Cell, Vol. 81, 467–470, May 19, 1995, Copyright © 1995 by Cell Press

Axis Formation in Plant Embryogenesis: Cues and Clues

Minireview

Gerd Jürgens
Lehrstuhl für Entwicklungsgenetik
Universität Tübingen
D-72076 Tübingen
Federal Republic of Germany

Imagine a textbook entitled *Developmental Biology* that focuses entirely on plants, mentioning animals only for their peculiar way of making germ cells by setting aside a group of precursor cells early in the embryo. The converse has been, and still is, common practice. It is true that the regenerative potential of plants, which is indeed impressive, sets them apart from the more familiar animal models: individual cells can give rise to embryos in culture; localized groups of stem cells called meristems make the adult plant in a seemingly autonomous fashion not only during normal development, but also by regeneration from lumps of undifferentiated cells in culture. These special features notwithstanding, plants do develop from a fertilized egg cell, the zygote, during the normal course of their life cycle and, like animals, have to establish the characteristic body organization of the multicellular adult form. Are the underlying mechanisms the same or different? In the past few years, the genetic and molecular analysis of flower development in two plant model species, Arabidopsis and Antirrhinum (snapdragon), has established a network of MADS domain transcription factors and others that regulate this best-characterized process in plant development (Weigel and Meyerowitz, 1994). However, flower development is like putting the finishing touches on the adult plant and may thus not give clues to mechanisms that underlie earlier processes such as axis formation and the generation of the overall body organization. By drawing largely on recent genetic studies in Arabidopsis, I will briefly discuss postembryonic development that eventually culminates in the formation of flowers, but mainly focus on pattern formation in the embryo that establishes the basic body organization of flowering plants.

The Shoot Meristem: Linking Up the Embryo with the Flower

Pattern formation in animals is largely confined to embryogenesis such that the future adult form is represented in the body organization of the mature embryo. By contrast, plant embryogenesis produces a juvenile form, the seedling, that lacks most structures of the adult plant. Embryogenesis in essence organizes two groups of stem cells at the opposite ends of the body axis, the primary meristems of the shoot and the root. These meristems then add new structures to the seedling, thus generating the species-specific adult form during postembryonic development (Steeves and Sussex, 1989). Regardless of the appearance of the adult plant, the shoot meristem is organized essentially the same way in different plant species. Two functional units can be distinguished within the meristem: a central zone, which is required for self-renewal and integrity of the meristem, and a peripheral zone, which makes primordia of lateral organs and their associated secondary shoot meristems, such as leaves and flowers (Steeves and Sussex, 1989). Whether leaves or flowers are produced depends on the physiological state of the meristem. In *embryonic flower* (*emf*) mutant seedlings, for example, the primary shoot meristem skips the vegetative phase of making leaves altogether, producing flowers directly (Sung et al., 1992). Shoot meristem identity seems to be conferred by the continuous expression of genes like the maize homeobox gene *Knotted1* (*Kn1*), whose ectopic expression can cause the formation of shoot meristems on leaves (Sinha et al., 1993; Jackson et al., 1994). A putative Arabidopsis homolog of *Kn1*, the *SHOOT MERISTEM-LESS* (*STM*) gene, is required for shoot meristem formation both in the embryo and during regeneration from tissue culture (Barton and Poethig, 1993). How the activity of such shoot meristem-specific genes is established and

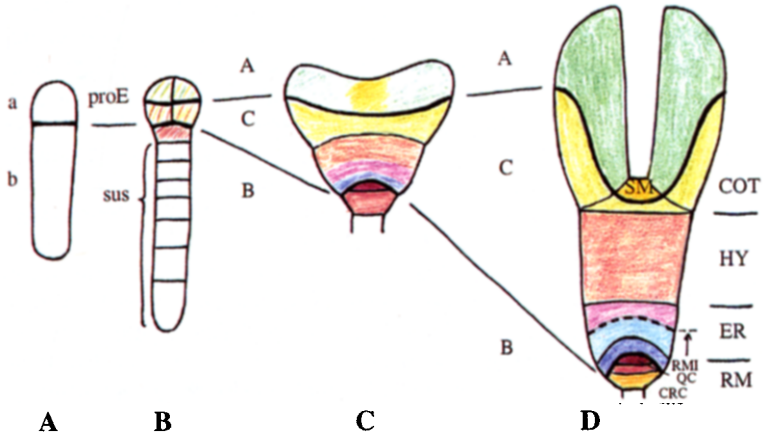

Figure 1. Formation of the Apical–Basal Axis in the Arabidopsis Embryo

(A) Asymmetric division of the zygote, giving a small apical (a) and a large basal (b) cell.

