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Embryogenesis in Higher Plants: An Overview

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INTRODUCTION

The sporophytic generation of higher plants is initiated with the double fertilization event that results in the formation of a single-celled zygote and a progenitor cell of the endosperm. Embryogenesis describes the subsequent period of development, during which the zygote undergoes a complex series of morphological and cellular changes resulting in the formation of a developmentally arrested mature embryo comprised of an embryonic axis with shoot and root poles and cotyledon(s), which often contains high levels of storage macromolecules such as proteins and lipids. Thus, the events that occur during embryonic development establish the organization of the plant body and prepare the embryo for both dormancy and germination.

Higher plant embryogenesis has been studied intensively during the past century. Studies using light and electron microscopy have provided detailed descriptions of the morphological and anatomical changes that characterize embryonic development (Maheshwari, 1950; Wardlaw, 1955; Natesh and Rau, 1984; Raghavan, 1986). Cellular differentiation has been studied largely in relationship to the biosynthesis and accumulation of storage proteins, lipids, and starch, macromolecular reserves that ultimately serve as nutrients for postgerminative seedlings (Jenner, 1982; Slack and Browse, 1984; Casey et al., 1986; Shotwell and Larkins, 1989). The focus of attention on these reserves reflects their agricultural importance and the fact that the abundance of these macromolecules facilitates studies of their accumulation. By contrast, much less is known about the processes that underlie embryo morphogenesis, although increasing attention is being devoted to this area.

In this review, we will provide an overview of the processes that occur during embryonic development, focusing primarily on dicotyledonous plants. Additionally, we will discuss some of the critical processes involved in embryo formation, about which relatively little is known. This review is meant to provide a conceptual summary of embryonic development rather than to cover all aspects of embryogenesis in detail or to describe events that occur in all species of plants. Readers are referred to other reviews on embryogenesis for additional coverage of the topic (Natesh and Rau, 1984; Dure, 1985; Raghavan, 1986; Crouch, 1987; Goldberg et al., 1989; Meinke, 1991a; De Jong et al., 1993; Lindsey and Topping, 1993).

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MORPHOLOGICAL DESCRIPTION OF EMBRYOGENESIS

A complete understanding of the early stages of embryogenesis requires knowledge of the preceding events that occur during the gametophytic generation. As shown in Figure 1A, the multicellular embryo sac that is embedded within the ovule has a polar organization along its micropylar-chalazal axis (Reiser and Fischer, 1993, this issue; Russell, 1993, this issue). The egg cell and synergids are located closest to the micropylar pole of the ovule, whereas the antipodal cells are situated at the opposite end of the embryo sac, closest to the chalazal pole (Jensen, 1965; Schulz and Jensen, 1968a; Mansfield et al., 1990). During pollination, the pollen tube penetrates the ovule through the micropyle and delivers a sperm nucleus that fuses with the haploid nucleus of the egg cell to produce the diploid embryo. A second sperm nucleus combines with the two polar nuclei of the central cell to produce an extraembryonic triploid endosperm. The endosperm provides nutrients for the developing embryo and either persists in the mature seed as a storage tissue or is absorbed during seed development (Vijayaraghavan and Prabhakar, 1984; Lopes and Larkins, 1993, this issue). Embryonic development proceeds within the confines of the protective maternal tissue of the ovule, which becomes the seed coat surrounding the developing embryo and endosperm.

Embryogenesis in higher plants can be divided conceptually into three overlapping phases. The first phase is one of morphogenesis, during which the polar axis of the plant body is defined with the specification of the shoot and root apices, and the embryonic tissue and organ systems are formed. The second phase is one of embryo maturation; it is characterized by the accumulation of storage reserves. During the final phase, the embryo prepares for desiccation, becomes desiccated, and enters a period of developmental arrest. Each of these phases is discussed below with particular emphasis applied to the morphogenetic phase.

