

Article Addendum

What's the physiological role of domain II-less Aux/IAA proteins?

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Arabidopsis has Aux/IAA proteins that lack domain II which is a binding site for the TIR1 auxin receptor. These proteins have been shown to be more stable than the canonical Aux/IAA proteins. We investigated the phenotypes of overexpression lines of domain II-less Aux/IAA proteins, IAA20 and IAA30, by the use of 35S promoter. The transgenic lines showed many aberrant phenotypes in auxin physiology. The most conspicuous phenotype was collapse of the root apical meristem as occurred in *plt1 plt2* double mutants. Because *IAA20* and *IAA30* were early auxin-inducible and were expressed in the root apical meristem, they may have a physiological role in maintaining the stem cell niche of root, by keeping the activity of MP/ARF5 and NPH4/ARF7 at an acceptable level. On the other hand, domain-II less Aux/IAA proteins are not present in black cottonwood tree or grapevine, suggesting wide diversification of Aux/IAA proteins in higher plants.

Aux/IAA genes play a pivotal role in auxin signal transduction since their products have been shown to be a direct target of the auxin F-box receptors including TIR1, which are components of SCF ubiquitin ligase E3 complexes.^{1,2} TIR1 binds the degron sequence of Aux/IAA proteins³ that is conserved in most of them and is called domain II since their finding. Importance of domain II is apparent because amino acid substitutions in its core sequence cause dominant mutations, which are supposed to occur due to accumulation of the mutated protein. However, 5 of the 29 Arabidopsis Aux/IAA proteins such as *IAA20* and *IAA30*, lack domain II. Another Aux/IAA protein, *IAA31*, has a G-to-D substitution in the degron sequence, which is similar to a G-to-E substitution in a dominant mutation of an *Aux/IAA* gene, *iaa3/shy2*.⁴ Consistent with these characteristics of domain II, *IAA20* and *IAA31* have been shown to be long-lived compared to the canonical *IAA17* protein.⁵

Because the physiological role of these noncanonical Aux/IAA proteins is unknown, we made transgenic Arabidopsis lines

overexpressing *IAA20*, *IAA30* or *IAA31* cDNA by means of 35S promoter, and investigated their phenotype. The three gene products are most related each other in amino acid sequence and form a single clade in the Arabidopsis Aux/IAA proteins.⁶ Each overexpression line (OX) showed qualitatively similar defects in auxin physiology, with *IAA20* OX being the most severely affected. *IAA20* OX showed low fecundity, low germination rate, short and agravitropic hypocotyls and shoots, agravitropic roots and defects in formation of vasculature. Most interestingly, growth of the primary roots was arrested soon after germination due to collapse of the root apical meristem (RAM). Lateral roots were formed and grew with fewer occasions of collapse than the primary roots. We also found that *IAA20* and *IAA30* were early auxin inducible, but *IAA31* was not induced by auxin. The collapse of RAM observed in the OXs is similar to that in *plt1 plt2* double mutants.⁷ *PLTs* encode AP2-class transcription factors, and are late auxin-inducible through the function of ARF5/MP and ARF7/NPH4. They act to establish the stem cell niche of root, and are essential for the root stem cell activity.^{7,8} Therefore, accumulated *IAA20* proteins may inhibit these ARFs, which would result in repression of *PLT* expression and ultimately bring about severe reduction of meristematic activity in roots of OX.