(B) The 8-cell stage. The proembryo (proE) consists of two tiers each of four cells (regions marked A for apical and and C for central) and is connected to the extraembryonic suspensor (sus) via the founder cell of the basal region (B) of the embryo.

(C) Embryo at heart stage. Approximate locations of cell groups that give rise to the primordia of seedling structures are indicated.

(D) Embryo at Torpedo stage. Clonal boundaries are marked by thick lines. The broken line indicates the upper end of the embryonic root derived from the root meristem initials (RMI). Below the quiescent center (QC) of the root meristem are the initials of the central root cap (CRC). Primordia of seedling structures: COT, cotyledons; HY, hypocotyl; ER, embryonic root; RM, root meristem; SM, shoot meristem.

maintained properly is not known. However, there are candidate genes in Arabidopsis for performing these functions. For example, the *ZWILLE* (*ZLL*) gene is specifically involved in establishing the primary shoot meristem in the embryo but not in any other (nonembryogenic) context (Jürgens et al., 1994). Mutations in another gene, *CLAVATA1* (*CLV1*), cause overgrowth of primary and secondary shoot meristems that also results in the formation of one or more supernumerary whorls in the center of the flower (Clark et al., 1993). This phenotype suggests that the organization of the meristem is altered, owing to an enlargement of the central zone required for self-renewal. Although more genes need to be identified to clarify this point, it is conceivable that the primary shoot meristem may acquire a specific organization in the embryo that is subsequently maintained by cellular interactions within the meristem.

Pattern Formation in the Arabidopsis Embryo

The primary meristems of the shoot and the root originate at distinct positions in the embryo as part of the overall body organization of the seedling (Barton and Poethig, 1993; Dolan et al., 1993). The latter may be viewed as the superimposition of an apical–basal pattern along the main body axis and a radial pattern perpendicular to this axis. The apical–basal pattern consists of a top-to-bottom array of elements: shoot meristem, cotyledons (embryonic leaves), hypocotyl (embryonic stem), radicle (embryonic root), and root meristem. The radial pattern is made up of concentric rings of tissue layers: epidermis, ground tissue (cortex and endodermis), and vascular tissue (pericycle, xylem, and phloem). The origins of the apical–basal and radial pattern elements have been traced back to cell groups of the early embryo in Arabidopsis where the very regular patterns of cell division facilitated these analyses (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994).

Formation of the Apical-Basal Axis

Apical–basal pattern formation starts with the asymmetric division of the zygote that gives two daughter cells of unequal sizes and different fates (Figure 1). The small apical cell generates, by cleavage divisions, an 8-celled proembryo that will give rise to most of the embryo. The large basal cell produces a file of 7–9 cells, of which all but the uppermost one will form the extraembryonic suspensor; the uppermost cell (hypophysis) joins the proembryo later to give rise to part of the root meristem. Within 1 day of fertilization, three embryonic regions are thus established along the axis: apical and central, which correspond to the upper and lower tiers within the proembryo, and basal, which is represented by the adjacent hypophysis (Figures 1A and 1B). The three embryonic regions differ in their cell division patterns: apical cells divide without preferential orientation; central cells produce cell files, thus expanding the axis; and the founder cell of the basal region undergoes a stereotyped program of division. However, the early regions (apical, central, and basal) do not correspond to primordia of seedling structures (Figures 1C and 1D). The apical region gives rise to the shoot meristem and most of the cotyledons; the central region also contributes to the cotyledons, but mainly produces hypocotyl, root, and root meristem initials; and the basal region gives the remainder of the root meristem, comprising the quiescent center and the initials of the central root cap. Data from clonal analysis support the view that cell ancestry does not play a role in generating apical–basal pattern elements: clone boundaries are variable and, moreover, can run across specific seedling structures, such as the cotyledons or the root meristem (Scheres et al., 1994; Dolan et al., 1994). Furthermore, the regularity of cell divisions in the early embryo is deceptive since mutations in the *FASS* (*FS*) gene totally alter the pattern of cell division, without affecting pattern formation (Torres Ruiz and Jürgens, 1994). Thus, the apical–basal pattern elements appear to be established by cellular interactions in a position-dependent manner.