Figures 1B to 1L show schematic diagrams of embryos at sequential stages of embryogenesis, and Figure 2 shows examples of the morphological changes that occur in a representative dicotyledonous plant, Arabidopsis. In many plants, the sequence of cell divisions early in embryogenesis occurs with such consistency that the cleavage patterns have been used to classify embryo ontogenesis into six distinct types (Johansen, 1950; Maheshwari, 1950). In particular, the crucifers Arabidopsis, *Brassica napus* (oilseed rape), and *Capsella*

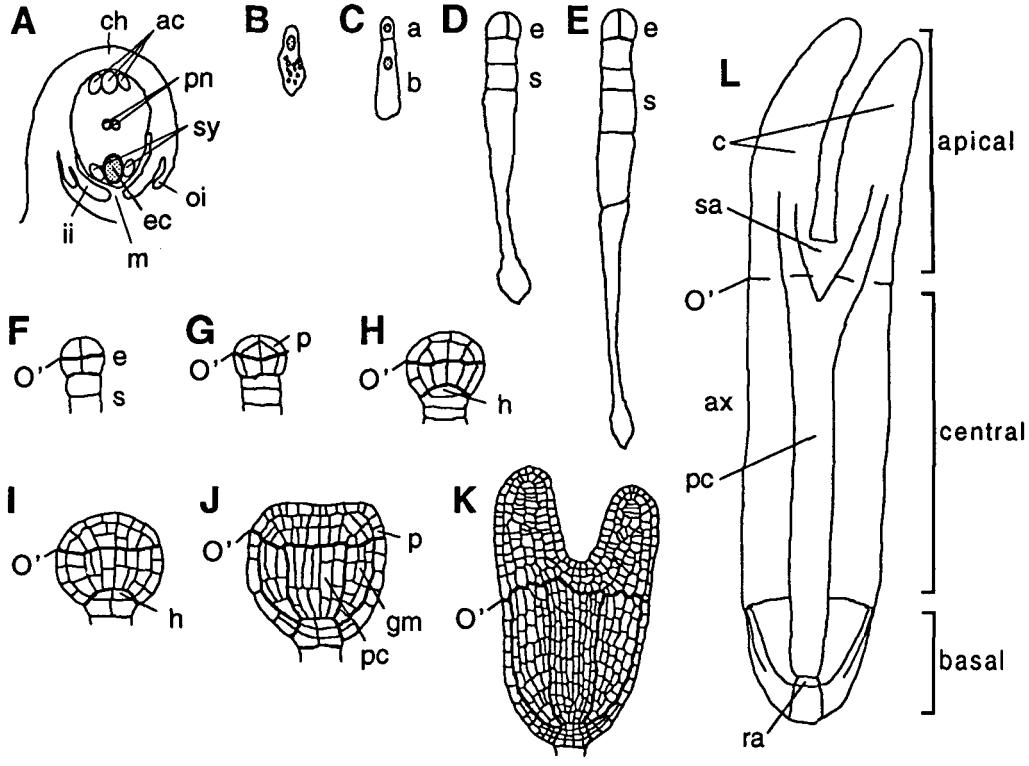

Figure 1. Embryonic Development in a Representative Dicotyledonous Plant.

(A) Ovule. The egg cell (ec) and synergids (sy) are located at the micropylar end (m) of the ovule, and the antipodal cells (ac) are at the chalazal end (ch). ii, inner integuments; oi, outer integuments; pn, polar nuclei.

(B) Zygote.

(C) One-celled embryo proper. The zygote has undergone a transverse cell division, producing a smaller apical cell (a) and a larger basal cell (b). The apical cell produces the embryo proper, and the basal cell develops into the suspensor and hypophysis.

(D) Two-celled embryo proper. The first division of the embryo proper (e) is longitudinal. The suspensor (s) has divided by transverse divisions.

(E) Quadrant stage embryo. The two-celled embryo proper divides by another longitudinal division, perpendicular to the plane of the previous division in the embryo, to produce a four-celled embryo proper. The suspensor has undergone additional transverse divisions.

(F) Octant stage embryo. The four quadrants have divided by transverse divisions to produce an eight-celled embryo proper. The transverse cell walls produced in this division form the indicated O′ line. The basal portion of the suspensor is not shown.

(G) Dermatogen stage. The cells of the octant stage embryo have divided by cleavages parallel to the surface to form a sixteen-celled embryo proper, setting apart the protoderm (p).

(H) Early globular stage. The cells of the protoderm have undergone divisions perpendicular to the surface. The interior cells of the embryo proper have undergone additional longitudinal divisions. The topmost cell of the suspensor has divided transversely to produce the hypophysis (h).

(I) Mid-globular stage. The cells of the hypophysis have divided longitudinally. The cells in the interior of the embryo proper have divided both longitudinally and transversely, while the protodermal cell divisions have continued.

(J) Transition stage. Cell divisions parallel to the surface indicate the emergence of the cotyledon buttresses as the apical pole of the embryo becomes broader. The developing procambium (pc) becomes visible as elongated cells at the center of the embryo. gm, ground meristem.

