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Article Addendum

What Makes each *Aux/IAA* Gene Unique in its Gene Family, Expression Pattern or Properties of the Gene Product?

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Addendum to:

Specificity and Similarity of Functions of the Aux/IAA Genes in Auxin Signaling of Arabidopsis Revealed by Promoter-Exchange Experiments Between MSG2/IAA19, AXR2/IAA3 and SLR/IAA14

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ABSTRACT

In the auxin signal transduction, two protein families, Aux/IAAs and auxin response factors, play a crucial role just downstream of auxin F-box receptors. Distinct and overlapping phenotypes of the dominant *Aux/IAA* mutants suggest some functional differentiation of the *Aux/IAA* genes in auxin signaling. Taking advantage of unique phenotypes of the *msg2/iaa19* mutants, we carried out promoter-exchange experiments, where cDNA of the *msg2*, *axr2/iaa7* or *slr/iaa14* gene was driven by the *MSG2* or *AXR2* promoter. The cDNAs were translationally fused to the green fluorescent protein gene to measure levels of expressed protein. Results showed that many abnormal phenotypes of the dominant *Aux/IAA* mutants were governed by their promoter activity, but some were dependent on their gene products. The latter result highlights the possible importance of Aux/IAA protein level controled by auxin F-box receptors.

Auxin exerts many physiological responses in different tissues, which has been a puzzle since its discovery in the 1920's. The discovery of two protein families, auxin response factors (ARFs) and Aux/IAAs, in the late 1990's was, thus, epoch-making because each physiological response might result from combinatorial interaction between a subset of the ARF and Aux/IAA families, which consist of 23 and 29 proteins in Arabidopsis, respectively.[1,2] Consequently, each *Aux/IAA* gene is thought to be differentiated in their physiological function, at least to some extent. Consistent with this idea, dominant Arabidopsis mutants of the *Aux/IAA* genes show both distinct and overlapping phenotypes. The next question is: What makes each *Aux/IAA* differentiated on a molecular level?

Theoretically there would be two extremes for this question. One is that the function of *Aux/IAAs* is solely decided by their expression pattern. In this case, all the dominantly mutated Aux/IAA proteins (mAux/IAAs) would produce similar defects if expressed in the same tissue. The other is that each Aux/IAA could interact with a distinct set of ARFs, leading to phenotypic defects characteristic to the repressed ARFs. In this case, each mAux/IAAs should induce qualitatively different defects even if expressed by the same promoter. This question has been addressed before by Knox et al.[3] and Weijers et al.[4] by the use of the promoter-swapping strategy. In the latest issue of Plant Physiology we also reported our results on this question[5] by taking advantage of the *msg2/iaa19* mutants, which exhibit fewer defects than the other dominant *Aux/IAA* mutants.[6,7] Figure 1 summarizes our results as well as those of Weijers et al.[4] In our experiments, cDNA of *msg2*, *axr2/iss7*[8] or *slr-1/iaa14*[9] was driven by the *MSG2* or *AXR2* promoter. Weijers et al. expressed the *bdl/iaa12*[10] or *shy2/iaa3*[11] cDNA by the *BDL* or *SHY2* promoters.

Of the 21 determined phenotypes in total, mAux/IAA proteins induce the same or qualitatively similar defects in 17 phenotypes (Fig. 1). In four cases, however, mAux/IAA did exert qualitatively different phenotypes, even when driven by the same promoter. This clearly shows that physiological function of *mAux/IAA* was determined by both the pattern of gene expression and the properties of gene products, but that gene expression may have a primary role. This conclusion is essentially the same as that reached by the previous study.[4]

The importance of gene expression has been widely recognized in studies of gene function. Thus, it would be surprising if each mAux/IAA protein had distinct characteristics, and the next question would be: What properties of the Aux/IAA proteins make them distinct from each other? The Aux/IAA proteins consist of three conserved regions, domain I, domain II and the carboxy-terminal domain (CTD). Domain II is a recognition site for auxin F-box receptors (AFBs).[12-14] AFBs ubiquitinate Aux/IAAs after auxin perception, leading to degradation of Aux/IAA. This relieves ARFs from repression of their transcriptional activities, which ultimately results in auxin responses.