What is the significance of the early regions along the apical–basal axis? Evidence comes from the embryonic phenotypes of mutations in three genes, *MONOPTEROS* (*MP*), *FACKEL* (*FK*), and *GURKE* (*GK*), which delete specific seedling structures (Mayer et al., 1991). In *mp* embryos, the cells in the central and basal regions divide abnormally, resulting in the absence of hypocotyl, root, and root meristem (Berleth and Jürgens, 1993). In *fk* embryos, the central region is affected while the basal region is not; in the seedling, the hypocotyl is missing such that the cotyledons are directly attached to the root (Mayer et al., 1991; Jürgens et al., 1994). Since the root meristem is composed of cells from both the basal and the central regions, the basal region appears to induce the adjacent cells of the central region to become root meristem initials that in turn produce the meristem-derived embryonic root (see Figures 1C and 1D). A similar argument can be made for the cotyledons, which are derived from both the apical and the central regions. The phenotype of *gk* mutants is essentially complementary to *mp*: the apical region of the embryo is altered, and, later on, *gk* seedlings lack shoot meristem and cotyledons (Mayer et al., 1991). This suggests that the cotyledons are initiated within the apical region, which then signals to adjacent cells of the central region to participate in cotyledon formation. Thus, the early regions may define genetically distinct groups of cells (compartments?) that generate the apical–basal pattern by cellular interactions.

How are the early regions established along the apical–basal axis? The boundary separating the central from the basal region originates with the first division of the zygote (Figures 1A and 1B). The basal daughter cell produces a file of cells of which the uppermost one becomes the founder cell of the basal region of the embryo. This file of cells is initially normal in *mp* embryos when the earliest defect becomes apparent in the proembryo, whereas later on both the central and the basal regions develop abnormally (Berleth and Jürgens, 1993). Thus, in normal development, the central region may induce the uppermost derivative of the basal daughter cell of the zygote to become the founder cell of the basal region. By contrast, the apical and the central regions are established by the transverse cell divisions within the proembryo that is derived from the apical daughter cell of the zygote.

Before dividing asymmetrically, the zygote elongates about 3-fold in the future axis, and concomitantly the corti-

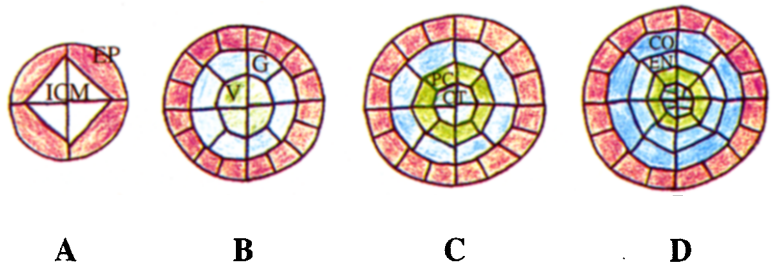

Figure 2. Formation of the Radial Pattern in the Arabidopsis Embryo

Schematic cross sections through the central region (see Figure 1) of embryo (A and B) and through the root primordium (C and D).

(A) Dermatogen stage. Periclinal (radial) divisions within the proembryo (Figure 1B) give the outer epidermis layer (EP) and an inner cell mass (ICM).

(B) Globular embryo. The inner cell mass has split into the ground tissue (G) and the centrally located vasculated primordium (V).

(C) Embryo at heart stage (Figure 1C). The pericycle layer (PC) surrounds the primordium of the conductive tissue (CT). The basic organization of the radial pattern is complete.

(D) Embryo at Torpedo stage (Figure 1D). Periclinal divisions in the ground tissue generate an outer cortex (CO) and an inner endodermis layer (EN).

cal microtubules, which were previously oriented at random, become aligned perpendicular to the axis (Webb and Gunning, 1991). This reorganization may reflect polarization of the zygote in response to some as yet unknown signal(s). Its significance for axis formation is suggested by mutations in the *GNOM* (*GN*) gene that affect the zygote and can completely abolish regional differentiation of the seedling axis (Mayer et al., 1991). The *gn* zygote expands but does not elongate, producing an enlarged apical cell at the expense of the basal cell (Mayer et al., 1993). Subsequent development is highly abnormal, and the region-specific *MP* gene was shown to be ineffective in *gn* embryos. The asymmetric division of the zygote seems to be involved in fixing the apical–basal axis of the embryo, but it is not known how the *GN* gene brings about its specific effect at the molecular level. Although the predicted 160 kDa GN (also called EMB30) protein shows sequence similarity to several other proteins over a stretch of 200 amino acids that is referred to as the Sec7 domain, the significance of this domain is not known, nor does the remainder of the GN protein readily suggest a specific function within the cell (Shevell et al., 1994). Since *GN* is the only Arabidopsis gene known to be specifically required for apical–basal pattern formation from the zygote on, determining the primary function of the GN protein will be an important step in further analysis of axis formation.