(K) Heart stage. Cotyledonary lobes continue to enlarge, making the change to bilateral symmetry more obvious. The O′ line is still recognizable.

(L) Linear cotyledon stage. The morphological organization of the embryo is shown. The apical domain comprises the cotyledons (c), the shoot apex (sa), and the upper axis; the central domain consists of the bulk of the axis (ax); and the basal domain includes the root apex (ra). The developing vascular tissue forks just below the O′ boundary.

(B) through **(L)** depict oilseed rape embryos and are adapted from Tykarska (1976, 1979). Drawings are not to scale.

bursa-pastoris represent plants in which the early divisions are virtually invariant (Schulz and Jensen, 1968a; Tykarska, 1979; Mansfield and Briarty, 1990). Other plants, such as cotton, have much more variable cleavage patterns (Pollock and Jensen, 1964). Thus, there is no universal pattern of early cell cleavages that describes embryonic development.

In many angiosperms, the initial division of the zygote is transverse and asymmetric, generating a small, chalazally oriented apical cell and a large basal cell, as shown in Figure 1C (Pritchard, 1964; Schulz and Jensen, 1968b; Tykarska, 1976; Mansfield and Briarty, 1990). In animal development, the asymmetric division of progenitor cells is a fundamental mechanism

by which cells with unequal cytoplasms and, consequently, different fates are generated (Gurdon, 1992; Horvitz and Herskowitz, 1992). Similarly, in cruciferous plants, the apical cell gives rise to the bulk of the embryo proper, including the cotyledons, shoot apex, and hypocotyl, whereas a part of the root apex and the suspensor originate from the basal cell (Schulz and Jensen, 1968a; Tykarska, 1976). In other plants, however, the first division of the zygote can be symmetrical, oblique, or longitudinal (Sivaramakrishna, 1978). Thus, a transverse and asymmetric division of the zygote is not a prerequisite for embryonic development in all plants.

As shown in Figures 1D, 1E, and 2B, the apical cell of cruciferous embryos next undergoes two longitudinal divisions to produce a four-celled embryo proper (Schulz and Jensen, 1968a; Tykarska, 1976; Mansfield and Briarty, 1990). A transverse division follows to produce two tiers of cells in an octant stage embryo proper, illustrated in Figures 1F and 2C. Evidence suggesting that the cell walls generated by this division, designated the O′ line, act as a boundary separating distinct domains within the embryo will be discussed in the following section. The next division of the octant stage embryo is periclinal, or parallel to the embryo surface. This division sets off the first histologically detectable tissue, the protoderm, which is the precursor of the epidermis, as shown in Figures 1G and 2D. The delineation of the protoderm establishes the globular stage embryo, which increases in size and cell number by anticlinal cell divisions of the protoderm (i.e., that are perpendicular to the embryo surface) and by longitudinal and, later, transverse divisions of interior cells (Figures 1H and 1I).

In crucifers, the basal cell of the two-celled embryo undergoes a series of transverse divisions, resulting in the formation of the hypophysis and the suspensor (Figures 1C to 1E). In

