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Report

Local Auxin Sources Orient the Apical-Basal Axis in *Arabidopsis* Embryos

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Summary

Establishment of the embryonic axis foreshadows the main body axis of adults both in plants and in animals, but underlying mechanisms are considered distinct. Plants utilize directional, cell-to-cell transport of the growth hormone auxin [1, 2] to generate an asymmetric auxin response that specifies the embryonic apical-basal axis [3–6]. The auxin flow directionality depends on the polarized subcellular localization of PIN-FORMED (PIN) auxin transporters [7, 8]. It remains unknown which mechanisms and spatial cues guide cell polarization and axis orientation in early embryos. Herein, we provide conceptually novel insights into the formation of embryonic axis in *Arabidopsis* by identifying a crucial role of localized tryptophan-dependent auxin biosynthesis [9–12]. Local auxin production at the base of young embryos and the accompanying PIN7-mediated auxin flow toward the proembryo are required for the apical auxin response maximum and the specification of apical embryonic structures. Later in embryogenesis, the precisely timed onset of localized apical auxin biosynthesis mediates PIN1 polarization, basal auxin response maximum, and specification of the root pole. Thus, the tight spatiotemporal control of distinct local auxin sources provides a necessary, non-cell-autonomous trigger for the coordinated cell polarization and subsequent apical-basal axis orientation during embryogenesis and, presumably, also for other polarization events during postembryonic plant life [13, 14].

Results and Discussion

Suspensor-Specific PIN7 Mediates Early Apical Auxin Response Maximum

An early step in embryonic axis formation, specification of the proembryo from the apical cell after zygote division, has been proposed to result from polarized auxin transport delivering auxin to this cell, thus activating downstream responses [3, 15, 16]. The in vivo visualization of PIN auxin transporters and asymmetric auxin responses in early embryos identified PIN7. PIN7 is suspensor specific and is polarized toward the proembryo, where the auxin response maximum is formed [3].

We assessed the role of PIN7 in the establishment of the apical auxin response by analyzing activity of *DR5rev::GFP* auxin response reporter [3, 17] in *pin7* [3] and *pin1/3/4/7* quadruple (examined due to potential functional redundancy among these PIN proteins in embryos [18, 19]) mutant embryos. In young wild-type embryos, a weak auxin response was observed in the proembryo but not in the suspensor (Figure 1A; n = 10) [3]. In contrast, in mutant embryos (*pin7*, 67%, n = 27; *pin1/3/4/7*, 41%, n = 17), a strong ectopic *DR5* signal was observed in suspensor cells (Figures 1D and 1G). Given the auxin transport function of PIN7 [7] and the predicted transport directionality [8] based on its polar localization toward the proembryo, these observations confirm previous findings [3] and strongly suggest that PIN7 mediates directional auxin flow from the suspensor to the proembryo, where auxin response maximum contributes to the proembryo specification. This scenario is entirely consistent with the proembryo specification defects characteristic for *pin7* mutant embryos [3].

Early Basal Auxin Source Is Required for Apical Embryo Development

An obvious question relates to the source of auxin transported by PIN7 from the suspensor to the proembryo. The indole-3-pyruvic acid (IPyA) tryptophan-dependent pathway seems the most prominent mechanism of auxin biosynthesis in *Arabidopsis* [10–12] and plays important developmental roles, including those in embryogenesis [10, 20]. We explored the kinetics of IPyA-dependent auxin production in *Arabidopsis* embryos by investigating expression patterns of two key enzymes, *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* (*TAA1*) and *YUCCA* (*YUC*) [9, 12, 21], of this auxin biosynthetic pathway. We used the available *TAA1::GFP-TAA1* translational fusion and *TAR2::GUS* transcriptional fusion [10], as well as generated new transcriptional fusions of the *YUC1* to *YUC11* promoters to a nuclear-targeted 3x*GFP* (*YUC1*, *YUC4*, and *YUC10*) or a cytosolic *GFP-GUS* (*YUC2*, *YUC5*, *YUC6*– *YUC9*, and *YUC11*) tag, and supported these visualizations by a bioinformatics approach (*YUC3* and *YUC9*) and previous reports [22].

Only the *YUC3*, *YUC4*, and *YUC9* genes were specifically expressed in the suspensor from the early stages on (Figures 2A and 2B and Figure S1 available online) [22]. To investigate the importance of this early basal auxin biosynthesis, we analyzed embryos defective in these *YUC* genes. Segregating the progeny of double-heterozygous mutant combinations (*yuc3*/+ *yuc9*/+ and *yuc4*/+ *yuc9*/+) did not display any defects in their basal expression domain (data not shown), but produced seedlings with apical defects manifested by an aberrant number and formation (in terms of shape and size) of embryonic leaves (cotyledons) with the penetrance of about 25% of double-homozygous progeny (Figures 2D–2H and Table S1). Such phenotypes are characteristic of developmental aberrations in embryonic apical regions where formation of cotyledons occurs [23–25]. Backcross experiments between

