Embryogenesis in Higher Plants: An Overview

Marilyn A. L. West and John J. Harada

Section of Plant Biology, Division of Biological Sciences, University of California, Davis, California 95616

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 develop-
ment, during which the zygote undergoes a complex series
of morphological and cellular changes resulting in the forma-
tion of a developmentally arrested mature embryo comprised
of an embryonic axis with shoot and root poles and cotyle-
don(s), which often contains high levels of storage macromolecules such as proteins and lipids. Thus, the events that
occur during embryonic development establish the organiza-
tion 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 morpho-
logical 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 accumu-
lation of storage proteins, lipids, and starch, macromolecular
reserves that ultimately serve as nutrients for postgermina-
tive seedlings (Jenner, 1982; Slack and Browse, 1984; Casey
et al., 1986; Shotwell and Larkins, 1989). The focus of atten-
tion on these reserves reflects their agricultural importance
and the fact that the abundance of these macromolecules facili-
tates studies of their accumulation. By contrast, much less is
known about the processes that underlie embryo morphogen-
esis, 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).

1 To whom correspondence should be addressed.

MORPHOLOGICAL DESCRIPTION OF EMBRYOGENESIS

A complete understanding of the early stages of embryogen-
esis 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 is-
sue). 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 pene-
trates 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 com-
bines with the two polar nuclei of the central cell to produce
an extraembryonic triploid endosperm. The endosperm pro-
vides 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
devolving embryo and endosperm.

Embryogenesis in higher plants can be divided conceptu-
ally 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 ex-
amples 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 (oileseed 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.

(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).

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 precur-
sor 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 struc-
ture 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 suspen-
sor 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 embry-
genesis 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
(Mansfield and Meinke, 1985; Mansfield and Briarty, 1990).

A dramatic transformation of embryo morphology occurs dur-
ing 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 for-
mation, 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 charac-
teristic 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
may 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 differen-
tiated at this stage vary in different species.

The latter stages of embryogenesis are concerned primar-
ily with preparing the embryo for developmental arrest and
germation (Crouch, 1967; 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 mor-
phogenetic phase occurs continuously; thus, there is no distinct
end to embryonic development or a definite beginning to postembyronic growth (Steeves and Sussex, 1989). By con-
trast, morphogenesis of higher plant embryos is interrupted
by a period of maturation during which storage reserve
macromolecules, including storage proteins, lipids, and car-
bohydrates, accumulate in virtually all cells of the embryo
(Walbot, 1978; Tykarska, 1987). These reserves are particu-
larly 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 macro-
molecules 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 for-
mation 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, dur-
ing 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 micropy-
lar 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 is-
sue). Following fertilization, the zygote is also characterized
by an asymmetric organization. In many plants, a redistribu-
tion of the endoplasmic reticulum, plastids, and mitochondria
that occurs after fertilization accentuates the polar organiza-
tion 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 unfer-
tilized 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, em-
byronic 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 em-
broys 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 gastrulation of zygote, which affects the gastrulation of embryos 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/hallus 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 zygoties, 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 cytoplasm is 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.

Establishment of Shoot and Root Apexes 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 1; 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). Histo logical 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 transceeds 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, 1966a; 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 the 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 internal cells of the embryo (Sterk et al., 1991). Similarly, mRNA encoding the Kunitz trypsin inhibitor, KT13, 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, *embryos 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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REFERENCES