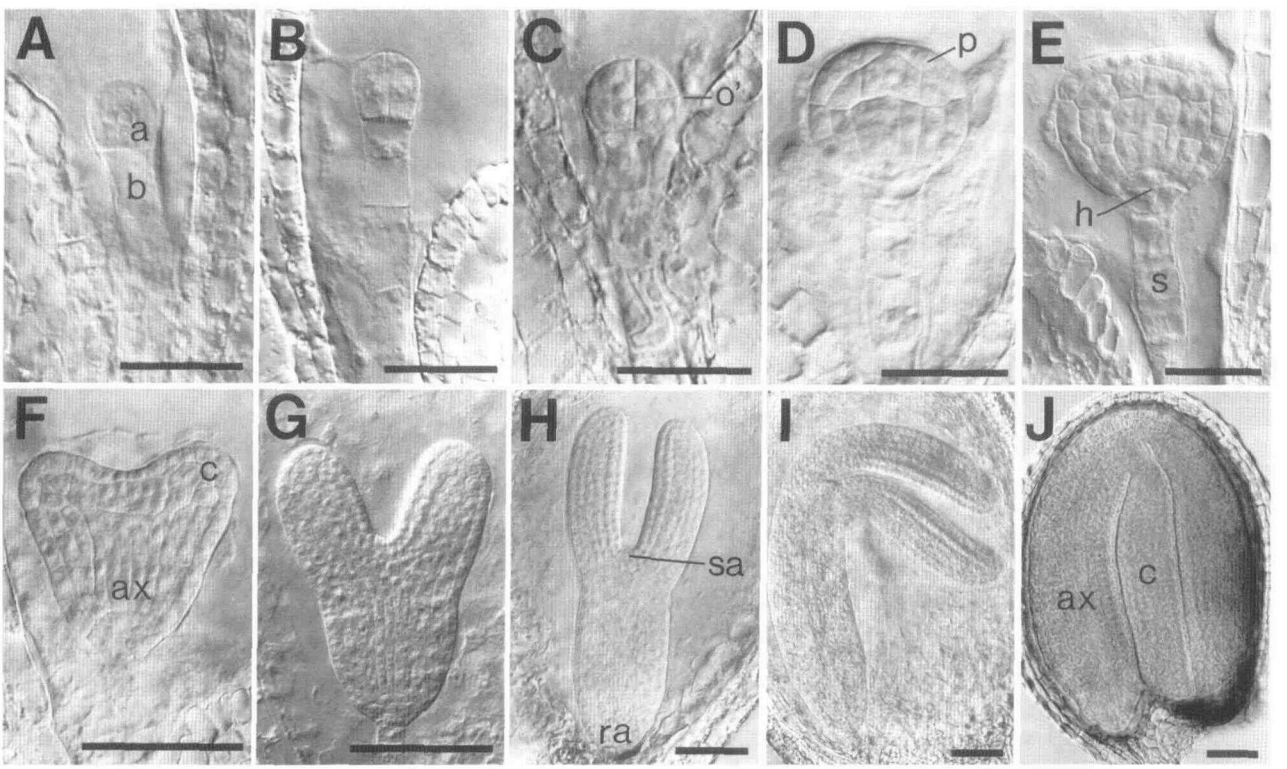

Figure 2. Arabidopsis Embryogenesis.

Developing Arabidopsis seeds were collected, processed, and photographed using differential interference contrast microscopy.
(A) *One-celled embryo proper.* a, apical cell; b, basal cell.
(B) Two- or four-celled embryo proper.
(C) Octant stage embryo. O′, O′ line.
(D) Early *globular stage embryo.* p, *protoderm.*
(E) Transition stage embryo. h, hypophysis; s, suspensor.
(F) Early heart stage embryo. ax, axis; c, cotyledon.
(G) Late heart stage embryo.
(H) Linear cotyledon stage embryo. ra, root apex; sa, shoot apex.
(I) Curled cotyledon stage embryo.
(J) Mature embryo.
Bars in **(A)** through **(E)** = 25 μm. Bars in **(F)** through **(J)** = 50 μm.

many plants, as shown in Figures 1H and 2E, the hypophysis, the uppermost derivative of the basal cell, serves as the precursor to the root cortex initials and the central region of the root cap (Schulz and Jensen, 1968a; Tykarska, 1979; Mansfield and Briarty, 1990). The suspensor is an ephemeral embryonic structure that, as shown in Figure 2E, comprises a single file of 6 to 11 cells in Arabidopsis and oilseed rape, although there are substantial variations in the number and layers of suspensor cells in different plants (Maheshwari, 1950; Wardlaw, 1955; Tykarska, 1976; Marsden and Meinke, 1985; Mansfield and Briarty, 1990). This structure is thought to function in embryogenesis by pushing the embryo into the nutrient-rich endosperm and, possibly, by serving as a conduit for nutrients and growth factors from the maternal tissue to the embryo (Yeung and Meinke, 1993, this issue). In crucifers, the suspensor reaches maximal cell number by the early globular stage of development and begins to senesce during the torpedo stage (Marsden and Meinke, 1985; Mansfield and Briarty, 1990).

A dramatic transformation of embryo morphology occurs during the transition from the globular to the heart stage. Figures 1J, 1K, and 2E to 2G show that cell divisions parallel to the surface occur at specific regions of the lateral margins of the globular stage embryo, resulting in the emergence of the two lobes of the cotyledons. The shift in embryo symmetry from radial at the globular stage to bilateral at the heart stage represents the initial delineation of the two major embryonic organ systems, the cotyledons and axis. Following their formation, the cotyledons and axis elongate rapidly as a result of cell division and cell expansion (Figures 1L, 2H, and 2I; Tykarska, 1979, 1980). Other tissues and structures characteristic of postembryonic plants can be discerned in a heart stage embryo (Tykarska, 1979; Mansfield and Briarty, 1990). For example, as shown in Figure 1J, the procambium, which is the precursor of the vascular tissue, and the ground meristem can be first identified histologically during the globular to heart stage transition. Furthermore, the cells that will form the root apex and, in some plants, the shoot apex can be distinguished by about this stage of embryogenesis. The extents to which the shoot apical meristem is activated and tissues are differentiated at this stage vary in different species.