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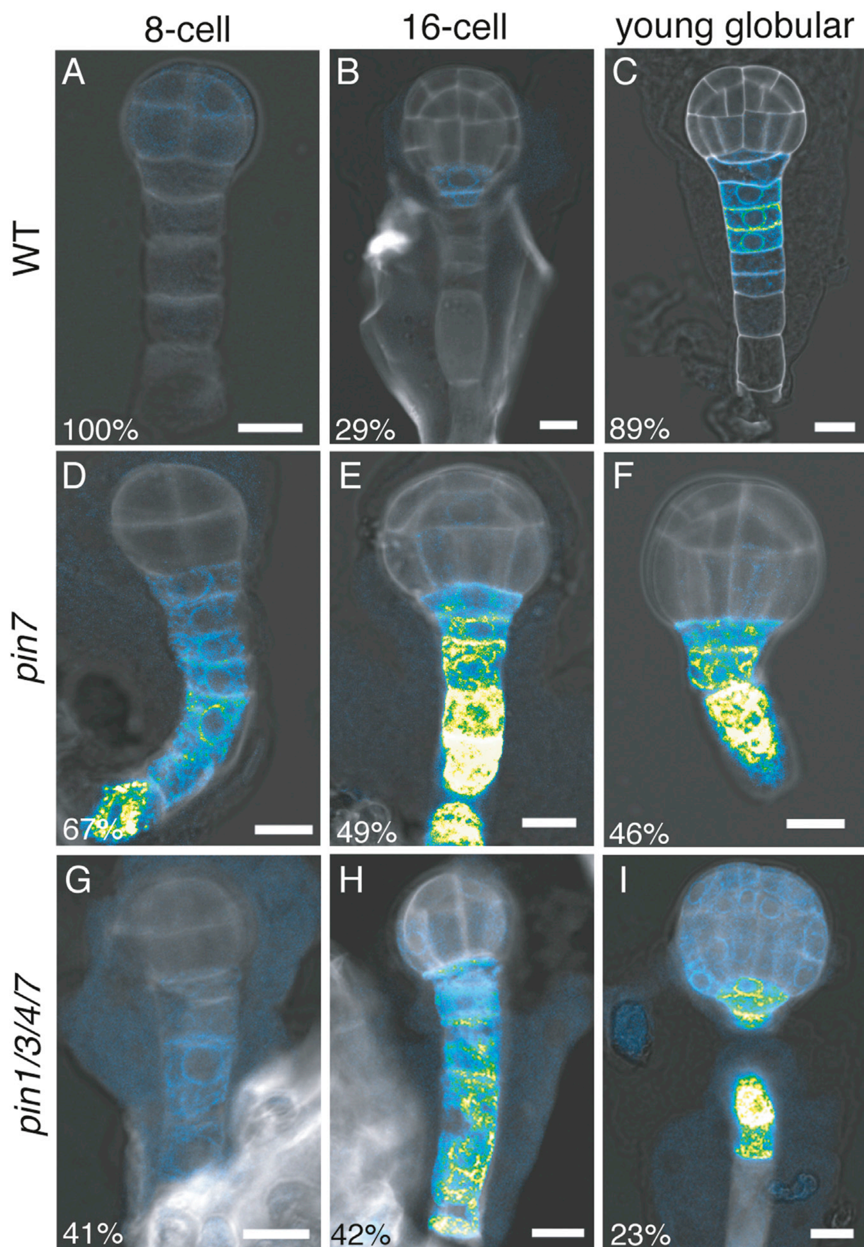

Figure 1. PIN7 Is Required for the Apical Auxin Responses in Early Embryos

The *DR5rev::GFP* reporter monitored the auxin response (blue-yellow intensity gradient) in wild-type (WT) (A–C), *pin7* (D–F), and *pin1/3/4/7* (G–I) in eight-cell (A, D, and G), 16-cell (B, E, and H), and young globular (C, F, and I) embryos. Scale bars represent 10 μm. Embryos are counterstained with Renaissance.

yuc3/9 or *yuc4/9* mutants as female plants and *DR5rev::GFP* as a pollen donor (Table S2) didn't reveal any defects in the resulting heterozygous embryos embedded in maternal tissues homozygous for the mutations indicating embryonic rather than maternal role of these genes in apical embryo patterning.

In summary, these observations reveal a non-cell-autonomous action of a basally localized auxin source in the development of apical embryo structures. Thus, at the base of early embryos, YUC3, YUC4, and YUC9 represent the localized auxin biosynthetic machinery and PIN7 represents the transport machinery that delivers auxin from the suspensor to specify the proembryo.

Later Apical Auxin Source Is Required for Basal Embryonic Patterning

The next step in the embryonic axis formation, the specification of basal embryo structures (the root pole) coincides with the rearrangement of PIN-polarity-driven auxin fluxes and the establishment of the auxin response maximum at the embryo base [3, 26, 27].

We tested the involvement of auxin biosynthesis in the basal embryo specification using the aforementioned expression marker lines for *TAA1* and *YUC*s. We detected a sharp onset of *TAA1* expression in the most apical cells of the 16-cell stage proembryos (Figures 3A and 3B). Additionally, after the globular stages, *YUC1* and *YUC4* were expressed in the same embryonic apical area, and *YUC8* was detected closer to the root pole (Figures 3C–3F). This onset of auxin biosynthesis in the embryonic apical region coincided remarkably with the establishment of the *DR5* auxin response maximum (Figure 3B) at the root pole [3].

