Early Embryogenesis in Flowering Plants: Setting Up the Basic Body Pattern

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Annu. Rev. Plant Biol. 2012. 63:483–506

First published online as a Review in Advance on January 3, 2012

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev-arplant-042811-105507

1543-5008/12/0602-0483$20.00

Keywords

zygote, apical-basal axis, root pole initiation, radial pattern, shoot meristem initiation, cotyledon initiation

Abstract

Early embryogenesis is the critical developmental phase during which the basic features of the plant body are established: the apical-basal axis of polarity, different tissue layers, and both the root pole and the shoot pole. Polarization of the zygote correlates with the generation of apical and basal (embryonic and extraembryonic) cell fates. Whereas mechanisms of zygote polarization are still largely unknown, distinct expression domains of WOX family transcription factors as well as directional auxin transport and local auxin response are known to be involved in early apical-basal patterning. Radial patterning of tissue layers appears to be mediated by cell-cell communication involving both peptide signaling and transcription factor movement. Although the initiation of the shoot pole is still unclear, the apical organization of the embryo depends on both the proper establishment of transcription factor expression domains and, for cotyledon initiation, upward auxin flow in the protoderm. Here we focus on the essential patterning processes, drawing mainly on data from *Arabidopsis thaliana* and also including relevant data from other species if available.

Contents

INTRODUCTION

Zygote: fertilization product of egg and sperm cell

The basic body pattern of a multicellular organism is established from the zygote—the fertilized egg cell—during embryogenesis. In flowering plants, embryogenesis lays down the basis for a stereotyped seedling displaying a simple body organization of two superimposed patterns. Along the main apical-basal axis of polarity, the apically located shoot meristem, which is usually flanked by one or two cotyledons, is linked with the basally located root meristem via the hypocotyl and seedling root. The perpendicular radial pattern comprises a series of concentrically arranged tissue layers, from the outermost epidermal tissue via the ground tissue to the centrally located vascular tissue. Although the body organization of the seedling looks similar in different flowering plant species, its developmental origin can vary between species. For example, members of the Brassicaceae family (such as *Arabidopsis thaliana*) display distinct, nearly stereotypic cell-division patterns in early embryogenesis, whereas embryos of other flowering plant species grow by seemingly random cell divisions (62, 63, 66, 94). In the former group of species, the origin of seedling tissues and organs can thus be easily traced back to specific cells or groups of cells in the early embryo (**Figure 1**). Although this correlation might suggest a causal link between the spatial regulation of cell divisions and pattern formation in the early embryo, *A. thaliana* mutants such as *fass* (*fs*) displaying altered cell-division planes nonetheless generate a normal body organization, whereas morphogenesis is compromised (147). Thus, the stereotypic cell-division pattern seen in *A. thaliana* embryos expresses, but is not instrumental to, developmental decisions and might facilitate such decisions in the early embryo comprising very few cells.

This review covers recent studies that address molecular mechanisms underlying the origin of the apical-basal axis of polarity, the initiation of both the root meristem and the shoot meristem as well as the cotyledons, and radial patterning. It also discusses the parental contributions to gene activity in early embryogenesis in regard to their potential role in early patterning events. For ease of reference, **Table 1** lists the gene abbreviations and full names referred to in this review.

ZYGOTE POLARITY AND ELONGATION

Zygote Polarity

In flowering plants, the zygote is formed by the fusion of the egg cell with one of the two sperm cells delivered by the pollen tube (reviewed in 25). Like the egg cell, the zygote is usually polarized with respect to the relative position of nucleus and vacuole. However, egg cell polarity and zygote polarity are different in some species, suggesting that the latter might be established independently of the former.

In many species, the egg cell has its nucleus located toward the chalazal end of the ovule (i.e., apically) and usually has a large vacuole located toward the micropylar end (i.e., basally). This is, for example, the case in *A. thaliana*, *Capsella bursa-pastoris*, and *Nicotiana tabacum* (tobacco), in all of which zygote organization resembles egg cell organization (94, 95, 103, 131, 170); polarity—as inferred from nucleus and vacuole position—appears thus to be maintained after fertilization. However, this was shown not to be the case in *A. thaliana* and probably *N. tabacum*. A transient symmetric stage, in which the nucleus is located centrally and smaller vacuoles are distributed rather evenly within the cell, developmentally separates the polarized egg cell from the similarly polarized zygote (29, 103, 151, 170). In *A. thaliana*, the transcription factor WRKY DNA-BINDING PROTEIN 2 (WRKY2) is involved in the polarization of the zygote by transcriptionally activating *WUSCHEL RELATED HOMEOBOX 8* (*WOX8*) and possibly *WOX9* (151). *WRKY2* is dispensable for the establishment or maintenance of egg cell polarity, which corroborates the notion that egg cell and zygote polarity are not intimately linked (151). Even stronger effects of fertilization on zygote polarity are, for example, observed in *Oryza sativa* (rice), *Zea mays* (maize), and *Papaver nudicaule*, in all of which egg cell polarity is reversed after fertilization. Whereas the nucleus localizes to the micropylar/basal end of the egg cell and the large vacuole to the chalazal/apical end, the opposite is the case in the zygote (25, 114, 123).

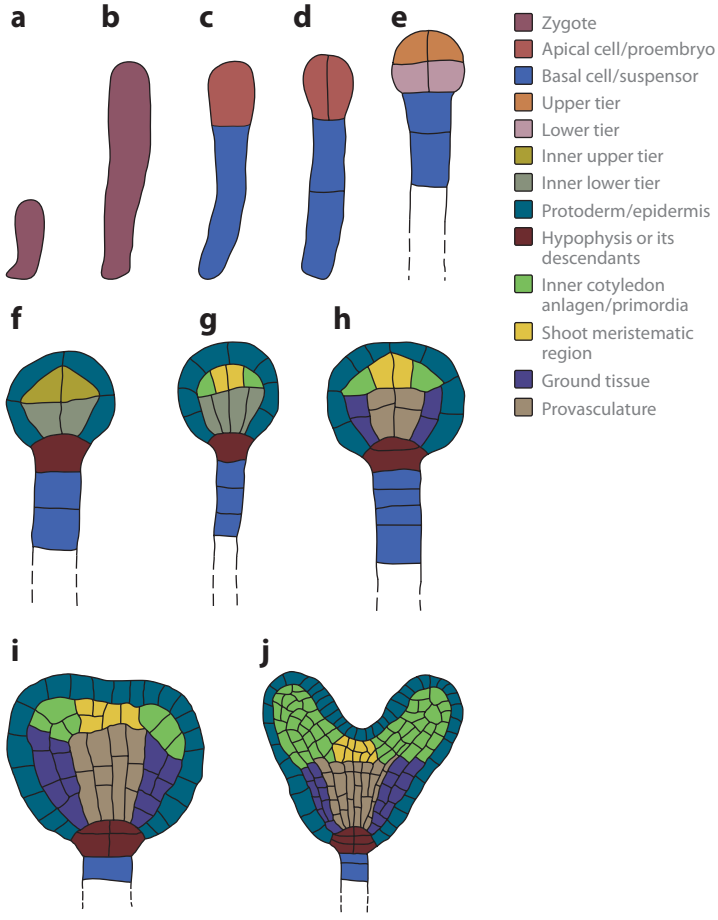

Figure 1

Early embryogenesis in *Arabidopsis thaliana*. Panels show longitudinal sections of embryos during consecutive developmental stages: (*a*) zygote, (*b*) elongated zygote, (*c*) one-cell stage, (*d*) two- or four-cell stage, (*e*) octant stage, (*f*) dermatogen stage, (*g*) early-globular stage, (*h*) mid-globular stage, (*i*) transition stage, and (*j*) heart stage. Groups of developmentally related cells are color-coded. Embryos not drawn to scale.