The latter stages of embryogenesis are concerned primarily with preparing the embryo for developmental arrest and germination (Crouch, 1987; Kermode, 1990; Galau et al., 1991; Thomas, 1993, this issue). Although outside the scope of this review, these stages are of particular interest because they are unique to seed plants. In lower vascular plants, the morphogenetic phase occurs continuously; thus, there is no distinct end to embryonic development or a definite beginning to postembryonic growth (Steeves and Sussex, 1989). By contrast, morphogenesis of higher plant embryos is interrupted by a period of maturation during which storage reserve macromolecules, including storage proteins, lipids, and carbohydrates, accumulate in virtually all cells of the embryo (Walbot, 1978; Tykarska, 1987). These reserves are particularly prevalent in the embryonic cotyledons of plants that do not store substantial reserves in their endosperm, and they are in large part responsible for a rapid increase in embryo mass and size (Mansfield and Briarty, 1992). These macromolecules subsequently serve as a nutrient source for the growing seedlings. During the final stages of embryogenesis, embryos also acquire the ability to withstand desiccation and eventually enter a period of metabolic quiescence (Kermode, 1990). The mature embryo remains dormant until it encounters conditions appropriate for germination.

MAJOR PROCESSES IN EMBRYONIC DEVELOPMENT

A striking characteristic of plants is that tissue and organ formation are repetitive processes that occur continuously (Steeves and Sussex, 1989). The majority of morphogenetic events occur postembryonically, such as the formation of leaves, stems, roots, and reproductive structures. However, during embryonic development, the polar axis of the plant is established, domains that set up the organization of the plant body are defined, and the primary tissue and organ systems are delineated. In this section, we summarize the information that is known about the processes involved in making the embryo.

Embryonic Polarity

The plant body is highly polarized, with a shoot–root axis. The first indication of polarity is seen in the unfertilized egg cell, which is present in an asymmetrically organized embryo sac and ovule (Reiser and Fischer, 1993, this issue). In many plants, the nucleus and much of the cytoplasm are confined to the chalazal pole, while a large vacuole is present at the micropylar end. Additionally, the cell wall of the unfertilized egg cell, which bounds the entire micropylar end, does not completely surround the chalazal border (Jensen, 1965; Schulz and Jensen, 1968b; Mansfield et al., 1990; Russell, 1993, this issue). Following fertilization, the zygote is also characterized by an asymmetric organization. In many plants, a redistribution of the endoplasmic reticulum, plastids, and mitochondria that occurs after fertilization accentuates the polar organization displayed in the egg cell (Jensen, 1968; Russell, 1993, this issue). More dynamic examples of zygote polarization have also been described. For example, in *Papaver nudicaule* and maize, the positions of the nucleus and vacuole in the unfertilized egg are exchanged following fertilization, with the nucleus becoming oriented toward the chalazal pole (Olson and Cass, 1981; van Lammeren, 1981). Because the asymmetry of the zygote reflects the polar organization of the egg, embryonic polarity may be established during embryo sac development.

The asymmetric cleavage of the zygote yields two cells with different cytoplasms. For example, the apical cell of cotton embryos has numerous plastids and large mitochondria, whereas the basal cell has many vacuoles (Jensen, 1968). However,

the observation that the first division of the zygote of some plants can be oblique or symmetrical suggests that the plane of division may be less important than the polar organization of the zygote.

What are the signals and processes that generate embryonic polarity? Genetic analyses of polarity and pattern formation in the fruit fly Drosophila have shown that localized maternal signals specify anterior–posterior and dorsal–ventral axes of the embryo (Ingham, 1988; St Johnston and Nusslein-Volhard, 1992). Although analyses of egg cell and zygote organization in plants suggest that polarity is fixed during embryo sac development, two observations seem to be inconsistent with this view. First, to our knowledge, the only known maternal-effect mutations of higher plants are the *shrunken endosperm* mutations of barley, which affect endosperm rather than embryo development (Felker et al., 1985). Although no other higher plant maternal-effect mutants have been identified, it is possible that such mutants could constitute a class of female-sterile mutants (Meinke, 1991b; Jürgens et al., 1991; Reiser and Fischer, 1993, this issue). As will be discussed, the only known mutation that affects the asymmetric cleavage of a zygote affects the zygotically acting gene, *GNOM*, of Arabidopsis (Mayer et al., 1993). Second, as reviewed by Zimmerman (1993, this issue), somatic cells of many plants can be induced to undergo embryogenesis, suggesting either that additional mechanisms that do not involve maternal components can generate polarity or that somatic cells can mimic the maternal environment.

