

## HOW AUXIN TURNS GENES ON – REGULATED PROTEIN DEGRADATION AT THE HEART OF AUXIN SIGNALLING

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### ABSTRACT

Auxin plays a pivotal role in many important aspects of plant growth and development. Central to auxin response are changes in gene expression that hinge on complex interactions between two families of transcription factors: On one hand, members of the Auxin Response Factor (ARF) family of DNA-binding proteins can bind the promoters of auxin-regulated genes to activate their transcription. On the other, members of the Aux/IAA family of transcriptional repressor proteins can dimerize with these activating ARF proteins thereby repressing transcription. Auxin induces the expression of such target genes by prompting the ubiquitin-dependent degradation of Aux/IAs in the 26S proteasome (Fig. 1). We have previously shown that the ubiquitination and destabilisation of Aux/IAA protein requires the ubiquitin ligase complex SCF<sup>TIR1</sup>. SCF-type ubiquitin ligases are common throughout the eukaryotes. They are multiprotein complexes consisting of the structural and catalytic core components Skp1, cullin and Rbx1 and an F-box protein that is responsible for the recruitment of the specific target proteins for ubiquitination by the complex. In the case of Aux/IAs, the F-box protein is TIR1 and we have been able to demonstrate a specific and auxin-dependent interaction between SCF<sup>TIR1</sup> and Aux/IAs using a simple pull-down assay in which tagged versions of an Aux/IAA are incubated with extracts of transgenic *Arabidopsis* plants expressing a myc-epitope-tagged version of TIR1.

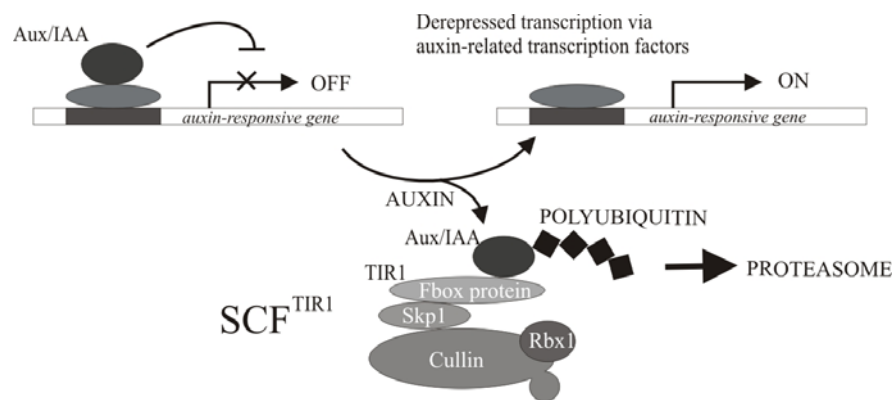


Fig. 1. Auxin-induced gene expression proceeds via the degradation of Aux/IAA proteins and thus transcriptional derepression in response to auxin. Components of the E3 ubiquitin ligase SCF<sup>TIR1</sup> collaborate with ubiquitin activating and conjugating enzymes to form a polyubiquitin chain on the target marking it for destruction in the proteasome. The F-box protein TIR1 is responsible for the specific selection of Aux/IAA proteins.

In terms of auxin-related changes gene expression, the auxin-induced increase in interaction between SCF<sup>TIR1</sup> and Aux/IAs is the earliest known event in the signal transduction cascade. Understanding the regulation of this interaction was thus an important step towards identifying

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the receptor for these genomic responses to auxin. In all previously documented cases, the regulation of interaction between SCF and target was dependent on modification of the target, most commonly by phosphorylation. We showed that this was not the case for Aux/IAAs but rather that their interaction with SCF<sup>TIR1</sup> was dependent of an auxin-induced modification of SCF<sup>TIR1</sup>. To understand the nature of this interaction we partially-purified TIR1 by immunoprecipitation and tested the ability of this purified TIR1 to interact with Aux/IAAs

(Figure 2a,b). Surprisingly, in pull-down assays, immunopurified TIR1 was able to interact with Aux/IAAs in an auxin-dependent manner suggesting that the proteins recovered in the TIR1 immunoprecipitation were sufficient to promote this interaction and hence included the auxin receptor for this response.