The relevance of these apical auxin biosynthetic genes for embryo development was analyzed in the *wei8 tar1/2* mutants, which lack functional *TAA1* and its homologs [10], and in the *yuc1/4/10/11* mutants [20]. *wei8 tar1/2* embryos already displayed strong developmental defects at the globular stage (Figure 3I), and *yuc1/4/10/11* embryos displayed abnormalities slightly later at transition stages (Figure 3L), consistent with the onset of their expression. The defects in embryos lacking the function of the apically expressed biosynthetic genes were similar and confined to the basal pole including defects in apical-basal boundary and ectopic proliferation at the embryonic root pole (Figures 3I–3L and Table S3). They are strongly indicative of aberrations in the apical-basal axis specification and reminiscent of mutants defective in auxin transport [3, 19, 28] or auxin response [5, 29]. These root pole specification defects also explain the postembryonic occurrence of seedlings lacking roots in these genotypes [10, 20]. These observations show that the onset of expression of the auxin biosynthetic machinery in the proembryo is required for the development of basal embryo structures, revealing a non-cell-autonomous action of apical auxin production in the specification of the basal end of the apical-basal embryo axis.

Onset of Apical Auxin Biosynthesis Is Required for PIN1 Polarization

The requirement of apical auxin production for basal embryo development is in line with the established notion of motile

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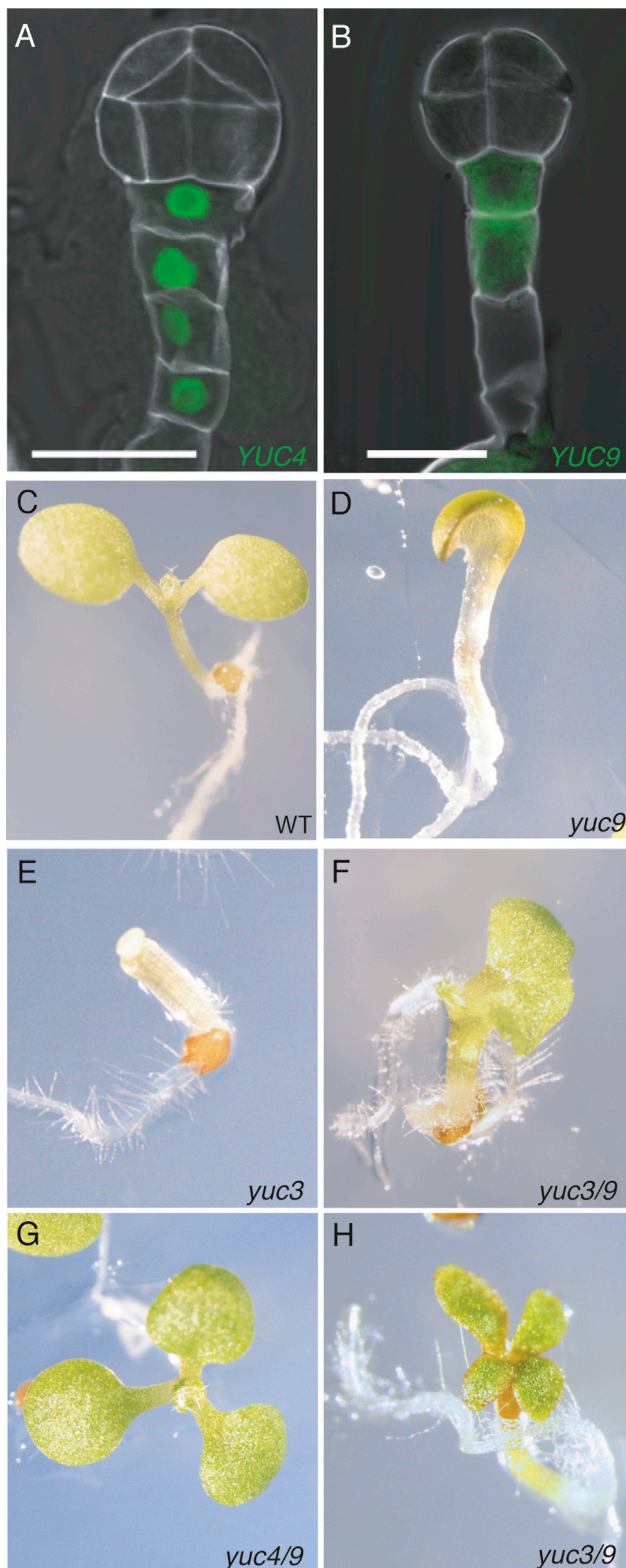

Figure 2. Local Auxin Production in the Suspensor Is Required for Apical Embryo Patterning

(A and B) *YUC4* (A) and *YUC9* (B) are expressed in the suspensor at 16-cell stage. Scale bars represent 10 μm. Embryos are counter-stained with Renaissance.

(C–H) WT Seedling (C). Panels (D)–(H) display representative phenotypes observed in *yuc3*, *yuc4*, and *yuc9* and of the progeny of *yuc3/+ yuc9/+* and *yuc4/+ yuc9/+*, e.g. monocotyledon (D), no cotyledon (E), fused cotyledon (F), tricotyledon (G), and quadricotyledon (H) seedlings.