Zygote Elongation

The *A. thaliana* zygote not only becomes polarized but also elongates approximately threefold along its apical-basal axis before it divides. This elongation depends on the GDP/GTP exchange factor for small G proteins of the ARF class (ARF-GEF) GNOM (GN). If *GN* is knocked out, elongation and asymmetric division are compromised, but GN targets in the zygote are not known (98, 132). Zygote elongation or its asymmetric division also depends

Shoot meristem: group of self-replenishing cells at the shoot apex that sustain shoot growth and the formation of lateral organs such as leaves and flowers

Table 1 Gene abbreviations and full names used in this review

Abbreviation	Full name
ACR4	*ARABIDOPSIS CRINKLY 4*
AGO1	*ARGONAUTE 1*
ALE1/2	*ABNORMAL LEAF-SHAPE 1/2*
ARR7/15	*ARABIDOPSIS RESPONSE REGULATOR 7/15*
AS1/2	*ASYMMETRIC LEAVES 1/2*
ATDEK1	*ARABIDOPSIS THALIANA DEFECTIVE KERNEL 1*
ATH1	*ARABIDOPSIS THALIANA HOMEOBOX 1*
ATHB8/15	*ARABIDOPSIS THALIANA HOMEOBOX 8/15*
ATML1	*ARABIDOPSIS THALIANA MERISTEM LAYER 1*
BBM/PLT4	*BABY BOOM/PLETHORA 4*
BDL/IAA12	*BODENLOS/INDOLE-3-ACETIC-ACID 12*
BIM1	*BES INTERACTING MYC-LIKE PROTEIN 1*
BOP1/2	*BLADE-ON-PETIOLE 1/2*
CLE40	*CLV3/ESR-RELATED 40*
CLV3	*CLAVATA 3*
CUC1/2/3	*CUP-SHAPED COTYLEDON 1/2/3*
CUP	*CUPULIFORMIS*
DCL1	*DICER-LIKE 1*
DRN	*DORNRÖSCHEN*
DRNL	*DORNRÖSCHEN-LIKE*
ENP/MAB4	*ENHANCER OF PINOID/MACCHI-BOU 4*
FDH	*FIDDLEHEAD*
FS	*FASS*
GN	*GNOM*
GRN/RKD4	*GROUNDED/RWP-RK DOMAIN 4*
HAN	*HANABA TARANU*
KAN1	*KANADI 1*
KN1	*KNOTTED 1*
KNAT1/BP	*KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1/BREVIPEDICELLUS*
LOG	*LONELY GUY*
LTP1	*LIPID TRANSFER PROTEIN 1*
MKK4/5	*MITOGEN-ACTIVATED PROTEIN KINASE KINASE 4/5*
MP/ARF5	*MONOPTEROS/AUXIN RESPONSE FACTOR 5*
MPK3/6	*MITOGEN-ACTIVATED PROTEIN KINASE 3/6*
NAM	*NO APICAL MERISTEM*
NPH4/ARF7	*NONPHOTOTROPIC HYPOCOTYL 4/AUXIN RESPONSE FACTOR 7*
OSH1	*Oryza sativa homeobox 1*
OSTF1	*Oryza sativa transcription factor 1*
PDF1/2	*PROTODERMAL FACTOR 1/2*
PHB	*PHABULOSA*
PHV	*PHAVOLUTA*

(*Continued*)

Table 1 (*Continued*)

Abbreviation	Full name
PID	*PINOID*
PID2	*PINOID 2*
PIN1/3/4/7	*PIN-FORMED 1/3/4/7*
PLT1/2/3	*PLETHORA 1/2/3*
PNF	*POUND-FOOLISH*
PNY	*PENNYWISE*
QHB	*quiescent-center-specific homeobox*
REV	*REVOLUTA*
RPK1	*RECEPTOR-LIKE PROTEIN KINASE 1*
SCR	*SCARECROW*
SHR	*SHORT-ROOT*
SSP	*SHORT SUSPENSOR*
STM	*SHOOT MERISTEMLESS*
TAA1	*TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*
TAR1/2	*TRYPTOPHAN AMINOTRANSFERASE RELATED 1/2*
TMO7	*TARGET OF MONOPTEROS 7*
TOAD2	*TOADSTOOL 2*
TPL	*TOPLESS*
WAG1/2	*WAG 1/2*
WOX1/2/3/5/8/9	*WUSCHEL RELATED HOMEOBOX 1/2/3/5/8/9*
WRKY2/33	*WRKY DNA-BINDING PROTEIN 2/33*
WUS	*WUSCHEL*
YDA	*YODA*
YUC1/4/10/11	*YUCCA 1/4/10/11*
ZLL/AGO10	*ZWILLE/ARGONAUTE 10*
ZMCUC3	*Zea mays CUP-SHAPED COTYLEDON 3*
ZMNAM1/2	*Zea mays NO APICAL MERISTEM 1/2*

on the interleukin-1 receptor-associated kinase (IRAK)/Pelle-like kinase SHORT SUSPENSOR (SSP), the MAPKK kinase YODA (YDA), MITOGEN-ACTIVATED PROTEIN KINASE 3 (MPK3), MPK6, and the RWP-RK family protein GROUNDED (GRN)/RWP-RK domain 4 (RKD4), which functions as a transcriptional regulator (7, 58, 89, 154, 155). There is evidence that *SSP*, *YDA*, *MPK3*, and *MPK6* as well as MITOGEN-ACTIVATED PROTEIN KINASE KINASE 4 (MKK4) and MKK5 act in the same pathway (7, 155), but the direct targets of this hypothetical kinase pathway in the zygote remain unknown. However, it might be meaningful that a close homolog of WRKY2, WRKY33, is phosphorylated by MPK3 and MPK6 (96, 162).

ZYGOTIC GENOME ACTIVATION

Zygotic genome activation already occurs in the zygote in flowering plants. For *N. tabacum*, evidence has been presented that deposited maternal transcripts are not sufficient for zygote elongation and division, but that this process requires zygotic de novo transcription (170). In *Z. mays* and *N. tabacum*, transcripts not present in egg and sperm cells accumulate in the zygote, which indicates that these transcripts

Cotyledon: leaf formed in the developing embryo

Root meristem: group of self-replenishing cells at the root tip that sustain root growth

Ground tissue: primordium that will give rise to two tissue layers, endodermis and cortex

are made de novo in the zygote (110, 125, 170). Comparable experiments have not been done in *A. thaliana*. However, in both *A. thaliana* and *Z. mays*, genes whose expression has not been detected in pollen are expressed in the zygote from the paternal allele (130, 151), implying zygotic genome activation at the zygote stage in these species.

