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Addendum

JLO regulates embryo patterning and organ initiation by controlling auxin transport

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In Arabidopsis, lateral organ initiation correlates with the formation of an auxin maximum in a group of cells at the periphery of the shoot apical meristem (SAM). This signal establishes founder cells that build the lateral organ. Primordia initiation is closely associated with the creation of a functional boundary that separates the newly formed primordium from the remainder of the meristem. In the June issue of *Plant Cell*, we have characterised the *JLO* (for *Jagged Lateral Organ*) gene of Arabidopsis, a member of the Lateral Organ boundary Domain gene family. *JLO* is expressed in boundaries and regulates both auxin transport, via a negative regulation of PIN auxin export carriers, and meristem fate by promoting the expression of the KNOX genes *SHOOTMERISTEMLESS* (*STM*) and *BP/KNAT1*. In this Addendum, we discuss the regulation of *PIN* genes by *JLO*, and propose a model for *JLO* function during embryonic and post-embryonic development.

The plant signalling molecule auxin plays a fundamental role in specifying the sites for lateral organ initiation.[1,2] The signal that induces protrusion of lateral organs is provided by the appearance of an auxin maximum at the flank of the shoot apical meristem. Polar auxin transport to the meristem is mediated by the coordinated action of influx and efflux carriers. Among the efflux carriers, *AtPIN1* represents the main actor of auxin transport in the aerial parts of the plant,[3,1] since loss-of-function mutation in the *PIN1* gene cause the formation of a needle-like shoot without organs.[4]

The expression of auxin response genes is regulated through the antagonistic action of auxin response factors (ARF) and auxin response inhibitors (Aux/IAA).[5,6] A role for developmental patterning during organ initiation and growth has been assigned to some ARFs such as *ARF5/MONOPTEROS (MP)* and its inhibitor *IAA12/BODENLOS* (*BDL*). Loss-of-functions mutations in *MP* and dominant mutations in *BDL* lead to the absence of an embryonic root, the formation of reduced vascular systems and flowerless shoots.[7,8] *MP* may regulate expression of the *PIN1* gene,[9] but it is so far unknown if MP promotes *PIN* expression directly or *via* mediator functions.

PIN1 controlled formation of auxin maxima at the primordia anlagen is tightly correlated with the down-regulation of class 1 *KNOX* (KNOTTED-Like homeobox) genes, such as *STM*, *BP/KNAT1* and *KNAT2* at these sites.[10,11] *STM* is specifically required for SAM formation and maintenance by keeping cells in an undifferentiated state.[12,11] *STM* and *PIN1* are expressed in a complementary pattern during organ formation,[13] suggesting that local down-regulation of *STM* may respond to auxin signalling.

In our article,[14] we describe a new arrested meristem mutant, called *jlo-D* for *Jagged Lateral Organ*. The dominant *jlo-D* mutant is strongly dwarfed, it forms serrate rosette leaves with short petioles and terminates shoot growth with the formation of a pin-like structure. Our molecular analysis revealed that the gene misexpressed in the *jlo*-D mutant belongs to the *Lateral organ Boundary Domain* (*LBD*) gene family. Proteins of this family are putative transcription factors that carry the conserved LOB domain, consisting of a Zn finger and a leucine zipper-motif.[15,16]

Formation of an arrested meristem in *jlo-D* mutants indicates that cells at the flanks of the meristem failed to transit from indeterminate to determinate fates. Consistent with this, we found expanded expression of *STM* in the terminated meristem. We used inducible misexpression of *JLO* to identify target genes *via* Affymetrix microarrays. This study revealed that *JLO* positively regulates *STM* and also *BP/KNAT1*, but represses members of the *PIN* family (see below). Interestingly, *JLO* was found to be mainly expressed in the boundary region that isolates the nascent lateral organ from the meristem.