See also Figure S1 and Tables S1 and S2.

proembryonic signals for the root pole specification [26, 30]. Apically produced auxin might be delivered through PIN1-mediated polarized transport to establish the basal auxin response maximum for the root pole specification [3]. At the onset of embryogenesis, PIN1 is expressed in the inner proembryo cells without clear polarity, but during early globular stages, PIN1 suddenly polarizes to the basal membranes of these cells, coinciding with the establishment of the auxin response maximum at the root pole [3]. Indeed, in *pin1/3/4/7* globular embryos, the *DR5* signal is retained to the proembryo (Figure 1I), instead of becoming focused at the basis as observed in WT (Figure 1C). It remains entirely unclear what signal(s) and mechanisms trigger this PIN1 polarization for the basal pole specification. Based on observations of the auxin effect on PIN polarity [31–33], and thus transport directionality [34], we hypothesize that apical auxin production itself triggers this PIN polarization.

We tested this notion by analyzing PIN1 polarity in the embryos defective in TAA1- or YUC-dependent auxin biosynthesis. Strikingly, in *wei8 tar1/2* embryos, PIN1 polarization in the inner cells of globular embryos never occurred (n = 40 for *wei8-1 tar1-1 tar2-1/+*, and n = 44 for *wei8-1 tar1-1 tar2-2/+*; Figure S2). And in both genotypes (*wei8 tar1/2* and *yuc1/4/10/11*), a gradual loss of overall PIN1 expression was observed at later stages of embryogenesis (Figure S2). This suggests that auxin biosynthesis in the proembryo is required for PIN1 polarization and for sustained PIN1 expression.

To further confirm that the onset of apical TAA1-dependent auxin production is required for the polarization of PIN1, we generated a partially rescued line, *wei8 tar1/2 TAA1::GFP-TAA1/+.* This line segregates 75% of *GFP-TAA1*-positive embryos (marked by GFP indicating rescued auxin biosynthesis) and 25% of *GFP*-negative, *TAA1*-deficient embryos. In this segregating embryo population, we analyzed PIN1 localization and in parallel monitored GFP-TAA1. Among the embryos analyzed at globular stage (n = 55), we observed an absolute co-occurrence of a GFP-TAA1 signal and polarized PIN1 in proembryonic inner cells (n = 34; Figures 4A and 4C). On the other hand, the lack of *GFP-TAA1* expression (n = 21) always correlated with the absence of PIN1 polarization (Figures 4B and 4C). Furthermore, cosegregation of the *GFP-TAA1* expression with polarized PIN1 in embryos arising from crosses between *wei8 tar1 tar2/+* and rescued *wei8 tar1/2 TAA1::GFP-TAA1* line (Table S4) confirmed that *TAA1* expression in the proembryo, but not in maternal tissues, is required for basal polarization of PIN1. These results show that the *TAA1* expression in the most apical proembryonic cells rescues the PIN1 polarization and other defects of *wei8 tar1/2* mutant (Figure 4 and Tables S2 and S4), suggesting that auxin supply is the signal triggering PIN1 polarization in the proembryonic inner cells.

We confirmed that auxin production in the proembryo, via PIN1 polarization, mediates polar auxin flow toward the root pole by examination of the *DR5*-visualized auxin response pattern in these mutants. In both *wei8 tar2* (82% [71.9% WT like, 10.1% defective embryos], n = 143) and *yuc1/4/8* (81.8% [74.8% WT like, 7% defective embryos], n = 89), the intensity of the basal *DR5* signal was highly reduced (Figures 4D–4J).