Table 1 **Shared and differentiated functions among the dominantly mutated Aux/IAA proteins**

Phenotype	Degree of Defects			Target Auxin Response Factors (ARF) (Putative)
	Dominant Mutants	Driven by MSG2 Promoter	Driven by AXR2 Promoter	
Size of mature plants	wt[1)] = *msg2* < *slr* < *axr2*	wt = msg2 = slr = axr2*	wt = msg2 < slr = axr2	ARF6 + ARF8[18]
Embryogenesis	wt = *msg2* = *slr* = *axr2* +NPH4/ARF7[16]	wt = msg2 < slr = axr2	wt = msg2 = slr = axr2*	MP/ARF5
Shape of etiolated hypocotyls	wt ≈ *msg2* < *slr*, *axr2* [2)]	wt ≈ msg2 = slr = axr2	wt ≈ msg2 < slr, axr2 [2)]	ARF6 + ARF8[18]
Hypocotyl gravitropism	wt < *msg2* < *slr* < *axr2*	wt < msg2< slr = axr2	wt < msg2< slr = axr2	NPH4 + ARF19[19,20]
Lateral root formation	wt < *msg2*, *axr2* [2)] < *slr*	wt < msg2 = slr = axr2	ND[1)]	NPH4 + ARF19[20,21]
Root gravitropism	wt = *msg2* < *slr* = *axr2*	wt = msg2 = slr = axr2*	ND	NPH4 + ARF19[20]
Root hair formation	wt = *msg2* < *slr* = *axr2*	wt = msg2 = slr = axr2*	ND	?

*This shows that *MSG2* or *AXR2* does not express in a tissue critical for the phenotype. Proteins appearing in a black background show the same function among the mutated Aux/IAA proteins; those in a grey background show quantitative differences in function among them; underlined names show qualitative differences; [1)]wt, wild type; ND, not determined; [2)]Two mutants exhibit qualitatively different phenotypes.

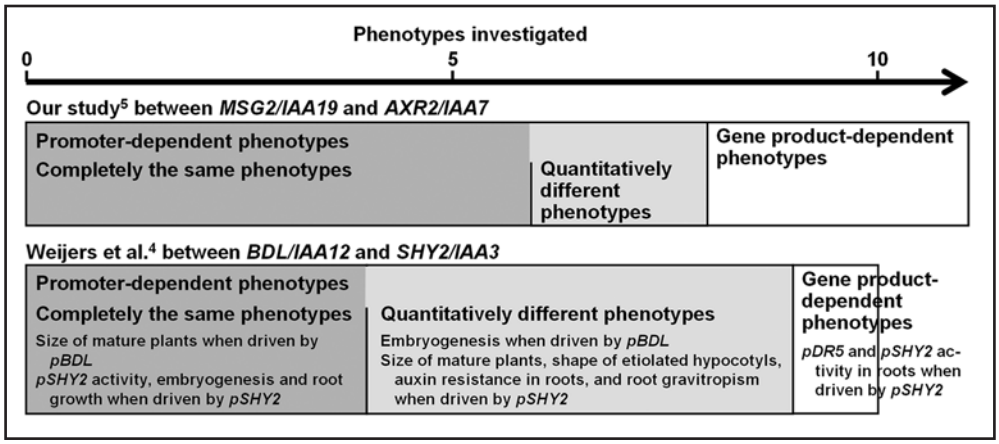

Figure 1. Promoter- and gene product-dependent phenotypes of the dominant *Aux/IAA* mutants as revealed by promoter-exchange experiments. *pBDL* represents promoter of the *BDL* gene.

Therefore, properties of Aux/IAAs are likely determined by the binding constants for ARFs through CTD and for AFBs through domain II. Because strength of interaction between Aux/IAAs and ARFs appears to be similar for pairs investigated so far with yeast two-hybrid assay[4,6,9,10,15,16] or fluorescence cross-correlation spectroscopy,[17] binding constants between Aux/IAAs and AFBs may be variable. In fact, when driven by the same *AXR2* or *MSG2* promoter, a protein level of msg2-1 estimated from fluorescence intensity of green fluorescent protein fused to msg2-1 was much lower than that of axr2-1,[5] suggesting that msg2-1 has a higher affinity to AFBs. This difference may cause a few msg2-specific defects independent of the promoter activities (Table 1, underlined). Even in the case where mAux/IAAs exhibited quantitatively different phenotypes, msg2-1 exerted weaker defects than did slr-1 and axr2-1 (Table 1, shaded). This may also be due to a lower msg2 level than the other two mAux/IAAs. Quantitative determination of the interaction between Aux/IAAs and AFBs will be needed to further understand functional differentiation of the Aux/IAA family.

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