Radial Pattern Formation

Radial pattern formation, which commences in the 8-celled proembryo, involves two choices of oriented cell division: periclinal (radial), which gives a new tissue layer, and anticlinal (circumferential), which increases the number of cells in a given layer. New tissue layers are successively formed from the periphery toward the center (Figure 2). Initially, an outer layer of epidermal precursor cells is separated from an inner cell mass. The epidermis layer then expands by anticlinal cell divisions, thus maintaining its integrity in the growing embryo. Further development of the radial pattern is confined to the central region of the axis that gives rise to hypocotyl, root, and root meristem initials; neither the apical nor the basal region is involved (Jürgens and Mayer, 1994; Scheres et al., 1995). The inner cell mass splits into an outer layer of ground tissue and the centrally located vascular precursor cells. The latter cells again divide periclinally to give a layer of pericycle cells surrounding the precursors of conductive tissue (phloem and xylem). This basic organization of the radial pattern is complete before the heart stage of embryogenesis (Figure 2C). One further subdivision occurs within the ground tissue, generating an outer cortex layer and an inner endodermis layer (Figure 2D).

By the time the root meristem has become active, clonal boundaries reflect the basic organization of the radial pattern (Dolan et al., 1994; Scheres et al., 1994). Are tissue-specific cell fates fixed early and then transmitted clonally? Mutant phenotypes suggest that this may be the case. For example, the epidermis cells are abnormally enlarged in early *keule* (*keu*) embryos (Mayer et al., 1991), and the endodermis layer is absent in *short root* (*shr*) embryos (Benfey et al., 1993; Scheres et al., 1995). Furthermore, the epidermis is marked by the tissue-specific expression of the *LTP* gene both in the embryo and during postembryonic development (Thoma et al., 1994). The genetic distinction of different tissues might involve blocking of plasmodesmata-mediated cell communication as has been observed between the epidermis and subepidermal tissues in the seedling (Duckett et al., 1994). While the available evidence seems to favor a model of genetically determined tissue types, oriented cell divisions do not appear to be required for radial pattern formation: cell divisions occur at random in early *fs* embryos such that the characteristic radial organization of tissues is not apparent, but later on the mutant seedlings display all tissues found in wild type (Torres Ruiz and Jürgens, 1994). This flexibility suggests that cellular interactions may play an important role in initiating and (possibly) maintaining tissue-specific gene expression in a position-dependent manner.

How is the radial pattern initiated in the early embryo? So far, only one gene has been identified that may be involved in this process: in *knolle* (*kn*) embryos, the epidermis is not separated from the inner cell mass by periclinal divisions within the proembryo, and the mutant seedlings appear to lack the characteristic epidermis layer (Mayer et al., 1991). How the *KN* gene relates, at the molecular level, to the periclinal divisions in the early embryo remains to be determined.

What Mechanisms May Underlie Pattern Formation in the Plant Embryo?

Pattern formation in the Arabidopsis embryo appears to depend largely on cell–cell communication, both in the apical–basal axis and in the radial dimension. This is for-

mally similar to pattern formation in the familiar animal models (e.g., vulva development in Caenorhabditis elegans or postblastoderm embryogenesis and imaginal disc development in Drosophila). However, the underlying mechanisms might be different in plants. For example, plant cells are cytoplasmically interconnected by plasmodesmata, allowing various kinds of molecules to pass freely from cell to cell. Unfortunately, it is not known whether this potential plant-specific means of intercellular communication is actually used and how it is regulated during development. Another serious problem in the analysis of plant pattern formation is the shortage of markers, both morphological (which cannot be changed) and molecular. As more genes with tissue-specific or region-specific expression patterns in the embryo will become available, some of the ideas presented here can be tested.