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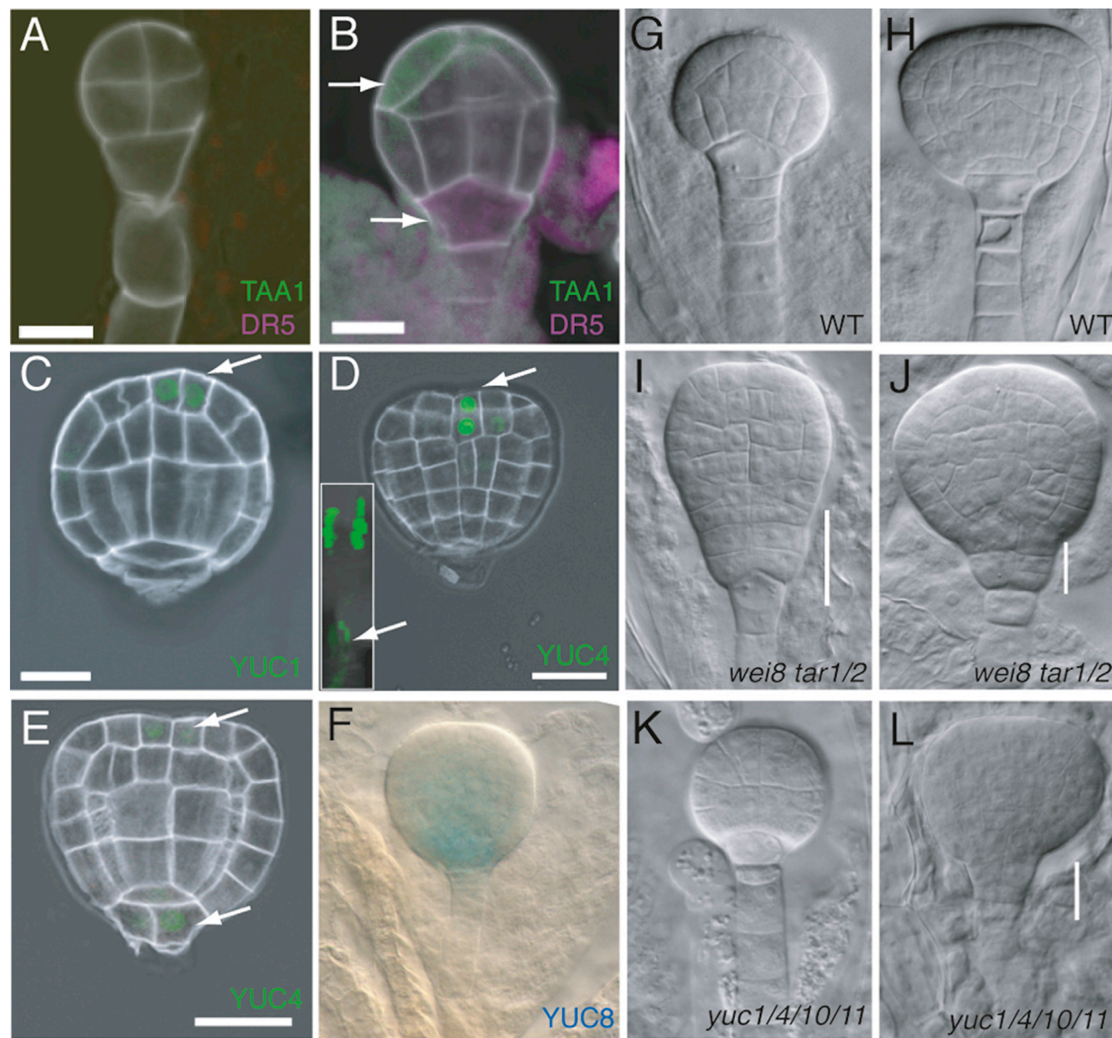

Figure 3. Apical Auxin Production Is Required for Basal Patterning

(A and B) *TAA1* (green) expression in apical protodermal cells coincides with the *DR5* auxin response (magenta) at the basal pole (*TAA1::GFP-TAA1 DR5::RFPer* embryos at eight-cell, A, and 16-cell, B, stages).

(C–E) *YUC1* (C) and *YUC4* (D, surface, and E, middle confocal sections) are expressed in apical protodermal cells from globular and transition stages. The inset in (D) shows a 90°-rotated three-dimensional projection of a z stack series showing the specificity of protodermal *YUC4* expression (green dots).

(A–D) White arrows indicate sites of expression. Renaissance is used as a counterstain. Scale bars represent 20 μm.

(F) *YUC8* is expressed in provascular cells at a late globular stage.

(G–L) WT embryo at globular (G) and transition (H) stages. Defects (white lines) are observed at globular (I and K) and transition (J and L) stages in *wei8 tar1/2* embryos (I and J) and at the transition stage only in *yuc1/4/10/11* embryos (K and L).

See also Table S3.

This supports the notion that the absence of both apical auxin supply and the accompanying PIN1 polarization leads to defects in auxin accumulation at embryo base.

Altogether, these experimental data highlight that the onset of localized auxin biosynthesis at the proembryo apex is required for the polarization of PIN1-mediated auxin fluxes toward the root pole.

Nonlocalized Auxin Production Does Not Rescue *wei8 tar1/2* Developmental Defects

To examine how important the spatiotemporal pattern of local auxin production is for embryo development, we used a transactivation system [35] and overexpression to analyze the spatial and temporal requirement of auxin biosynthesis for embryonic axis establishment.

Two activator lines, RPS5A and Q0990, drove expression of *TAA1* and *iaaM* (a bacterial auxin biosynthetic gene [36]), in the whole embryo or inner proembryo cells, respectively [35, 36]. Whereas expression of *TAA1* under its own promoter fully rescued *wei8 tar1/2* phenotypes (Figure 4 and Tables S2 and S4), ectopic expression didn't (Figure 4K and Table S2). Notably, ectopic expression of *TAA1*, but not *iaaM*, displayed a subtle partial rescue, possibly because downstream YUC activity [9, 12, 21] is providing spatial aspects due to its localized expression. This analysis shows that a spatial regulation of auxin biosynthesis is important to mediate correct embryo patterning.

Furthermore, overexpression of *TAA1* under the *35S* promoter, known to be strongly active only at postglobular embryonic stages [37], showed rescue of *wei8 tar1/2*

