Too SHY 2 Repress

Jason W. Reed[1] and Bastiaan O. R. Bargmann[2]

[1] The University of North Carolina at Chapel Hill, Department of Biology

[2] Virginia Polytechnic Institute and State University, School of Plant and Environmental Sciences

The hormone auxin regulates many aspects of plant development, in part by regulating transcription (Powers and Strader, 2020). Two types of proteins control canonical auxin-regulated transcription: class A Auxin Response transcription Factors (ARFs) bind to and activate promoters of auxin-responsive genes; and Aux/IAA proteins interact with ARFs to repress gene activation. In angiosperms, multiple paralogous genes encode both ARF and Aux/IAA proteins. Differences in expression patterns or biochemical properties among these paralogs likely underlie diverse auxin response characteristics in different cellular contexts. In this issue, Cho *et al*. show that a difference in a single amino acid between different Aux/IAA proteins confers strikingly divergent effects on auxin-regulated root hair growth (Cho et al., 2024). In particular, whereas the Aux/IAA protein AXR2/IAA7 always represses auxin response and root hair growth, SHY2/IAA3 can act bimodally to either activate or repress auxin response, depending on its level relative to other Aux/IAA proteins.

Root hairs protrude from epidermal cells called trichoblasts and play crucial roles in water and nutrient absorption. Various environmental and hormonal signals control length and density of root hairs. Auxin promotes root hair growth by stimulating Aux/IAA protein turnover and thereby likely activating transcription through ARF proteins. However, an earlier study found that gain-of-function mutations in different *IAA* genes, which stabilize the encoded Aux/IAA proteins even in presence of auxin, had opposite effects on root hairs (Knox et al., 2003). The current work explains this paradox as arising from differences in biochemical properties among these Aux/IAA proteins, and in how these interact in the context of the regulatory environment in root hair cells.

Aux/IAA proteins interact with ARF transcription factors through their shared C-terminal Phox and Bem1 (PB1) domains, which allow for front-to-back oligomerization and enable stacking and recruitment of multiple Aux/IAAs at a single ARF-binding locus (Korasick et al., 2014). Aux/IAA proteins cause transcriptional repression by recruiting TOPLESS/TOPLESS-RELATED (TPL/TPR) co-repressors. Aux/IAAs are typically viewed as negative regulators that counteract class A ARF activity, thus preventing the transcription of auxin-responsive genes until the Aux/IAA proteins are degraded in response to increased auxin levels.

However, the study by Cho *et al*. challenges this simple conventional view by demonstrating that the Aux/IAA protein SHY2/IAA3 can exhibit a dose-dependent converse function, acting either to activate or repress gene expression (and consequently promote or inhibit root-hair elongation) when at lower or higher levels, respectively. The authors posit that SHY2/IAA3 derepresses gene expression by interacting comparatively weakly with the TPL/TPR co-repressors. Moderate levels of SHY2/IAA3, either the wild-type or a stabilized mutant protein, can compete for ARF binding with other Aux/IAAs (such as AXR2/IAA7) that interact more strongly with TPL/TPR co-repressors. Hence, presence of SHY2/IAA3 would decrease TPL/TPR co-repressor recruitment at auxin-responsive loci, and thereby relieve repression and increase transcription rates. However, when SHY2/IAA3 levels exceed a

threshold, oligomerization through PB1 domains allows the stacking of multiple Aux/IAA repressors and TPL/TPR co-repressors, re-establishing conventional repression of gene expression.