The initial events of pattern formation in the Arabidopsis embryo are different from the later phases since they cannot involve cell–cell communication: the asymmetric division of the zygote as well as the periclinal divisions in the 8-celled proembryo appear to segregate fates within cells. How is position translated into cell fate in these cases? One possible mechanism is suggested by the observation that in C. elegans, the unequal division of the zygote is associated with the differential degradation of a specific uniformly distributed maternal mRNA, thus establishing territories of differential gene expression along the main body axis of the early embryo (Evans et al., 1994). A potentially plant-specific alternative for embryonic axis fixation is suggested by recent results from the brown alga, Fucus. The Fucus zygote also divides asymmetrically, giving an upper thallus cell and a lower rhizoid cell. If the cell wall of the zygote is removed, the axis can be formed, but not fixed (Quatrano et al., 1991). Moreover, cell ablation studies on early embryos suggest that the different cell fates of thallus versus rhizoid may be imprinted into the cell wall (Berger et al., 1994). Which mode, if either, of cell-fate segregation applies in the early embryo of Arabidopsis remains to be determined. The two proteins thus far identified do not appear to regulate gene expression directly, unlike the transcription factors that play decisive roles in flower development. Does axis formation in higher-plant embryos involve an extracellular detour on its way to the nucleus?

References

Barton, M. K., and Poethig, R. S. (1993). Development *119*, 823–831.

Benfey, P. N., Linstead, P. J., Roberts, K., Schiefelbein, J. W., Hauser, M.-T., and Aeschbacher, R. A. (1993). Development *119*, 57–70.

Berger, F., Taylor, A., and Brownlee, C. (1994). Science *263*, 1421–1423.

Berleth, T., and Jürgens, G. (1993). Development *118*, 575–587.

Clark, S. E., Running, M. P., and Meyerowitz, E. M. (1993). Development *119*, 397–418.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., and Scheres, B. (1993). Development *119*, 71–84.

Dolan, L., Duckett, C. M., Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, S., and Roberts, K. (1994). Development *120*, 2465–2474.

Duckett, C. M., Oparka, K. J., Prior, D. A. M., Dolan, L., and Roberts, K. (1994). Development *120*, 3247–3255.

Evans, T. C., Crittenden, S. L., Kodoyianni, V., and Kimble, J. (1994). Cell *77*, 183–194.

Jackson, D., Veit, B., and Hake, S. (1994). Development *120*, 405–413.

Jürgens, G., and Mayer, U. (1994). In Embryos: Colour Atlas of Development, J. B. L. Bard, ed. (London: Wolfe Publishing), pp. 7–21.

Jürgens, G., Torres Ruiz, R. A., Laux, T., Mayer, U., and Berleth, T. (1994). In Plant Molecular Biology: Molecular–Genetic Analysis of Plant Development and Metabolism, G. Coruzzi and P. Puigdomènech, eds.(Berlin: Springer-Verlag), pp. 95–103.

Mansfield, S. G., and Briarty, L. G. (1991). Can. J. Botan. *69*, 461–476.

Mayer, U., Torres Ruiz, R. A., Berleth, T., Miséra, S., and Jürgens, G. (1991). Nature *353*, 402–407.

Mayer, U., Büttner, G., and Jürgens, G. (1993). Development *117*, 149–162.

Quatrano, R. S., Brian, L., Aldridge, J., and Schultz, T. (1991). Development *113* (Suppl. 1), 11–16.

Scheres, B., Wolkenfelt, H., Willemsen, V., Terlouw, M., Lawson, E., Dean, C., and Weisbeek, P. (1994). Development *120*, 2475–2487.

Scheres, B., Di Laurenzio, L., Willemsen, V., Hauser, M.-T., Jaanmat, K., Weisbeek, P., and Benfey, P. N. (1995). Development *121*, 53–62.

Shevell, D. E., Leu, W.-M., Gilimor, C. S., Xia, G., Feldmann, K. A., and Chua, N.-H. (1994). Cell *77*, 1051–1062.

Sinha, N., Williams, R., and Hake, S. (1993). Genes Dev. *7*, 787–795.

Steeves, T. A., and Sussex, I. M. (1989). Patterns in Plant Development (Cambridge: Cambridge University Press).

Sung, Z. R., Belachew, A., Shunong, B., and Bertrand-Garcia, R. (1992). Science *258*, 1645–1647.

Thoma, S., Hecht, U., Kippers, A., Botella, J., de Vries, S. C., and Somerville, C. (1994). Plant Physiol. *105*, 35–45.

Torres Ruiz, R. A., and Jürgens, G. (1994). Development *120*, 2967–2978.

Webb, M. C., and Gunning, B. E. S. (1991). Planta *184*, 187–195.

Weigel, D., and Meyerowitz, E. M. (1994). Cell *78*, 203–209.