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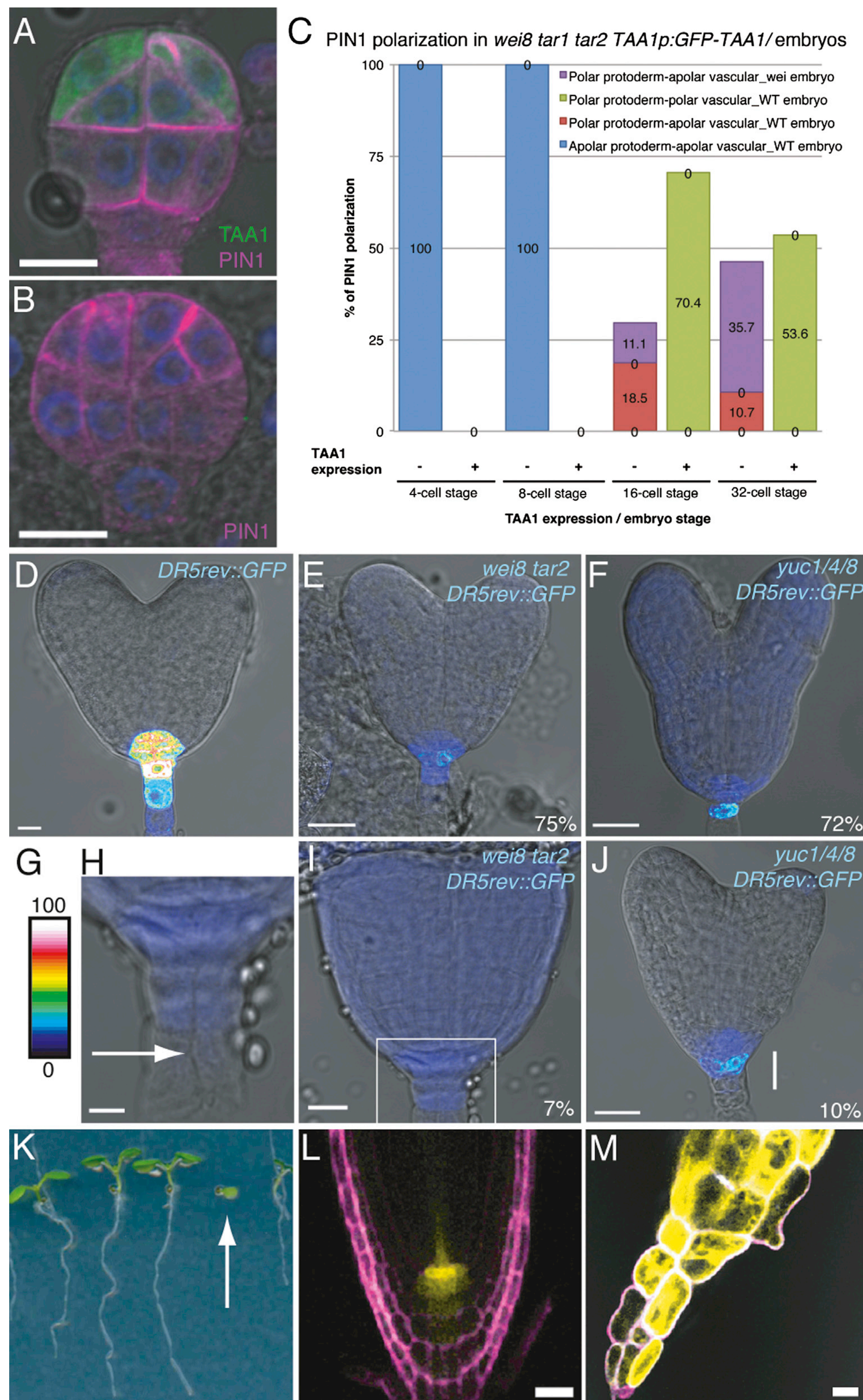

Figure 4. Local Auxin Production at Proembryo Apex Is Required for PIN1 Polarization and Basal Auxin Response Maximum

(A and B) PIN1 (magenta) was immunolocalized in a *wei8 tar1/2 TAA1::GFP-TAA1*/+ segregating line. Both rescued (A; with *TAA1-GFP* expression [green] and polarized PIN1) and nonrescued (B; nonpolar PIN1) embryos are shown at the 16-cell stage. Nuclei are marked by DAPI (blue).

(C) Correlation between *TAA1* expression and PIN1 polarization in *wei8 tar1/2 TAA1::GFP-TAA1*/+ embryos.

(D–J) *DR5* expression pattern in heart-stage embryos of *DR5rev::GFP* (D), *wei8 tar2*/+ *DR5rev::GFP* (E, H, and I), and *yuc1/4/8 DR5rev::GFP* (F and J). Normal-looking embryos (E and F) and embryos with root-pole-related phenotypes (H–J) are shown. The white box (I) indicates the closeup shown in (H).

(legend continued on next page)

postembryonic phenotypes, but with reduced fertility (data not shown). Nevertheless, such completely normal plants still segregated aberrant *wei8 tar1/2* seedlings at high frequencies (Table S2). A subset of these affected seedlings carried *TAA1-GFP* or *GFP-TAA1* transgenes as observed by GFP fluorescence (Figure 4M and Table S2), indicating that, despite being expressed, the functional GFP-tagged TAA1 proteins didn't rescue *wei8 tar1/2* embryo development when expressed under the *35S* promoter.

These observations indicate that auxin biosynthesis at a given location and at a given development stage is important for the establishment of PIN polarization and, consequently, for the apical-basal axis formation.