Fucoid algae zygotes have been used as a model system to study the induction of cellular polarity in plant embryogenesis (Quatrano, 1978; Kropf, 1992; Goodner and Quatrano, 1993, this issue). Selection and fixation of a rhizoid/thallus axis can occur in response to a variety of environmental stimuli in a process that involves microfilament networks and the cell wall. By contrast to higher plant zygotes, axis fixation of the algal zygote is a dynamic process because the egg cytoplasm is apolar. Thus, in the absence of clues about the maternal factors that may generate asymmetry in the egg cell, insights into the establishment of polarity may be obtained by studying plants whose cytoplasms are organized after fertilization.

Pattern Formation: Establishing Embryonic Domains

The polarity of the embryo defines an axis upon which the body plan of the plant is elaborated. Continued embryonic development may be viewed conceptually as a series of partitioning events that sequester increasingly more specialized regions. An early compartmentation step appears to involve the creation of three spatial domains along the longitudinal axis of the embryo, as shown diagrammatically in Figure 1L (Jürgens et al., 1991; Mayer et al., 1991). The apical domain is composed of the cotyledons, shoot apex, and upper hypocotyl; the central domain includes the majority of the hypocotyl; and the basal domain consists primarily of the root.

Initial evidence for these domains was derived from studies of Arabidopsis mutants defective in the specification of the plant's spatial organization (Mayer et al., 1991). Mutations in four of nine complementation groups identified in saturation mutagenesis experiments resulted in the deletion of domains along the apical–basal axis. Specifically, (1) the apical domain mutant, *gurke*, lacks cotyledons and the shoot apical meristem; (2) in a mutant lacking the central domain, *fackel*, cotyledons appear to be fused directly to the roots; (3) the basal deletion mutant, *monopteros*, lacks both the hypocotyl and root; and (4) the terminal mutant, *gnom*, has deletions in both apical and basal domains. These mutant phenotypes have been interpreted to indicate that there are three distinct domains within the embryo. Mutations in the five remaining complementation groups affect radial pattern, which is defined as tissue organization, and embryo shape. Based on this analysis, it has been estimated that 40 zygotically active genes may be sufficient to establish the body organization of Arabidopsis (Mayer et al., 1991). However, because mutants were identified only at the seedling stage, this may be a minimal estimate if other mutants defective in pattern formation are unable to complete embryogenesis and produce viable seedlings.

Although pattern formation mutants were originally identified as seedlings, the morphological consequences of the mutations can be first observed at an early embryonic stage, suggesting that the domains are established early in embryogenesis (Mayer et al., 1991). For example, cotyledon primordia do not form in embryos of the apical deletion mutant *gurke*. Similarities between the position of the boundary separating the apical and central domains and the O′ line have been interpreted to indicate that these domains are established with the first transverse division of the embryo proper at the octant stage (see Figure 1; Tykarska, 1976; Mayer et al., 1991). This interpretation does not indicate whether cell lineage or positional information plays the primary role in establishing these domains because the early cell divisions are critical in determining position, particularly in plants that undergo a consistent pattern of early cleavages (Poethig, 1987, 1989; Dawe and Freeling, 1991). Detailed studies of the *gnom* mutant provide additional support for the early determination of these domains (Mayer et al., 1993). Rather than undergoing an asymmetric cleavage, the mutant zygote often produces nearly equal-sized daughter cells, and subsequent cleavages of the *gnom* embryo are also abnormal. One interpretation of *GNOM* gene activity is that it promotes the asymmetric division of the zygote required for the formation of apical and basal domains. Alternatively, the gene may be required to establish the polarized organization of the zygote, which, in turn, leads to an asymmetric cleavage.