Recently *IAA30* has been shown to be specifically expressed in the quiescent center cells (QC) of RAM. *IAA20* is also expressed in the columella root cap and QC, but *IAA31* was expressed at a low level without specificity in RAM.⁹ Using *IAA20* or *IAA30 promoter-GUS* reporter genes, we also confirmed the expression of both genes in QC and the columella root cap (unpublished data). On the other hand, MP activity, through which *PLTs* are transcribed, is regulated through twin canonical Aux/IAA proteins, *IAA12/BDL* and *IAA13* in RAM formation during embryogenesis.^{10,11} Therefore, we propose an auxin signaling pathway that acts to maintain the stem cell niche of root as shown in Figure 1. Because *IAA20* and *IAA30* do not contain domain II, they should not act directly downstream of TIR1. Furthermore, we postulate that they are auxin-inducible through the function of MP and NPH4. Then, what is the role of *IAA20* and *IAA30*, which can not interact with the TIR1 auxin receptor? Ectopic expression of *PLT* in the embryo causes homeotic transformation of the apical region to root and hypocotyl identity,⁷ suggesting that strict control of their expression is needed for correct formation and maintenance of RAM. Because *IAA20* and *IAA30* are not degraded through the function of the TIR1 auxin receptor, their levels tend to remain the same, and may only increase in response to auxin. These characteristics of *IAA20* and *IAA30* suggest that they

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act as damper in the auxin signaling pathway that lessen the fluctuations of the MP and NPH4 levels and ensures that expressions of the late auxin-inducible *PLT* genes remain at acceptable levels. Recently *IAA30* has been also reported to express through a transcription factor, *LEC2* and has been suggested to be involved in somatic embryogenesis.^{12,13} Though the DNA binding domain of *LEC2* (B3 domain) is related to that of ARFs,¹⁴ it binds to the RY motif, which is different from the auxin response element.¹²

We investigated the presence of the noncanonical Aux/IAA proteins in four other plant species for which complete genome sequences were available (Table 1). Each has Aux/IAA proteins with the dominant mutant-type domain II. Domain II-less Aux/IAA proteins also often lack domain I. Black cottonwood tree and grapevine do not have Aux/IAA proteins that lack only domain II. Thus, domain II-less Aux/IAA proteins like *IAA20* or *IAA30* of Arabidopsis are found only in Arabidopsis and rice. *OsIAA8* (Os02g49160) of rice appears to be an orthologue of *IAA20* or *IAA30* of Arabidopsis.¹⁵ However, it is not auxin-inducible.¹⁶ *OsIAA4* (Os01g13030) has the dominant mutant-type domain II like Arabidopsis *IAA31*. However, unlike *IAA31*, it is auxin-inducible.¹⁶ Moss *Physcomitrella patens* does not have any noncanonical Aux/IAA proteins. These results suggest that Aux/IAA proteins diversified extensively in both structure and function in higher plants. Thus, no consistent roles could be assigned to the noncanonical Aux/IAA proteins at present.