Several lines of experimental evidence support this model of the bimodal function of SHY2/IAA3. Analysis of gain-of-function mutants *shy2-1*, *axr2-1*, and *axr3-1*, which express stabilized versions of SHY2/IAA3, AXR2/IAA7, or AXR3/IAA17, respectively, shows that AXR2-1 and AXR3-1 repressed root-hair growth, whereas SHY2-1 enhanced it. When expressed behind different root-hair-specific promoters, SHY2 and SHY2-1 enhanced root hair growth when expressed at relatively low levels (e.g., under the native SHY2 promoter) but inhibited root hair growth when expressed at higher levels (under the EXPANSIN A7 promoter). Expression of AXR2-1 or AXR3-1 under either of these promoters always inhibited growth. Additionally, by controlling nuclear translocation using SHY2-1-GFP fused to the glucocorticoid receptor, the authors could enhance or inhibit hair growth using low and high levels of the dexamethasone ligand, respectively. Co-immunoprecipitation and microscale thermophoresis indicates that SHY2/IAA3 indeed interacts more weakly with TPL than does AXR2/IAA7. Interestingly, the difference in Km is only about two- to three-fold, so it is likely that the combined effect of multiple tandemly interacting Aux/IAA proteins is needed to produce the opposing responses seen. Furthermore, domain swaps and site-directed mutations indicated that a specific C-to-R amino acid variation in the EAR motif within domain I (R in SHY2/IAA3 and C in AXR2/IAA7) causes the different interaction strengths. Analyses of predicted protein structures explain the differential affinities, and experiments with other R- and C-containing Aux/IAAs corroborate this model.

Thus, the authors propose that SHY2/IAA3 is a partially dominant-negative protein, in which weak interaction with TPL/TPR effectively reduces repression compared to the effect of other co-expressed strong repressors. This is analogous to a sports team in which a weaker player can hamper a team's performance when substituted in place of a stronger player, despite both being skilled. By uncovering the dose-dependent bimodal function of SHY2/IAA3, and providing a plausible mechanism, the study shows that the influence of this protein depends strongly on the regulatory context. It seems likely that similar competition mechanisms underlie evolution of transcriptional regulation in other eukaryotic systems as well.

Several questions remain open for further investigation. For example, according to the model presented here, multimerization of Aux/IAA proteins through PB1 domains is required for the transition from activation to repression of transcription upon higher SHY2/IAA3 expression levels. This could potentially be tested using known single-face PB1 domain mutations that allow dimerization but not oligomerization (Korasick et al., 2014). Such experiments could be conducted in plants, or in heterologous systems, such as yeast, where expression levels of combinations of Aux/IAAs could be assessed for their effect on ARF-mediated reporter gene activation (Pierre-Jerome et al., 2017). Creation of mutants lacking all but a small subset of Aux/IAA proteins might also help to isolate effects of the remaining ones in intact plants. Perhaps in the absence of C-containing Aux/IAAs, SHY2/IAA3 would lack its gene activation activity.

The results also underscore the relevance of relative levels of different Aux/IAA proteins to the output dynamics. In this work, by studying the comparatively simple response of root hair growth, the authors were able to isolate effects of particular Aux/IAA proteins. Responses in whole organs such as roots may need to explore responses simultaneously in different cell types (Bargmann et al.,

2013), and would also need to take into account the effects of regulatory feedback on the auxin-inducible *IAA* genes.

Several other Aux/IAAs have "RG" EAR motifs, such as AXR5/IAA1 and MSG2/IAA19, and these can confer similar effects on root hairs as does SHY2/IAA3. Do these proteins normally confer bimodal auxin response in other tissues? What biochemical and regulatory properties do other Aux/IAAs with other EAR motifs have? For example, *iaa18-1* gain-of-function mutants have long hypocotyls (Ploense et al., 2009), in contrast to both *shy2* and *axr2-1* gain-of-function mutants. IAA18 has a distinct pattern of amino acids in its EAR motif, whose properties have not been assessed and which might underlie this unusual activity.

Lastly, *IAA* genes encoding Aux/IAA proteins are often retained after genome duplications (Remington et al., 2004), suggesting selection in favor of balance among dosage of these genes. Further studies of Aux/IAA proteins may reveal whether additional biochemical subfunctionalizations may underlie evolutionary expansion and persistence of *IAA* genes. Clearly there is much still to learn about how plants use this core auxin response system.

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