This de novo expression of paternal genes in the zygote also indicates that the paternal genome is not generally silenced in the zygote or early embryo. This idea has received support from other studies (120, 156, 165), although in these cases it cannot be clearly distinguished between transcripts delivered by the pollen and de novo transcription from the paternal alleles in the zygote. However, whereas *Z. mays* displays an equivalent parental contribution in the zygote and during early embryo development (101), in *A. thaliana* maternal transcripts appear to predominate during early embryogenesis (5). This maternal predominance is thought to result from the downregulation of the paternal alleles by the maternal chromatin small interfering RNA (siRNA) pathway, whereas the activation of the paternal alleles during the course of embryogenesis is thought to be mediated by maternal histone chaperone complex CAF1 (5). However, it cannot be excluded that the maternal predominance during early *A. thaliana* embryogenesis is mainly or also due to transcript carryover from the egg cell rather than specific downregulation of the paternal alleles. Hence, the two aforementioned mechanisms (the chromatin siRNA pathway and activity of the CAF1 complex) could generally be involved in zygotic genome activation. In conjunction with a supposed stronger transcript contribution of the egg cell as compared with the sperm cell, mechanisms delaying the zygotic genome activation would prolong the predominance of transcripts derived from the maternal alleles.

Some observations argue against general differences between paternal and maternal alleles in *A. thaliana*. For example, both paternal and maternal histone H3 variants are replaced by de novo synthesized H3 variants in the zygote (50, 51). And although imprinting is quite common in the angiosperm endosperm, only a few genes imprinted in the embryo have been reported so far (56, 90, 118). The maternal-to-zygotic transition thus appears to already commence in the zygote. In contrast to animals, however, because there is pronounced postmeiotic gene expression in both female and male gametophytes followed by postfertilization gene expression, the maternal-to-zygotic transition might more appropriately be called the gametophytic-to-sporophytic transition. This transition might be completed sooner or later, presumably depending mainly on species-specific velocities of development during early embryogenesis. In this view, the longer it takes for the zygote and its progeny to divide, the earlier in developmental time the gametophytic-to-sporophytic transition might occur.

ZYGOTE DIVISION AND SEPARATION OF APICAL AND BASAL CELL FATE

In the vast majority of flowering plant species, the zygote divides transversely, generating an apical daughter cell and a basal daughter cell, whereas in some species oblique or longitudinal divisions occur (62, 133). When the zygote divides transversely, the two daughter cells may be quite different in size, depending on the position of the plane of cell division. In *Ricinus communis* and *Triticum aestivum* (wheat), for example, the zygote divides "symmetrically," generating two daughter cells of equal size (74, 133). In other species, zygotes divide asymmetrically. Whereas in *Coriaria nepalensis* and *Anethum graveolens*, for example, the apical daughter cell is larger than the basal one, in *A. thaliana* the apical daughter cell of the zygote is smaller than the basal one (94, 133). There seems to be no general rule regarding the size ratio of the apical daughter cell and the basal daughter cell of angiosperm zygotes (133).

Nonetheless, the division of the zygote might still—directly or indirectly—separate apical and basal cell fate and hence might also consolidate or establish the apical-basal axis of

polarity, which is then maintained throughout plant life. Some evidence supports this view. In both *Z. mays* and *N. tabacum*, the apical daughter cell of the zygote exhibits a transcriptional profile distinct from the basal counterpart (48, 113). In *A. thaliana*, two developmental pathways, in addition to the YDA pathway mentioned above, have been linked to apical-basal axis establishment after zygote division: One involves the transcription factors WOX8, WOX9, and WOX2, whereas the other is auxin dependent, involving the auxin efflux regulator PIN-FORMED 7 (PIN7) as well as the transcriptional regulators MONOPTEROS (MP)/AUXIN RESPONSE FACTOR 5 (ARF5) and BODENLOS (BDL)/INDOLE-3-ACETIC-ACID 12 (IAA12) (see below) (**Figure 2**).

Besides *WOX8*, whose expression in the zygote is induced by WRKY2, *WOX2* is also expressed in the zygote (40, 151). After zygote division, though, these two genes are not coexpressed anymore; *WOX2* is expressed in the apical daughter cell of the zygote, and *WOX8* (together with *WOX9*) is expressed in the basal (40). *WOX9*, which is assumed to be

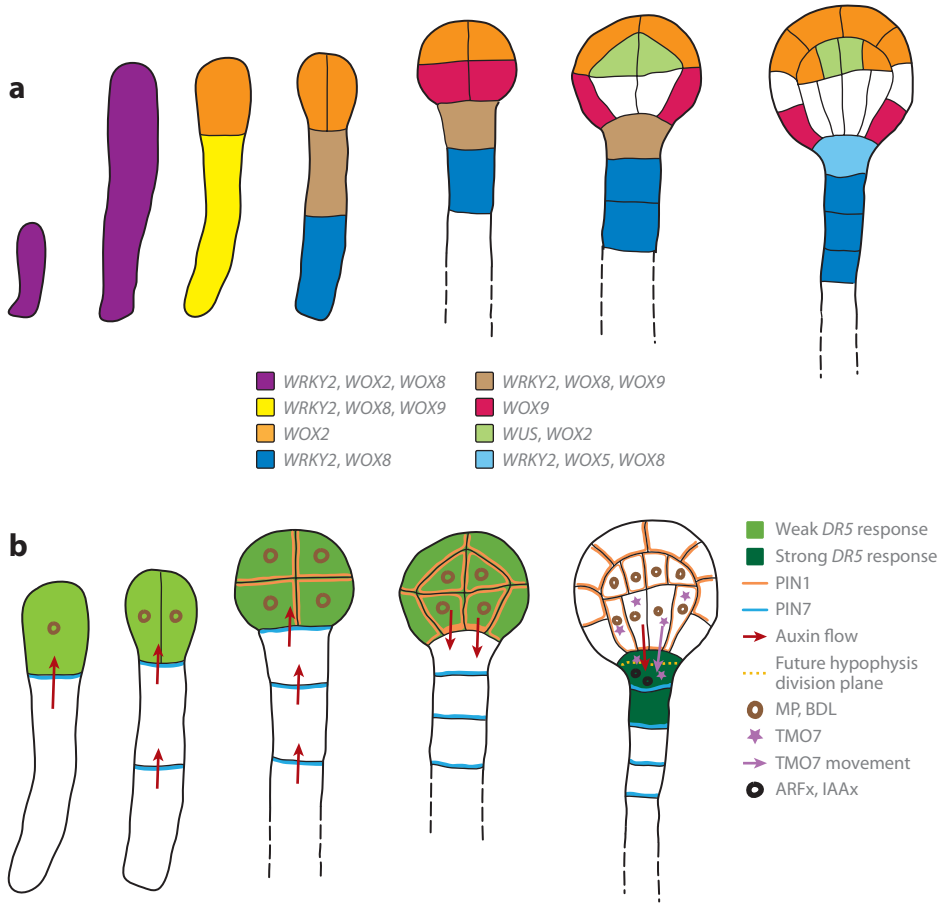

Figure 2

Apical-basal patterning and hypophysis specification in early embryogenesis of *Arabidopsis thaliana*. (*a*) Expression patterns of *WRKY2* and early-expressed *WOX* genes. (*b*) Auxin signaling and hypophysis specification. Embryos not drawn to scale.