References

- Dharmasiri N, Dharmasiri S, Estelle M. The F-box protein TIR1 is an auxin receptor. *Nature* 2005; 435:441-5.
- Kepinski S, Leyser O. The Arabidopsis TIR1 protein is an auxin receptor. *Nature* 2005; 435:446-51.
- Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* 2007; 446: 640-5.
- Tian Q, Reed JW. Control of auxin-regulated root development by the *Arabidopsis thaliana* *SHY2/IAA3* gene. *Development* 1999; 126:711-21.
- Dreher KA, Brown J, Saw RE, Callis J. The Arabidopsis Aux/IAA protein family has diversified in degradation and auxin responsiveness. *Plant Cell* 2006; 18:699-714.
- Remington DL, Vision TJ, Guilfoyle TJ, Reed JW. Contrasting modes of diversification in the *Aux/IAA* and *ARF* gene families. *Plant Physiol* 2004; 135:1738-52.
- Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B. The *PLETHORA* genes mediate patterning of the Arabidopsis root stem cell niche. *Cell* 2004; 119:109-20.
- Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B. *PLETHORA* proteins as dose-dependent master regulators of Arabidopsis root development. *Nature* 2007; 449:1053-7.
- Nawy T, Lee JY, Colinas J, Wang JY, Thongrod SC, Malamy JE, Birnbaum K, Benfey PN. Transcriptional profile of the Arabidopsis root quiescent center. *Plant Cell* 2005; 17:1908-25.
- Hamann T, Benkova E, Bäurle I, Kientz M, Jürgens G. The Arabidopsis *BODENLOS* gene encodes an auxin response protein inhibiting *MONOPTEROS*-mediated embryo patterning. *Genes Dev* 2002; 16:1610-5.
- Weijers D, Benkova E, Jäger KE, Schlereth A, Hamann T, Kientz M, Wilmoth JC, Reed JW, Jürgens G. Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators. *EMBO J* 2005; 24:1874-85.
- Braybrook SA, Stone SL, Park S, Bui AQ, Le BH, Fischer RL, Goldberg RB, Harada JJ. Genes directly regulated by *LEAFY COTYLEDON2* provide insight into the control of embryo maturation and somatic embryogenesis. *PNAS* 2006; 103: 3468-73.
- Stone SL, Braybrook SA, Paula SL, Kwong LW, Meuser J, Pelletier J, Hsieh TF, Fischer RL, Goldberg RB, Harada JJ. Arabidopsis *LEAFY COTYLEDON2* induces maturation traits and auxin activity: Implications for somatic embryogenesis. *Proc Natl Acad Sci USA* 2008; 105:3151-6.
- Ulmasov T, Hagen G, Guilfoyle TJ. ARF1, a transcription factor that binds to auxin response elements. *Science* 1997; 276:1865-8.
- Kalluri UC, DiFazio SP, Brunner AM, Tuskan GA. Genome-wide analysis of Aux/IAA and ARF gene families in *Populus trichocarpa*. *BMC Plant Biol* 2007; 7:59.
- Jain M, Kaur N, Garg R, Thakur JK, Tyagi AK, Khurana JP. Structure and expression analysis of early auxin-responsive *Aux/IAA* gene family in rice (*Oryza sativa*). *Funct Integr Genomics* 2006; 6:47-59.

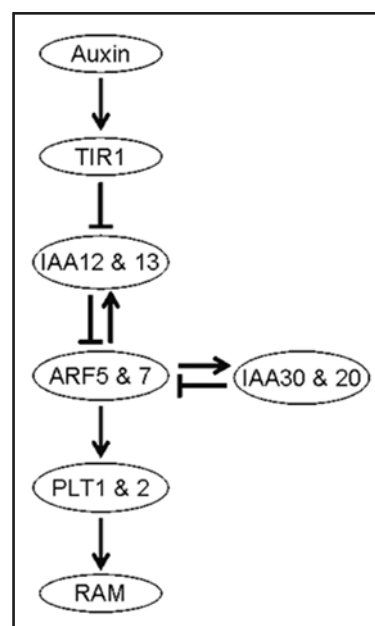


Figure 1. Model for domain II-less Aux/IAA proteins (*IAA30* and *IAA20*) in maintenance of the root apical meristem (RAM).

Table 1 Noncanonical Aux/IAA proteins in a few plant species

Species	Total number of Aux/IAA proteins	Domain II-less proteins	Dominant mutation-type proteins*	References
<i>Arabidopsis thaliana</i>	29	5 (1)**	1 (0)**	5
<i>Oryza sativa</i>	31	5 (3)	1 (0)	15***, 16
<i>Populus trichocarpa</i>	35	3 (3)	2 (0)	15
<i>Vitis vinifera</i>	31	6 (6)	3 (1)	This study
<i>Physcomitrella patens</i>	2	0	0	This study

*Aux/IAA proteins with the domain II sequences which are comparable to those of dominantly-mutated Aux/IAA proteins of Arabidopsis. **Atypical Aux/IAA proteins often also lack domain I. Numbers in parenthesis show proteins without domain I and domain II. ***Type of domain II was classified according to alignment of amino acid sequences reported by Kalluri et al.,¹⁵ and subsequently corrected manually by us. Thus, this classification is not completely the same as that shown by them.¹⁵