Establishment of Shoot and Root Apices and Embryonic Organs

The polar axis of the embryo is defined with the specification of the shoot and root apical meristems. Formation of the embryonic root apex occurs in a process that involves derivatives

of both the basal and apical cells of a two-celled embryo (Figure 1C). The hypophyseal region, derived from the topmost cell of the suspensor, is incorporated into the embryo proper, where it gives rise to part of the root cap and its initial cells and the ground meristem initial cells (Figures 1H and 1I; Tykarska, 1979). The remainder of the root apex, including the ground meristem and procambium, is contributed by the apical cell. The organization of the root apical meristem is evident by the heart stage (Figures 1J and 1K).

By contrast, delineation of the embryonic shoot apex is much more cryptic. Clonal analyses in cotton and maize suggest that the embryonic region that gives rise to the shoot apical meristem, the epiphysis, is determined in an early globular stage embryo (Christianson, 1986; Poethig et al., 1986). Histological analysis indicates that the epiphysis is segregated early from the progenitor cells of the cotyledons. For example, the epiphysis of *Downingia* can be distinguished from adjacent cells by its differential staining in an early globular stage embryo (Kaplan, 1969). At the transition stage of embryogenesis, the centrally localized epiphyseal cells divide more slowly than the cotyledon cell progenitors, resulting in the formation of a bilaterally symmetrical heart stage embryo (Swamy and Krishnamurthy, 1977).

In relationship to the embryonic domains discussed previously, epiphysis formation may be viewed as a partitioning event in the apical domain that segregates cells with the potential to become the shoot apical meristem. Support for this view can be inferred from the analysis of 2S and 12S storage protein mRNA accumulation in oilseed rape embryos (Fernandez et al., 1991). Storage protein gene expression is tightly regulated during embryogenesis and, therefore, is an excellent marker of cellular differentiation (Perez-Grau and Goldberg, 1989; Thomas, 1993, this issue). At one embryonic stage, the storage protein mRNAs are not detected in epiphyseal cells but are prevalent in cotyledonary cells, indicating that the two regions are segregated functionally within the apical domain. Moreover, the lower boundary of the epiphysis is indicated by a distinct line of cell walls that separates adjacent cells with and without storage protein mRNA. This boundary transcends tissue types, extending through the ground tissue and into the protoderm, and coincides with the position of the O′ line. This observation is consistent with the idea that the progenitors of the shoot apex are partitioned early in embryogenesis, because the first transverse cell division of the embryo proper that generates the O′ line occurs before the cell cleavage that delineates the protoderm (see Figures 1F and 1G).

Morphological changes during the globular stage to heart stage transition are the first visible sign of the formation of the two embryonic organ systems, the cotyledons and the axis. The emergence of the cotyledons from a radially symmetrical globular stage embryo represents another partitioning event in the apical region, indicating that groups of cells are induced to proliferate at specific sites. A recent report suggests that polar auxin transport may be involved in directing these localized cell divisions (Liu et al., 1993). Globular stage embryos, either treated with auxin transport inhibitors or derived from plants genetically defective in polar auxin transport, do not produce two cotyledonary lobes but, rather, form fused cotyledons that develop as ring-shaped structures above shoot apices. This requirement for polar auxin transport is specific to the globular stage because auxin transport inhibitors applied to heart-shaped embryos do not induce fused cotyledons. This work implies that a conduit for auxin transport must be formed in the globular stage embryo to signal the site of cotyledon formation or that auxin is perceived differently by distinct groups of cells in the apical region.

Tissue Differentiation

Another level of partitioning within the embryo is the organization of the primary embryonic tissues, the protoderm, the ground tissue, and the procambium. The first tissue that can be identified histologically is the protoderm (Figure 1G); progenitors of the ground tissue and procambium can generally be discerned during the transition from the globular stage to the heart stage of embryogenesis (Figure 1J; Schulz and Jensen, 1968a; Tykarska, 1979; Mansfield and Briarty, 1990). Ultrastructural evidence for the differentiation of embryonic tissues is first observed in early heart stage embryos, as plastids in cells of the ground meristem and protoderm become more differentiated than those in procambial cells, and ground meristem and procambial cells become more vacuolated than cells of the protoderm.

Other data suggest that the fate of the tissues can be distinguished even before they can be discerned ultrastructurally. For example, an early indication of protoderm differentiation is that the divisions of cells that comprise this tissue occur primarily by cleavages perpendicular to the embryo surface. The expression of specific genes also provides evidence for the functional organization of tissues early in embryogenesis. Protodermal cells in globular stage carrot embryos specifically accumulate mRNA encoding a lipid transfer protein, EP2, indicating that these cells are distinct from interior cells of the embryo (Sterk et al., 1991). Similarly, mRNA encoding the Kunitz trypsin inhibitor, KTi3, which accumulates in the ground meristem cells of soybean heart stage embryos, is detected initially in a small subset of cells at the micropylar end of globular stage embryos that have been interpreted to be ground meristem initials (Perez-Grau and Goldberg, 1989). These studies suggest that cells destined to become specific embryonic tissues begin to differentiate by the globular stage.