Embryo proper: cells forming the embryo

Suspensor: extraembryonic, often filamentous structure anchoring the embryo proper to the ovule wall

Hypophysis: in *Arabidopsis thaliana*, a cell basally adjacent to the embryo proper and involved in root pole formation

a target of WRKY2 as well, might already be expressed in the zygote and possibly also in the apical daughter cell of the zygote (40, 151, 163). WOX8 and WOX9 are supposed to signal from the basal to the apical daughter cell for proper *WOX2* expression to occur (10). However, because there are stronger defects in *wox8 wox9* or *wox9* alone than there are in *wox2* mutant embryos, WOX8 and WOX9 appear to have additional, WOX2-independent functions in early development (10, 40, 163). *WRKY2* is coexpressed with *WOX8* and partially with *WOX9* during the earliest stages of embryogenesis (40, 151) (**Figure 2*a***), which could account for the early expression of these two *WOX* genes in the basal lineage. The problem of the separation of apical and basal cell fate, however, would not be solved with this extension of the WOX pathway; instead, the problem would be shifted from understanding *WOX2*, *WOX8*, and *WOX9* transcript distribution to understanding *WRKY2* transcript distribution.

The auxin-dependent pathway implicated in apical-basal axis establishment during *A. thaliana* embryogenesis becomes relevant immediately after zygote division, when auxin is transported from the basal to the apical daughter cell via PIN7 (30) (**Figure 2*b***). The auxin response in the apical descendant of the zygote triggered by this directional auxin transport might be important for its proper specification, as evidenced by its transverse instead of longitudinal division in *bdl*, *mp*, *mp bdl*, and *pin7* mutant embryos (30, 42). *MP* encodes an ARF, *BDL* encodes an AUXIN (AUX)/IAA inhibitor, and both are expressed in the apical cell lineage (41, 43); *MP* and *BDL* form a system of two interconnected feedback loops that can be modulated by auxin via the degradation of BDL protein (76). The initial transport of auxin to the apical cell(s) might thus be sufficient to establish expression of these two important developmental regulators. But, comparable to the WOX/WRKY case, the next step on the hierarchy ladder has to be taken now, and how PIN7-mediated basal-to-apical auxin transport is set up must be determined.

HYPOPHYSIS SPECIFICATION AND ROOT POLE FORMATION

Importance of Auxin in Hypophysis Specification and Root Pole Formation

The root pole is the basal end of the angiosperm embryo. In *A. thaliana*, the specification of the founder cell of the root meristem is not the result of a (spatially) isolated developmental program, but the consequence of developmental events that take place in the apically adjoining cells (157).

One of these events is the overall reversal of the above-mentioned basal-to-apical auxin flow from the dermatogen stage onward. The PIN1 auxin efflux regulator formerly nonpolarly distributed in the cells of the embryo proper starts to become localized predominantly to the basal side of the lower inner cells, and the formerly apically localized PIN7 becomes localized to the basal side of the suspensor cells. In consequence, auxin accumulates in the hypophysis and the subhypophyseal cell as indicated by the auxin response reporter *DR5* (30) (**Figure 2*b***).

This accumulation of auxin in the hypophysis appears to be crucial for its specification and subsequent root pole formation, as suggested by the fact that impairment of auxin biosynthesis and transport as well as auxin signaling interfere with these processes. The auxin-biosynthesis multiple mutants *yucca 1* (*yuc1*) *yuc4 yuc10 yuc11* and *tryptophan aminotransferase of arabidopsis 1* (*taa1*) *tryptophan aminotransferase related 1* (*tar1*) *tar2* as well as the auxin transport quadruple mutant *pin1 pin3 pin4 pin7* are rootless, just like seedlings in which the phosphorylation status-dependent polar PIN1 localization is reversed from the basal to the apical side in the inner cells of the embryo proper by the misexpression of the PIN1-phosphorylating serine/threonine kinase PINOID (PID) (19, 30, 31, 102, 139). Moreover, the regulation of *PIN1* expression involves MP and its inhibitor BDL (157). This might explain why the knockout of *MP*, or mutations causing the stabilization of BDL, lead to the non- or misspecification of the hypophysis and subsequent failure to form

a root (157). Thus, MP-BDL-dependent auxin signaling in the cells of the embryo proper indirectly ensures the accumulation of auxin in the hypophysis, where signaling through another ARF-AUX/IAA pair presumably mediates the actual specification process (157) (**Figure 2*b***). Recently, detailed expression analysis revealed several *ARF* candidates expressed in the hypophysis (117).

Additional Factors Involved in Hypophysis Specification and Root Pole Formation

In addition to auxin, other molecules likewise serve as mobile signaling cues for hypophysis specification. TARGET OF MONOPTEROS 7 (TMO7), a small transcriptional regulator whose expression is regulated by MP and BDL, also moves from the provascular cells into the hypophysis and contributes to its specification (128) (**Figure 2*b***). SHORTROOT (SHR) might also move there, as inferred from the expression of *SCARECROW* (*SCR*) in the hypophysis (106, 164). Although SCR does not appear to be necessary for hypophysis specification itself—as indicated by the apparently normal hypophysis division in the *scr* mutant—SCR is subsequently required for proper root pole formation (164). Similar considerations apply to the *PLETHORA* (*PLT*) genes *PLT1*, *PLT2*, *PLT3*, and *BABY BOOM* (*BBM*)/*PLT4* and to *WOX5*. The expression of some of them depends on *MP* and its close homolog *NONPHOTOTROPIC HYPOCOTYL 4* (*NPH4*)/*ARF7* or is initiated in the hypophysis in an *MP-BDL*-dependent fashion, but at least *WOX5* is mainly required for root organization of later developmental stages and root stem cell maintenance (3, 34, 40, 122).

Although auxin signaling is of central importance for root pole initiation, it is not the only plant hormone signaling pathway involved. The brassinosteroid signaling component *BES INTERACTING MYC-LIKE PROTEIN 1* (*BIM1*) and the AP2 transcription factors DORNRÖSCHEN (DRN) and DORNRÖSCHEN-LIKE (DRNL), which interact with BIM1, are required for proper hypophysis division and root formation, suggesting that auxin-brassinosteroid crosstalk is involved in root pole initiation (16, 17, 169). In addition, the requirement of two feedback repressors of cytokinin signaling, *ARABIDOPSIS RESPONSE REGULATOR 7* (*ARR7*) and *ARR15*, for the same process indicates the necessity to dampen cytokinin signaling (105). This dampening happens specifically in the lower derivative of the hypophysis via *ARR7* and *ARR15*, whose expression depends on auxin (105) and hence possibly also indirectly on MP-BDL-dependent signaling.

Provasculature: cells that will give rise to the vasculature (the conductive tissue)

Positional Information During Root Initiation

The fate of the hypophysis thus appears to be determined by its position at the basal end of the early embryo rather than its descent from the basal daughter cell of the zygote. Indeed, the clonal origin of the hypophysis might not be relevant for root pole initiation. In the *hanaba taranu* (*han*) mutant, expression domains of genes are shifted apically so that genes normally expressed only in the suspensor replace "apical" genes in the lower half of the embryo proper. As a consequence, it is not the histologically still-discernable hypophysis that becomes the founder cell of the future root pole, but rather cell(s) from the lower-tier descendants (108). As in the wild type, the cell(s) to be recruited for root pole formation appear to be those closest to cells with an apical cell fate.