Conclusions

Apical-basal body axis in the embryo emerges at early stages from postfertilization events, rather than being a direct interpretation of the egg cell asymmetry [38]. Our results suggest a simple and elegant mechanism for the initiation of the embryo axis and the specification of its apical and then the basal end. Key *YUC* auxin biosynthetic genes [9, 12, 21] are expressed at the base of young embryos, and the corresponding mutants show defects in the apical embryonic regions. At the one-cell stage, PIN7, on behalf of its auxin transport activity and feedback-based polarization away from the basal source toward the apical end of the embryonic axis [3], mediates the formation of the apical auxin response maximum for proembryo specification. At the globular stage, a new focus of auxin production appears at the proembryo apex, as reflected by the onset of *TAA1/YUC* expression. This activity is required for the polarization of PIN1 in the inner proembryonic cells, which reverts auxin fluxes toward the embryo base and leads to the formation of a new auxin response maximum there that is required for the specification of the future root pole. The accompanying theoretical work [39] shows, by means of modeling young embryos, that spatially localized, dynamic auxin sources coupled to a feedback between auxin and PIN polar localization are sufficient to generate polarity and asymmetric auxin distribution during embryogenesis, confirming all results described in the present paper.

We propose that the spatiotemporal auxin biosynthesis and its feedback on auxin transport polarity generate a dynamic local auxin response that specifies sequentially the apical and the basal end of the embryonic axis. Thus, local auxin sources in conjunction with polar auxin transport represent the earliest known positional cues and the mechanism to orient the plant growth axes during embryonic and, presumably, postembryonic development.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, two figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.09.039.

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References

1. Chapman, E.J., and Estelle, M. (2009). Mechanism of auxin-regulated gene expression in plants. Annu. Rev. Genet. *43*, 265–285.
2. Grunewald, W., and Friml, J. (2010). The march of the PINs: developmental plasticity by dynamic polar targeting in plant cells. EMBO J. *29*, 2700–2714.
3. Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature *426*, 147–153.
4. Swarup, R., and Bennett, M.J. (2003). Auxin transport: the fountain of life in plants? Dev. Cell *5*, 824–826.
5. Weijers, D., Schlereth, A., Ehrismann, J.S., Schwank, G., Kientz, M., and Jürgens, G. (2006). Auxin triggers transient local signaling for cell specification in Arabidopsis embryogenesis. Dev. Cell *10*, 265–270.
6. Ueda, M., Zhang, Z., and Laux, T. (2011). Transcriptional activation of Arabidopsis axis patterning genes WOX8/9 links zygote polarity to embryo development. Dev. Cell *20*, 264–270.
7. Petrásek, J., Mravec, J., Bouchard, R., Blakeslee, J.J., Abas, M., Seifertová, D., Wiśniewska, J., Tadele, Z., Kubes, M., Covanová, M., et al. (2006). PIN proteins perform a rate-limiting function in cellular auxin efflux. Science *312*, 914–918.
8. Wiśniewska, J., Xu, J., Seifertová, D., Brewer, P.B., Růzicka, K., Blilou, I., Rouquié, D., Benková, E., Scheres, B., and Friml, J. (2006). Polar PIN localization directs auxin flow in plants. Science *312*, 883.
9. Mashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H., Natsume, M., Hanada, A., Yaeno, T., Shirasu, K., Yao, H., et al. (2011). The main auxin biosynthesis pathway in Arabidopsis. Proc. Natl. Acad. Sci. USA *108*, 18512–18517.
10. Stepanova, A.N., Robertson-Hoyt, J., Yun, J., Benavente, L.M., Xie, D.-Y., Dolezal, K., Schlereth, A., Jürgens, G., and Alonso, J.M. (2008). TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell *133*, 177–191.
11. Tao, Y., Ferrer, J.-L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., et al. (2008). Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell *133*, 164–176.
12. Won, C., Shen, X., Mashiguchi, K., Zheng, Z., Dai, X., Cheng, Y., Kasahara, H., Kamiya, Y., Chory, J., and Zhao, Y. (2011). Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. Proc. Natl. Acad. Sci. USA *108*, 18518–18523.
13. Ikeda, Y., Men, S., Fischer, U., Stepanova, A.N., Alonso, J.M., Ljung, K., and Grebe, M. (2009). Local auxin biosynthesis modulates gradient-directed planar polarity in Arabidopsis. Nat. Cell Biol. *11*, 731–738.
14. Eklund, D.M., Ståldal, V., Valsecchi, I., Cierlik, I., Eriksson, C., Hiratsu, K., Ohme-Takagi, M., Sundström, J.F., Thelander, M., Ezcurra, I., and Sundberg, E. (2010). The Arabidopsis thaliana STYLISH1 protein acts

(K) *RPS5A>>TAA1 wei8 tar2/+* didn't rescue *wei8 tar2/+* seedling phenotypes (arrow).
(L and M) *TAA1* expression pattern in *wei8 tar1/2 TAA1::GFP-TAA1* (L) or *wei8 tar1/2 35S::GFP-TAA1* (M) roots.
Scale bars represent 5 μm (H), 10 μm (A, B, and D), and 20 μm (E, F, I, J, L, and M). A semiquantitative color-coded heatmap is provided (G). See also Figure S2 and Tables S2 and S4.

as a transcriptional activator regulating auxin biosynthesis. Plant Cell *22*, 349–363.