Genetic approaches may yield significant insights into the processes involved in the formation of embryonic tissues, apical meristems, and organs. Embryonic mutants of Arabidopsis and maize have been described whose phenotypes suggest that morphogenetic processes have been altered (Meinke, 1986, 1991b; Clark and Sheridan, 1991). For example, *embryo lethal* (*emb*) mutations that confer leaflike characteristics to embryonic cotyledons have been identified in Arabidopsis (Meinke, 1992; M.A.L. West and J.J. Harada, unpublished results). The products of these defective genes appear to be

key embryonic regulators because cotyledon identity is altered and the maturation program is modified such that storage proteins do not become prevalent in cotyledons of mutant embryos. Furthermore, other Arabidopsis *emb* mutations have been identified that arrest morphological development at an early embryonic stage but allow cellular differentiation, as defined by the expression of genes normally expressed during the maturation and desiccation stages, to continue (M.A.L. West, R. Yadegari, K.L. Matsudaira, J.L. Zimmerman, R.L. Fischer, R.B. Goldberg, and J.J. Harada, unpublished results). These studies indicate that cell differentiation can be uncoupled from morphogenesis. Other *emb* mutations may represent disruptions in housekeeping functions, such as an Arabidopsis mutant that is a biotin auxotroph (Schneider et al., 1989).

SUMMARY AND PROSPECTS

A simple perspective that emerges from this review is that embryogenesis can be seen as a hierarchy of partitioning events that culminates in the production of a morphologically complex organism. The polarity of the embryo, which may reflect the asymmetric organization of the egg cell, establishes an axis upon which the plant body is elaborated. An early compartmentation of the embryo sets off domains that appear to be involved in establishing the organization of the plant body. Other events organize the embryonic tissue and organ systems and partition progenitors of the shoot and root apices early in embryogenesis. Boundaries separating some of the embryonic regions coincide with cell cleavages that occur at an early embryonic stage. However, it is difficult to distinguish the roles of cell lineage and positional information in determining cell fate in this instance because the consistent patterns of cleavages in cruciferous embryos largely establish cell position.

Although histological, morphological, and biochemical studies have provided descriptive information about embryogenesis, a mechanistic understanding of the processes that underlie embryonic development is lacking. Among the critical questions that remain to be addressed are the following.

First, how is the polarity of the embryo established? In many higher plants, the polarity of the embryo is thought to reflect the nonrandom distribution of cytoplasmic components in the unfertilized egg cell. What is responsible for the cytoplasmic organization within this cell? Does the microenvironment of the ovule or of the embryo sac influence the polarization of the egg cell? How is polarity established in somatic embryos that develop independent of maternal tissue?

Second, how are embryonic domains and compartments partitioned? The establishment of these regions is of obvious importance in embryo morphogenesis, yet very little is known about the mechanisms involved. Do early cell divisions partition these domains, and, if so, how are the cleavage planes determined? What are the inductive signals involved, and how is the competence of the receptive cells determined? Do plasmodesmata, which are connections between the adjacent cells, play a role in creating these domains (Lucas et al., 1993)?

Third, what genes are involved in early embryonic development? As we discussed previously, important clues about the processes involved in morphogenesis may be obtained from analyses of embryonic mutants. These mutations also provide a means to identify the genes that participate in these processes, for example, when modified T-DNAs and transposable elements are used as insertional mutagens (Herman and Marks, 1989; Yanofsky et al., 1990; Aarts et al., 1993; Bancroft et al., 1993). In addition, in plants with detailed genetic and physical maps, such as Arabidopsis, it is also possible to isolate genes based upon their map positions (Arondel et al., 1992; Giraudat et al., 1992; Leyser et al., 1993). A more detailed understanding of morphogenetic processes awaits the isolation and analysis of these crucial genes.

We anticipate that the answers to these and other questions will provide significant insight into the processes involved in embryogenesis. Because many of the mechanisms uncovered are likely to be unique to plants, this new information will add to our understanding of the diversity of mechanisms that underlie embryonic development.

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