In an even more extraordinary case of atypical embryonic root initiation, which occurs in the *topless-1* (*tpl-1*) mutant, a root is initiated not only basally but also apically and, interestingly, like in *han*, in an MP-independent fashion (87, 108). TPL, a cosuppressor that binds to BDL and probably other AUX/IAAs as well as indirectly to jasmonate ZIM-domain (JAZ) repressor proteins and directly to WUSCHEL (WUS), might recruit histone deacetylases to repress gene expression (70, 86, 115, 141; reviewed in 73). The *tpl-1* mutation is a dominant negative mutation relieving the repression

Protoderm: outermost cell layer of the embryo proper that differentiates into the epidermis

of TPL targets; especially derepression of the TPL targets *PLT1* and *PLT2* leads to the formation of a secondary root pole (135).

Many angiosperm species—including various monocots and, e.g., *Pisum sativum* (pea)—do not exhibit a cell that clearly corresponds to the *A. thaliana* hypophysis, i.e., a single uppermost derivative of the basal daughter cell of the zygote that invariably divides into a smaller upper lens-shaped and a larger lower cell to give rise to the quiescent center and the columella of the root meristem, respectively (reviewed in 59). Nevertheless, these species of course also form a root, and they may do so by employing signaling pathways similar to those in *A. thaliana*, which specify the hypophysis in a position-dependent manner. In *O. sativa*, the *WUS*-type homeobox gene *quiescent-center-specific homeobox* (*QHB*) is—similar to *WOX5* in *A. thaliana*—expressed in a few cells at the basal pole of the embryo; in *Z. mays* and *O. sativa*, an *SCR* homolog might play a role in root patterning (40, 67, 68, 82, 83). The developmental significance of the singular hypophysis in *A. thaliana* might thus mainly relate to the minimal number of cells that constitute the embryo at the very early stage when the root pole is initiated.

RADIAL PATTERNING AND PROTODERM SPECIFICATION

Separation of Inner and Outer Fate in the Early Proembryo

In *A. thaliana*, the beginning of radial patterning is marked by the tangential divisions of the cells of the embryo proper in the octant-stage embryo. The eight outer cells thus formed are the founder cells of the protoderm, and during embryogenesis the eight inner cells will give rise to, e.g., the provasculature and the ground tissue (66, 94, 126) (**Figure 1**). Like apical-basal axis establishment, these tangential divisions have been linked to the action of *WOX* genes and *MP*. In *wox2* and, with a higher penetrance, in *wox2 mp*, *wox2 wox8*, and *wox1 wox2 wox3*, some cells of the octant-stage embryo proper do not divide tangentially, so that a "continuous" protodermal layer is not formed (10, 40). How *WOX* genes and MP-dependent auxin signaling mediate the proper orientation of these cell-division planes is not known.

An early difference between protodermal and inner cells is the divergence of transcriptional activities. The GLABRA 2 (GL2) family homeodomain transcription factors *ARABIDOPSIS THALIANA MERISTEM LAYER 1* (*ATML1*) and *PROTODERMAL FACTOR 2* (*PDF2*) are initially expressed throughout the early embryo proper, but immediately after the tangential divisions have occurred their expression becomes confined to the protodermal cells (1, 88) (**Figure 3*a*,*b***). Conversely, the expression of *ZWILLE* [*ZLL*, also called *ARGONAUTE 10* (*AGO10*)], which is expressed in the apical cells from the four-cell stage on and is involved in shoot meristem maintenance, becomes confined to the inner cells (91, 104) (**Figure 3*a*,*b***). Remarkably, in *Z. mays* and *O. sativa*, where the cell-division planes after the zygotic division appear randomly oriented, the expression of *ATML1* homologs also becomes confined to the protoderm, and these homologs might serve a similar function during protoderm development as their *A. thaliana* counterparts (52–54, 167).

In *atml1 pdf2* double-mutant seedlings, cotyledons seem devoid of an epidermis and the shoot apex lacks distinct cell layers (1). The *ATML1* promoter and the *PDF2* promoter each contain a potential binding site for WUS, the founding member of the WOX family (1, 40, 143), and thus the expression of *ATML1* and *PDF2* could be directly regulated by WOX transcription factors, including those involved in the tangential divisions of the octant-stage embryo (**Figures 2*a*** and **3*c***). Furthermore, both the *ATML1* promoter and the *PDF2* promoter contain an eight-nucleotide sequence termed the L1 box, which is also present in the promoters of other epidermally expressed genes such as *PDF1*, *FIDDLEHEAD* (*FDH*), *LIPID TRANSFER PROTEIN 1* (*LTP1*), and—almost perfectly matching—the *O. sativa ATML1* homolog *Oryza sativa transcription*

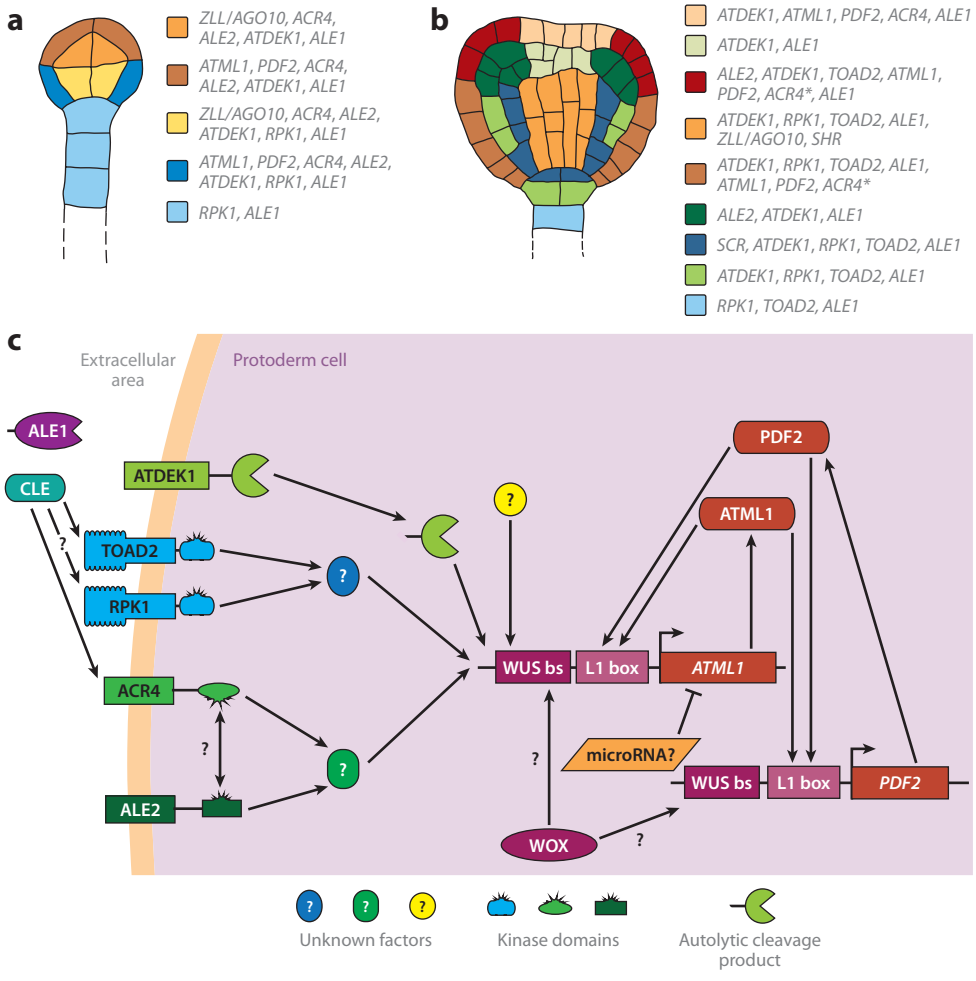

Figure 3

Radial patterning in early embryogenesis of *Arabidopsis thaliana*. (*a*,*b*) Expression patterns of genes important for radial patterning. Panel *a* shows the dermatogen stage; panel *b* shows the transition stage. Asterisk indicates that weak *ACR4* expression was detected ubiquitously in embryos. (*c*) Signaling pathways maintaining protoderm identity. Abbreviation: WUS bs, WUS binding site. Embryos not drawn to scale.