15. Vanneste, S., and Friml, J. (2009). Auxin: a trigger for change in plant development. Cell *136*, 1005–1016.
16. Kieffer, M., Neve, J., and Kepinski, S. (2010). Defining auxin response contexts in plant development. Curr. Opin. Plant Biol. *13*, 12–20.
17. Ulmasov, T., Murfett, J., Hagen, G., and Guilfoyle, T.J. (1997). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell *9*, 1963–1971.
18. Vieten, A., Vanneste, S., Wiśniewska, J., Benková, E., Benjamins, R., Beeckman, T., Luschnig, C., and Friml, J. (2005). Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. Development *132*, 4521–4531.
19. Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I.A., Friml, J., Heidstra, R., Aida, M., Palme, K., and Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature *433*, 39–44.
20. Cheng, Y., Dai, X., and Zhao, Y. (2007). Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. Plant Cell *19*, 2430–2439.
21. Stepanova, A.N., Yun, J., Robles, L.M., Novak, O., He, W., Guo, H., Ljung, K., and Alonso, J.M. (2011). The Arabidopsis YUCCA1 flavin monooxygenase functions in the indole-3-pyruvic acid branch of auxin biosynthesis. Plant Cell *23*, 3961–3973.
22. Le, B.H., Cheng, C., Bui, A.Q., Wagmaister, J.A., Henry, K.F., Pelletier, J., Kwong, L.W., Belmonte, M., Kirkbride, R., Horvath, S., et al. (2010). Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. Proc. Natl. Acad. Sci. USA *107*, 8063–8070.
23. Treml, B.S., Winderl, S., Radykewicz, R., Herz, M., Schweizer, G., Hutzler, P., Glawischnig, E., and Ruiz, R.A.T. (2005). The gene ENHANCER OF PINOID controls cotyledon development in the Arabidopsis embryo. Development *132*, 4063–4074.
24. Torres-Ruiz, R.A., Lohner, A., and Jürgens, G. (1996). The GURKE gene is required for normal organization of the apical region in the Arabidopsis embryo. Plant J. *10*, 1005–1016.
25. Furutani, M., Vernoux, T., Traas, J., Kato, T., Tasaka, M., and Aida, M. (2004). PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in Arabidopsis embryogenesis. Development *131*, 5021–5030.
26. Zhang, Z., and Laux, T. (2011). The asymmetric division of the Arabidopsis zygote: from cell polarity to an embryo axis. Sex. Plant Reprod. *24*, 161–169.
27. Peris, C.I., Rademacher, E.H., and Weijers, D. (2010). Green beginnings - pattern formation in the early plant embryo. Curr. Top. Dev. Biol. *91*, 1–27.
28. Dhonukshe, P., Tanaka, H., Goh, T., Ebine, K., Mähönen, A.P., Prasad, K., Blilou, I., Geldner, N., Xu, J., Uemura, T., et al. (2008). Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. Nature *456*, 962–966.
29. Berleth, T., and Jürgens, G. (1993). The role of the monopteros gene in organising the basal body region of the Arabidopsis embryo. Development *118*, 575–587.
30. Yoshida, S., Saiga, S., and Weijers, D. (2013). Auxin regulation of embryonic root formation. Plant Cell Physiol. *54*, 325–332.
31. Sauer, M., Balla, J., Luschnig, C., Wisniewska, J., Reinöhl, V., Friml, J., and Benková, E. (2006). Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes Dev. *20*, 2902–2911.
32. Balla, J., Kalousek, P., Reinöhl, V., Friml, J., and Procházka, S. (2011). Competitive canalization of PIN-dependent auxin flow from axillary buds controls pea bud outgrowth. Plant J. *65*, 571–577.
33. Wabnik, K., Kleine-Vehn, J., Balla, J., Sauer, M., Naramoto, S., Reinöhl, V., Merks, R.M.H., Govaerts, W., and Friml, J. (2010). Emergence of tissue polarization from synergy of intracellular and extracellular auxin signaling. Mol. Syst. Biol. *6*, 447.
34. Berleth, T., and Sachs, T. (2001). Plant morphogenesis: long-distance coordination and local patterning. Curr. Opin. Plant Biol. *4*, 57–62.
35. Weijers, D., Van Hamburg, J.-P., Van Rijn, E., Hooykaas, P.J.J., and Offringa, R. (2003). Diphtheria toxin-mediated cell ablation reveals interregional communication during Arabidopsis seed development. Plant Physiol. *133*, 1882–1892.
36. Weijers, D., Sauer, M., Meurette, O., Friml, J., Ljung, K., Sandberg, G., Hooykaas, P.J.J., and Offringa, R. (2005). Maintenance of embryonic auxin distribution for apical-basal patterning by PIN-FORMED-dependent auxin transport in Arabidopsis. Plant Cell *17*, 2517–2526.
37. Johnson, K.L., Degnan, K.A., Ross Walker, J., and Ingram, G.C. (2005). AtDEK1 is essential for specification of embryonic epidermal cell fate. Plant J. *44*, 114–127.
38. Jeong, S., Bayer, M., and Lukowitz, W. (2011). Taking the very first steps: from polarity to axial domains in the early Arabidopsis embryo. J. Exp. Bot. *62*, 1687–1697.
39. Wabnik, K., Robert, H.S., Smith, R.S., and Friml, J. (2013). Modeling framework for the establishment of the apical-basal embryonic axis in plants. Curr. Biol. Published online November 27, 2013. http://dx.doi.org/10.1016/j.cub.2013.10.038.