There is a close resemblance between the terminated meristems of *jlo-D* mutants and those of *pin1* mutants,[4] suggesting that *JLO* may interfere with auxin transport. Induced overexpression of *JLO* caused a reduction of auxin transport to 30% of that measured in wild-type, and decreased expression of the auxin reporter DR5rev:GFP. Supporting our microarray data, we observed decreased *PIN1* mRNA levels (down to 15% within 3 hours) correlating with a decrease of PIN1 proteins (Fig. 1) upon *JLO* induction. Importantly, the polarity of PIN1 proteins is not affected after induction, and residual PIN1-GFP protein is still localized at the plasma membrane (Fig. 1B, arrowhead). However, *PIN1* cannot be the only target that is regulated by *JLO*, because plants expressing *PIN1* from the constitutive *CaMV* 35S promoter show the typical *JLO* overexpression

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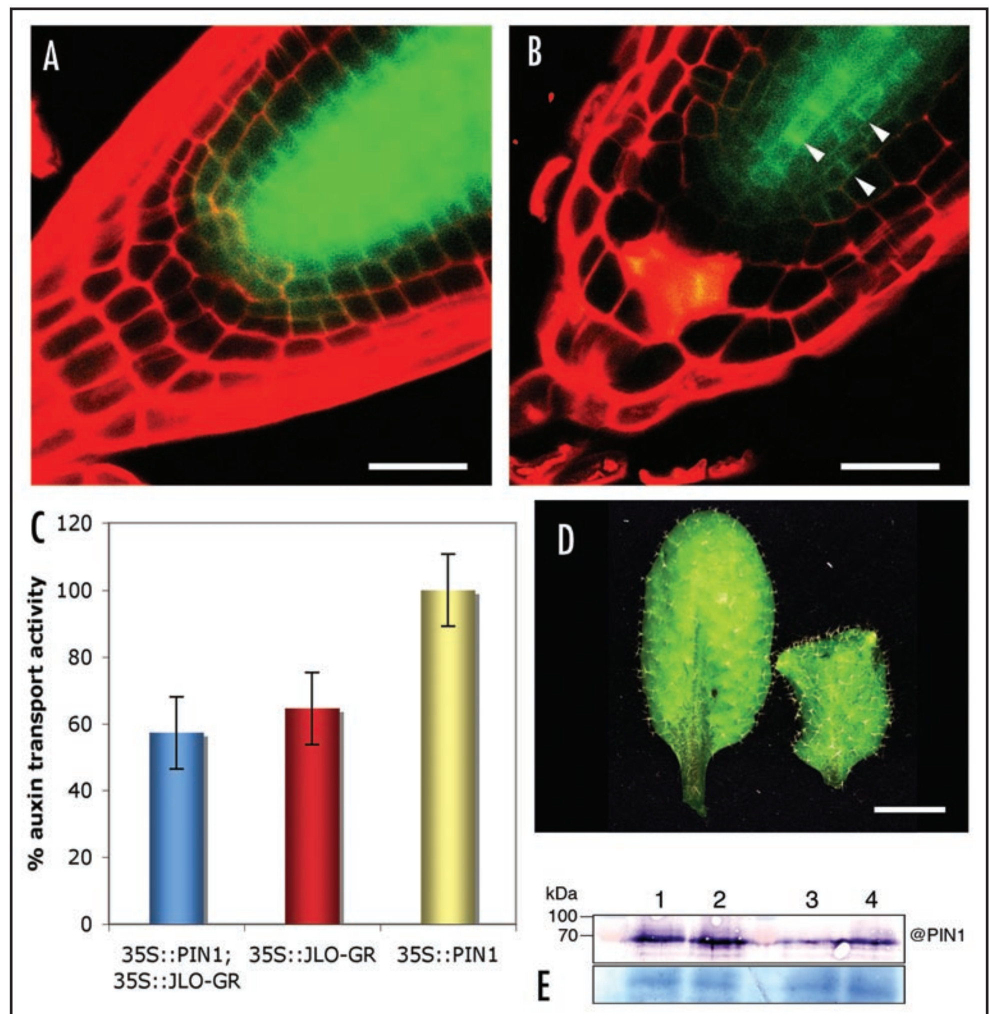