factor 1 (*OSTF1)* (1, 2, 167). Because ATML1 and PDF2 bind to the L1 box in vitro, it is conceivable that these two transcription factors establish a positive feedback loop that regulates the transcription of genes expressed in the epidermis (1, 2) (**Figure 3*c***). In the case of *ATML1*, however, the L1 box and the WUS binding site do not appear to be the only important regulatory regions. Although the L1 box is essential for the expression of *PDF1*, this is not the case for *ATML1* (2, 143). Rather, the L1 box controls expression redundantly with the WUS binding site in the *ATML1* promoter, but even when both elements are deleted, a hexameric copy of an *ATML1* promoter fragment still confers weak expression (143). In addition, *ATML1* is still expressed in the *atml1 pdf2* and *wox8 wox9* double-mutant backgrounds (10, 143). Thus, although these two "pathways" might converge on *ATML1* expression, other factors are probably involved in the regulation of this gene. Because the *ATML1* promoter

confers expression in the suspensor but the messenger RNA (mRNA) is detected there only in the *dicer-like 1* (*dcl1*) mutant, a microRNA might regulate the *ATML1* expression in the suspensor (111, 143) (**Figure 3*c***).

The inner cells of the *A. thaliana* embryo give rise to the various concentric tissue layers that have been described in the root and are laid down during embryogenesis (126, 127). The GRAS transcription factor SHR is one of the best-described players involved in radial patterning. It is expressed in the provasculature and moves out to the neighboring cell layer, where it activates the transcription of another GRAS transcription factor gene, *SCR* (46, 106). *SCR* is expressed in the ground tissue and the hypophysis at the globular stage of embryogenesis. When the cells of the ground tissue of the hypocotyl and the embryonic root pole divide periclinally between the triangular stage and the heart stage to generate the inner layer of endodermis and the outer layer of cortex cells, *SCR* continues to be expressed in the inner layer (164) (**Figure 3*b***). These periclinal cell divisions depend on both *SHR* and *SCR* (46, 164). SHR and SCR activate microRNA165/166 in the endodermis of the mature root, from where the microRNAs feed back onto the vasculature to control its patterning. Because the two microRNAs are already expressed during embryogenesis, they might contribute to embryonic patterning as well (14).

Maintenance of Radial Patterning

RECEPTOR-LIKE PROTEIN KINASE 1 (RPK1) and TOADSTOOL 2 (TOAD2), two closely related leucine-rich-repeat receptor-like kinases (LRR-RLKs), are redundantly required for the maintenance of radial patterning (112) (**Figure 3*c***). The protoderm marker *ATML1* as well as the central domain markers *ZLL/AGO10* and *SHR* are correctly expressed only initially in *rpk1 toad2* embryos, which have enlarged protoderm cells (112). At the late-globular stage of embryogenesis, the expression of *ATML1* is (almost) lost, and the expression of *ZLL/AGO10* and *SHR* extends over the entire basal domain in *rpk1 toad2*, suggesting that *RPK1* and *TOAD2* play an essential role in the maintenance but not the establishment of the radial pattern in *A. thaliana* (112).

The ligands binding to RPK1 and TOAD2 during embryogenesis are unknown, although it was recently suggested that the signaling peptide derived from CLAVATA 3 (CLV3) binds to TOAD2 (71). Because this signaling peptide is functionally similar to other signaling peptides of the CLV3/ESR-RELATED (CLE) family (109), any of these might be the endogenous ligand for RPK1 and TOAD2 (**Figure 3*c***). Hence, at least some of these signaling peptides might play a role during early embryogenesis, an assumption that receives support from the analysis of the RLK ARABIDOPSIS CRINKLY 4 (ACR4). ACR4 might bind the signaling peptide CLE40, which is the closest homolog of CLV3, and is involved in protoderm specification, where it acts together with ABNORMAL LEAF-SHAPE 2 (ALE2), another RLK (138, 145) (**Figure 3*c***). Although neither the single mutants nor the double mutant appear to show severe protodermal defects during embryo development, in mutant combinations with *ale1* the protoderm is misspecified (36, 145). Accordingly, *ale1 ale2* and *ale1 acr4* double mutants do not properly express *ATML1* (145). *ALE1* encodes a protease that is predominantly expressed in the endosperm, and thus ALE2 and ACR4 might perceive a signal from the endosperm to ensure proper protoderm specification (144, 145) (**Figure 3*c***). However, toxin-dependent endosperm ablation rather suggests that the endosperm is not involved in embryo patterning, and the feasibility of somatic embryogenesis also argues against essential peptide signals from the endosperm (158; reviewed in 168). In addition to its expression in the endosperm, *ALE1* is weakly expressed in the early embryo itself (144), and this might be relevant for embryogenesis.

Protoderm formation and *ATML1* expression are prevented in *arabidopsis thaliana defective kernel 1* (*atdek1*) mutant embryos, which arrest at the globular stage (60, 81, 150).

ATDEK1 encodes a calpain protease that undergoes autolytic cleavage (**Figure 3*c***) and is expressed in the embryo (60, 61, 81). In *ATDEK1* knockdown lines, seedlings show a transformation of epidermal to mesophyll-like cell fate in the cotyledons, similar to what has been observed in *atml1 pdf2* double mutants (1, 60). In conclusion, although a number of key players have been analyzed, the overall genetic program of setting up the radial pattern or only the protoderm is still largely unexplored.

SHOOT MERISTEM SPECIFICATION AND COTYLEDON INITIATION

The Organizing Center

The *A. thaliana* shoot meristem can be morphologically delineated for the first time during embryogenesis at the late-torpedo stage (6, 78). In the mature embryo, the shoot meristem consists of a few small cells with big nuclei and small vacuoles, and its first molecular mark is the onset of *WUS* expression in the four inner cells of the apical embryo region at the dermatogen stage (78, 97) (**Figure 2*a***). *WUS* encodes a homeodomain transcription factor, and its expression remains confined to a subset of cells close to the shoot apex during later stages of development (**Figure 4*a***), defining an organizing center that keeps the neighboring stem cells in a pluripotent state (97). The *wus* mutation results in the lack of a functional shoot meristem and the formation of a flat and enlarged shoot apex consisting of aberrant cells (78). *WUS* orthologs seem to play similar roles in dicots like *Petunia hybrida* and *Antirrhinum majus*, but possibly not in monocots like *O. sativa* and *Z. mays* (70, 107, 140).

