a receptor is currently auxin binding protein 1 (ABP1) [43].

Conclusions
The discovery that F-box proteins function as hormone receptors is an exciting and unexpected development in the complex story of auxin regulation. As with any significant discovery, this one leads to a number of important questions. Foremost among these is how auxin binding promotes the TIR1–Aux/IAA interaction. One possibility is that auxin binds to TIR1 and promotes a conformational change that facilitates substrate binding. Alternatively, it is possible that auxin acts to stabilize an existing interaction between TIR1 and its substrate, perhaps by contacting both proteins.

Because auxin acts directly on the ubiquitin E3 enzyme with no intervening signaling steps, hormone-dependent degradation of Aux/IAA proteins is extremely rapid. Does nature use this fast and economical mechanism to regulate the degradation of other SCF substrates? In plants at least, the answer is likely to be yes. The hormone gibberellin (GA) promotes the degradation of transcriptional repressors called DELLA proteins through an SCF (SCF<sub>GID2</sub> in rice or SCF<sub>Sly</sub> in Arabidopsis) [44]. Recent studies in rice show that a protein called GID1 functions as a GA receptor. Upon GA binding, GID1 interacts with the DELLA protein SLR1 and promotes its degradation by SCF<sub>GID2</sub> [45**]. In this case, the receptor GID1 appears to act as a bridge between the SCF and SLR1. In another example from plants, response to the hormone jasmonic acid (JA) requires the F-box protein COI1 [46,47]. Since COI1 is closely related to TIR1 it is
possible that COI1 binds JA or a JA derivative. Of course these are but two examples. Both animals and plants have large families of (mostly uncharacterized) F-box proteins [29,30]. In the future it will be interesting to see how many of these are also directly regulated by small ligands.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


5. In this paper and [2*], the authors show that [1*] IAA interacts with SCFTIR1 in GST-IAA7 pulldown. Further, they show that TIR1 expressed in either insect cells [1**] or Xenopus embryos [2**] binds IAA7 in an auxin-dependent manner.


29. Several Aux/IAA and ARF proteins act in a specific pair-wise fashion to regulate different developmental processes.


32. Ruegger M, Dewey E, Gray WM, Hobbie L, Turner J, Estelle M: *The TIR1* protein of *Arabidopsis* functions in auxin response and is...

The authors demonstrate that the interaction between TIR1 and IAA7 is probably not dependent on an auxin-mediated post-translational modification of IAA7.

The auxin response is mediated by a family of TIR1/AFB proteins that function throughout plant growth and development. Genetic analyses show that these proteins function redundantly. A fraction of plants deficient in four members of the family exhibit severe embryonic growth defects similar to those seen in the mp or bdl mutants.
The authors provide compelling evidence that the soluble protein GID1 is a GA receptor. When GID1 binds GA it interacts with the DELLA protein SLY and promotes its degradation by SCFGID2.