Figure 1. PIN1 expression after JLO misexpression. (A and B) Expression of PIN1:PIN1-GFP in roots of 35S:JLO-GR plants grown on GM medium (A) or GM medium supplemented with 1 µM dexamethazone to induce JLO activity (B). In un-induced plants (A), PIN1-GFP is mainly expressed in vascular tissues and pericycle cells. After induction of *JLO* expression, PIN1 is limited to the central cells of the stele. Note that PIN1-GFP fusion protein is still membrane localized after induction (arrowheads) of *JLO* expression and that *JLO* expression does not appear to alter PIN1 localisation. (C) auxin transport measurement in 35S:PIN1; 35S: JLO-GR (blue), 35S:JLO-GR (red) and 35S: PIN1 (yellow). Note that inhibition of auxin transport is not overcome by an overexpression of *PIN1*. (D) Leaves of *35S:PIN1;35S: JLO-GR* plants before (left) and after (right) *JLO* induction. Leaves show a JLO overexpressing phenotype, showing that 35S:PIN1 cannot suppress JLO overexpression effects. (E) Top panel: Expression level of *PIN1* proteins detected by Western blot using anti-PIN1 antibodies. Lanes 1 and 2: Two independent 35S:PIN1;35S: JLO-GR lines after JLO induction; Lane 3: proteins extracted from wild-type; Lane 4: proteins extracted from 35S:PIN1 overexpressing line. PIN1 protein remains highly expressed in 35S: PIN1;35S:JLO-GR plants. Bottom: Coomassie blue stained loading control. Bars: (A and B) 50 µm, (D) 1 cm.

phenotype after *JLO* misexpression, and decreased auxin transport (Fig. 1C and D).

Together, our data show that *JLO* acts upstream of *PIN1* and regulates *PIN1* expression at the transcriptional level. The suggested role for *JLO* in regulating auxin export carriers should also be reflected in a loss-of-function phenotype. T-DNA insertion mutants in *JLO* (*jlo*-1 or *jlo*-2) are embryo lethal, and *jlo-1* embryos arrest development at the globular stage where they lack provascular cell specification and fail to initiate cotyledons. Thus, *JLO* is required for normal embryo development. Embryonic apical-basal polarity is specified by local auxin gradients. The partially redundant activities of PIN proteins are central for setting up these patterns, and quadruple *PIN* mutants (*pin1pin3pin4pin7)* fail to specify the axis.[17] We had found that these 4 *PIN* genes are also downregulated upon *JLO* misexpression. We therefore analysed auxin distribution in *jlo-1* using a DR5rev:GFP reporter line. In globular stage *jlo*-1 mutants, auxin accumulates in basal cells of the suspensor, but not in apical cells or the hypophysis, where an auxin maximum is usually observed prior to root meristem specification.[17]

Several observations strengthen the role of *JLO* in auxin dependent patterning processes: (1) phenotypes similar to that of *jlo-1* were previously described for *monopteros (mp)* and *bodenlos* (*bdl)* mutants; dominant *bdl* mutations that cause increased stability of the BDL protein and inhibit MP activity cause a lack of auxin accumulation in the hypophysis,[9] (2) constitutive expression of *MP* induces the formation of a needle-like inflorescence shoot[18] similar to *pin1* and *jlo*-D mutants; (3) it was recently shown that ARF genes induce expression of several LBD genes that are required for lateral root initiation.[19]

We propose that *JLO* expression is activated by ARF proteins that control embryo patterning, such as MP. We noted that the *JLO* promoter contains several auxin response elements, but where and when this activation takes place remains to be investigated. Differential *JLO* activation in the embryo may then serve to control and restrict local PIN gene expression, thus permitting a patterned distribution of auxin. During postembryonic development, *JLO* remains expressed in boundaries, where it serves to activate KNOX gene expression and repress *PIN1*.

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