Despite considerable efforts to identify regulators and downstream targets of this master regulator (11; reviewed in 24), our knowledge is scant about the mechanism(s) of initiation and early confinement of *WUS* expression and about the identity of the *WUS*-dependent non-cell-autonomous signal(s) maintaining stem cell fate in the shoot meristem. In postembryonic development, however, cytokinin signaling activates *WUS* expression (37). Because WUS inhibits the expression of several type-A *ARR*s that are negative regulators of cytokinin signaling, a positive feedback mechanism involving *WUS* and cytokinin signaling might thus operate in the shoot meristem to maintain

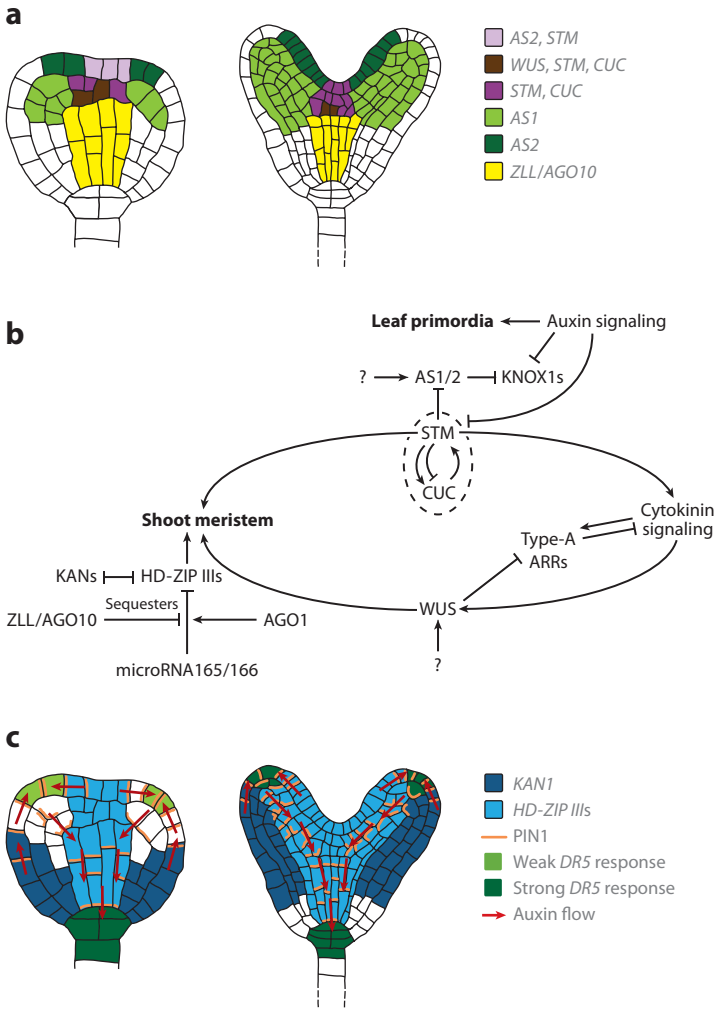

Figure 4

Shoot meristem and cotyledon initiation in *Arabidopsis thaliana*. (*a*) Expression patterns of genes important for establishment of the shoot meristem and initiation of cotyledons in *A. thaliana* during the transition stage and the heart stage. *CUC1–3* expression is generalized as *CUC*. (*b*) Pathways and hormonal regulation in shoot meristem and cotyledon initiation. (*c*) Expression patterns of *KAN1* and *HD-ZIP III* genes (exemplarily shown for *REV*, which includes domains of all other members), auxin flow mediated by PIN1 (idealized representation), and *DR5* response. Embryos not drawn to scale.

its integrity (37, 79) (**Figure 4*b***). This crosstalk may already operate during embryogenesis. In *O. sativa*, the *LONELY GUY* (*LOG*) gene, which encodes a cytokinin-activating enzyme and is specifically expressed in the shoot meristem region, is important for shoot meristem maintenance (75).

Shoot Meristem Indeterminacy and the Shoot Meristem–Cotyledon Boundary Region

The class I KNOTTED-like homeodomain transcription factor SHOOT MERISTEMLESS (STM) might indirectly activate *WUS* expression via its induction of cytokinin biosynthesis and signaling (37, 57, 85, 166) (**Figure 4*b***), and in addition to its cytokinin-related effects, it restricts gibberellic acid levels (45, 57). Similar to its *Z. mays* ortholog *KNOTTED 1* (*KN1*) and its *O. sativa* ortholog *Oryza sativa homeobox 1* (*OSH1*), *STM* is expressed in the presumptive shoot meristem from the globular stage onward (85, 124, 134) (**Figure 4*a***); in addition, in the oil palm *Elaeis guineensis* an *STM* ortholog is expressed in the shoot meristem, at least during vegetative development (64). Together with WUS, STM is required to maintain the shoot meristem: WUS acts as the instructor of the organizing center, and STM acts as a repressor of differentiation across the entire shoot meristem (80). In differentiated tissue, simultaneous expression of WUS and STM can induce meristematic activity, with WUS non-cell-autonomously triggering divisions in *STM*-expressing tissue (35).

Being a transcription factor, STM functions in the nucleus, and this localization depends on BEL1-like homeodomain transcription factors (22, 121). Shoot meristem initiation is consistently inhibited in the *stm* mutant and the *arabidopsis thaliana homeobox 1* (*ath1*) *pennywise* (*pny*) *pound-foolish* (*pnf*) triple mutant, and also in the *cup-shaped cotyledon 1* (*cuc1*) *cuc2* double mutant, which fails to express *STM* in the presumptive shoot meristem (4, 6, 121). The NAC transcription factors CUC1–3 are redundantly required for shoot meristem establishment as well as cotyledon separation. At early embryonic stages, their expression domains partially overlap with the *STM* expression domain (**Figure 4*a***), whereas *CUC1–3* expression domains in general surround the *STM* expression domain at later stages (4, 47, 142, 152). How this expression pattern evolves is not clear. However, there appears to be mutual regulation involving positive and negative feedback loops (**Figure 4*b***): Not only are the CUCs required for *STM* expression, but STM regulates the expression of *CUC1–3* and the expression of microRNA164, which in turn targets *CUC1* and *CUC2* transcripts for degradation (4, 77, 92, 137). The *P. hybrida* and *A. majus CUC* orthologs *NO APICAL MERISTEM* (*NAM*) and *CUPULIFORMIS* (*CUP*) are also expressed at organ boundaries, and they are important for both boundary establishment and shoot meristem development (136, 159). In *Z. mays*, the putative *CUC1/2* orthologs *Zea mays NO APICAL MERISTEM 1/2* (*ZmNAM1/2*) and the *CUC3* ortholog *Zea mays CUP-SHAPED COTYLEDON 3* (*ZmCUC3*) are in part initially coexpressed with a shoot meristem marker, and later in a ringlike pattern around the shoot meristem (173), hinting at a strong conservation of *CUC* gene function at least among flowering plants.