These data suggested that the SCF<sup>TIR1</sup> -Aux/IAA interaction might involve direct auxin binding. This idea was tested by including radiolabelled IAA in the TIR1 pull-down assays and assaying the ability of increasing concentrations of unlabelled IAA to reduce the recovery of radiolabel. Figure 2c shows clear evidence of saturable auxin binding in this respect, indicating that auxin is incorporated directly into the SCF<sup>TIR1</sup> -Aux/IAA complex to promote its formation. Importantly, Aux/IAA protein alone was not able to bind radiolabelled IAA. Although this and other data provided circumstantial evidence that TIR1 might be the protein that was actually binding auxin, it was possible that other proteins might could be the protein that was actually binding auxin, it was possible that other proteins might be involved. To test this possibility, we expressed TIR1 in a non-auxin responsive host using a *Xenopus* (frog) embryo expression system. TIR1 expressed in this way was able to interact with Aux/IAAs in an auxin-dependent manner in pull-down assays (Figure 3a,b). Because it is highly unlikely that *Xenopus* has an auxin receptor capable of interacting with and brokering the interaction between TIR1 and Aux/IAAs, we conclude that TIR1 is the auxin receptor for the process of auxin-induced Aux/IAA destabilization.

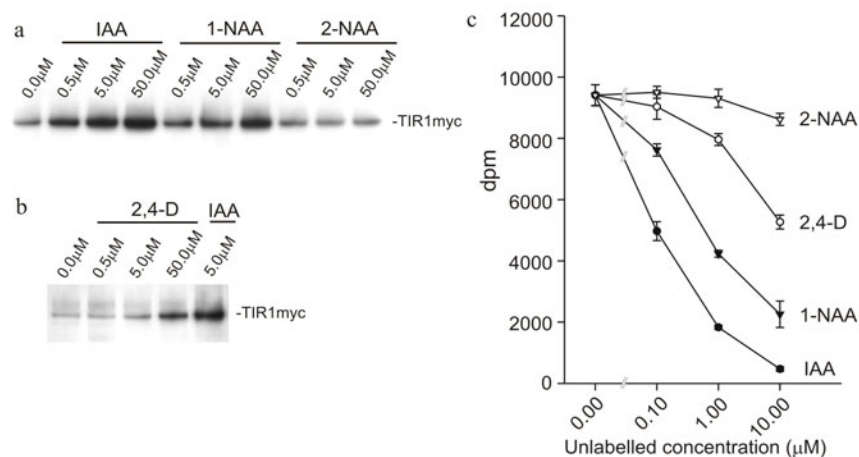


Fig.2. An auxin receptor activity copurifies with TIR1 and the SCF<sup>TIR1</sup> – Aux/IAA interaction involves direct auxin binding. a,b. Partially-purified TIR1 retains the ability to interact with Aux/IAAs in an auxin-dependent manner. The interaction is specific for active auxins. c. Active auxins are able to reduce the recovery of radiolabelled IAA in pull-down assays suggesting that the SCF<sup>TIR1</sup> – Aux/IAA interaction involves direct auxin binding.

This work defines a new class of receptor in plant hormone signaling, one in which the F-box protein component of an SCF-type ubiquitin-ligase binds IAA to promote the destruction of Aux/IAA transcription factors. With more than 700 F-box proteins in Arabidopsis and similarly high numbers in other species, it will be interesting to see to what extent this type of mechanism might apply to the perception of other small molecules.

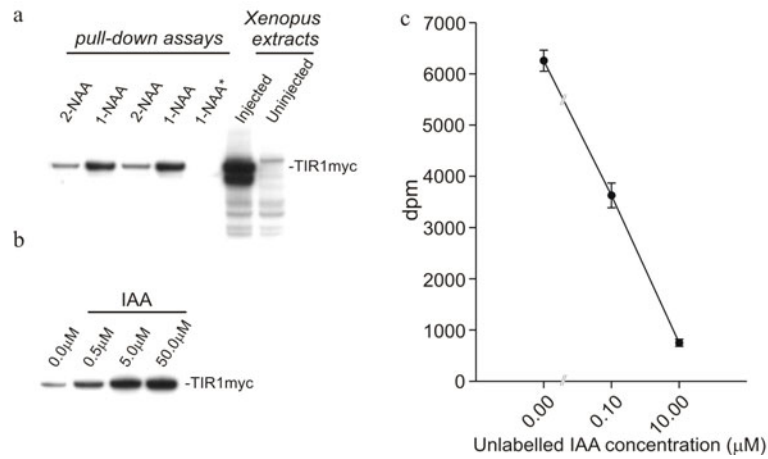


Figure 3. Xenopus-expressed TIR1 is able to interact with Aux/IAAs in an auxin dependent manner. a.b. Pull-down assays using TIR1 expressed in Xenopus embryos. c. Radiolabelled IAA pull-down assays using extracts of embryos expressing TIR1.