Meristem Establishment

A general prerequisite for shoot meristem identity seems to be the presence of class III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) transcription factors. This family consists of PHABULOSA (PHB), PHAVOLUTA (PHV), REVOLUTA (REV), ARABIDOPSIS THALIANA HOMEOBOX 8 (ATHB8), and ATHB15. Expression of all but *ATHB8* is already detectable from early embryonic stages onward, and in part there is overlap with the future site of the shoot meristem, whereas especially *PHB*, *REV*, and *ATHB15* expression domains partially coincide with the *ZLL/AGO10* provascular expression domain as well; ATHB8 mRNA is detectable from the heart stage onward (26, 91, 100, 116). Conversely, expression domains of members of the *KANADI* (*KAN*)

gene family could be regarded as complementary to those of the *HD-ZIP III*s, which they are supposed to antagonize (26–28, 69) (**Figure 4*c***). The *phb rev* double, *phb phv rev* triple, and other loss-of-function mutant combinations involving *athb8* and *athb15* lack the embryonic shoot meristem and in severe cases fail to establish bilateral symmetry (26, 116). The dominant mutation *phb-1d* leads to ectopic meristems that express the shoot meristem marker *STM* on the lower side of leaves, and also causes an enlarged embryonic shoot meristem and partially suppresses the *stm* mutant phenotype (99). Two recent findings further support a pivotal role for HD-ZIP III transcription factors in shoot meristem formation. First, exclusion of HD-ZIP III proteins from the embryonic root pole is necessary for its proper establishment (38). Second, dominant *HD-ZIP III* mutants suppress the *tpl-1* double-root phenotype, possibly by excluding PLT1 and PLT2 from the future shoot meristem cells. Conversely, misexpression of dominant *HD-ZIP III*s can lead to (homeotic) root-pole-to-shoot-pole transformations during embryogenesis (135). It is not clear at present whether the HD-ZIP IIIs directly regulate *STM* and/or *WUS* in ectopic shoot meristem formation.

HD-ZIP III transcripts are targeted by microRNA165/166, and the dominant *HD-ZIP III* mutations reside in the microRNA pairing sites, rendering the HD-ZIP mRNAs resistant to degradation (93, 119, 146, 160, 171). The microRNA-dependent degradation involves the AGO proteins AGO1 and ZLL/AGO10, which both bind microRNA165/166 (172). It was suggested that ZLL/AGO10 and AGO1 act in an antagonistic fashion (**Figure 4*b***), with ZLL/AGO10 positively regulating HD-ZIP III transcript levels through competition with AGO1—possibly by sequestering microRNA165/166. Such a sequestration could ensure sufficiently high HD-ZIP III levels during shoot meristem establishment and maintenance (172). Given that *ZLL/AGO10* expression in the provasculature is necessary for embryonic shoot meristem maintenance, a non-cell-autonomous signal could, in principle, instruct the shoot meristem from the cells underneath (149). In this scenario, the two primary meristems of shoot and root would be initiated as WUS- and WOX5-positive cell groups, respectively, in response to inductive signals, at the opposite ends of the provasculature in early embryogenesis.

Initiation of Cotyledon Primordia

When the cotyledon primordia start to emerge in *A. thaliana*, the embryo organization shifts from radial to bilateral symmetry. The sites of cotyledon initiation correlate with auxin accumulation at subapical foci in the protoderm, as indicated by the auxin response reporter *DR5* (8) (**Figure 4*c***). Auxin might therefore directly cause cotyledon initiation in the apical margins of the globular embryo (8). In addition, *STM* and *CUC* expression have to be excluded from those sites (see below). Auxin transport toward the incipient primordia is mediated by PIN auxin efflux regulators, probably mainly by PIN1 (8) (**Figure 4*c***). PIN1 is apically localized in the protoderm, and the apical localization of PIN proteins is generally brought about by PID and its homologs PID2, WAG1, and WAG2, three of which have been shown to directly phosphorylate PINs (20, 23, 31, 49, 102). For example, the *pid wag1 wag2* triple mutant and the *pin1 pid* double mutant lack cotyledons (20, 33), as does the *pid enhancer of pinoid* (*enp*) double mutant (148). *ENP/MACCHI-BOU 4* (*MAB4*) encodes an NPH3-like protein that is involved in the regulation of PIN1 localization (32, 148). It is noteworthy that in both double mutants (*pin1 pid* and *pid enp*) the expression domains of *CUC* genes and *STM* are enlarged, and that cotyledon formation is partially restored when *CUC* genes or *STM* are knocked out in *pin1 pid* (33, 148); this highlights both the importance of directional auxin transport to the cotyledon initiation sites and the requirement to exclude specific transcripts/proteins from there. This view is supported by cotyledon formation defects in the auxin response mutants *mp* and *bdl* (9, 42). However, it might also be relevant in this context that MP directly activates the

expression of *DRN*—especially because *DRN* and *DRNL* redundantly act in cotyledon formation (16, 21). Additionally, DRN and DRNL are involved in the establishment and maintenance of boundary and shoot meristem gene expression domains, and they act together with PIN1 and PID (16, 18, 72). Auxin-related processes might be involved in cotyledon initiation in other flowering plant species as well, including monocots, but this has barely been investigated so far (reviewed in 15).

Another factor involved in cotyledon development, *ASYMMETRIC LEAVES 1* (*AS1*), which encodes a MYB domain protein and orthologs of which are present in *Z. mays* and *A. majus*, is initially expressed mainly subepidermally in the incipient cotyledon primordia, whereas *AS2*, which encodes a LATERAL ORGAN BOUNDARY (LOB) domain protein, is expressed protodermally before cotyledon outgrowth and later at the adaxial cotyledon side (12, 55, 84, 129, 153) (**Figure 4*a***). The loss of *AS1* or *AS2* makes *STM* dispensable for shoot meristem initiation and maintenance, suggesting that STM negatively regulates *AS1* and *AS2* (12, 13). Studies in primarily adult leaves suggest that *KNOX* genes are negatively regulated by AS1/2 and that AS1/2 possibly converge with auxin signaling to repress the *KNOX* member *KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1* (*KNAT1*)/*BREVIPEDICELLUS* (*BP*) (12, 39, 44) (**Figure 4*b***). The expression of *AS2* itself is negatively regulated by KAN1 and positively by BLADE-ON-PETIOLE 1/2 (BOP1/2), the expression of the latter in turn being directly or indirectly repressed by *STM* (65, 161). How exactly *AS1* and *AS2* are linked to auxin, however, has not been resolved.

PERSPECTIVES

Considerable progress has been made in the analysis of mechanisms underlying specific events in early embryogenesis, notably in *A. thaliana*. For example, we now have a clear conceptual framework for the initiation of the root meristem in the early embryo. However, although the main regulators have been identified and characterized, it is still rather obscure how these early events relate to the establishment of the molecular system for self-maintenance of the functional root meristem at the heart stage of embryogenesis. The initiation and establishment of the self-maintenance system are even less clear for the shoot meristem. Large-scale approaches combining expression profiling of specific embryo regions with functional characterization of putative developmental regulators might contribute to closing the gap.

Another unsolved problem is the origin of the apical-basal pattern. Although genes encoding developmental regulators are expressed in either the apical or the basal daughter cell of the zygote, it is not known how the expression of these regulators is ultimately established. This also relates to the mode of division of the zygote: Is it truly unequal, reflecting an intrinsic polarity of the zygote before division? Alternatively, the division might be equal, and only the two daughter cells would be exposed to different environments and thus might perceive different signals.

The contribution of the gametes to early embryogenesis still needs to be assessed. Although differentially regulated genes have been identified, their role in early patterning has not been clarified. And the significance of epigenetic regulation of patterning is still an open question.

Finally, most studies have focused on a few species, notably *A. thaliana*. Considering the differences in cell-division patterns between early embryos from different species, exploring orthologous developmental regulators might reveal to what extent their actions and regulatory networks are conserved among the flowering plant species when the cellular contexts of developmental events are not.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Martin Bayer for critical reading of the manuscript.

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Annual Review of Plant Biology
Volume 63, 2012

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