Plant Embryogenesis: Zygote to Seed

Robert B. Goldberg,* Genaro de Paiva, Ramin Yadegari

Most differentiation events in higher plants occur continuously in the postembryonic adult phase of the life cycle. Embryogenesis in plants, therefore, is concerned primarily with establishing the basic shoot-root body pattern of the plant and accumulating food reserves that will be used by the germinating seedling after a period of embryonic dormancy within the seed. Recent genetics studies in *Arabidopsis* have identified genes that provide new insight into how embryos form during plant development. These studies, and others using molecular approaches, are beginning to reveal the underlying processes that control plant embryogenesis.

A major problem in plant development is to unravel the mechanisms operating during embryogenesis that enable a plant to specify its body plan and tissue differentiation patterns. Although progress with a variety of animal systems has been spectacular in this regard (*1*), a detailed understanding of the events that govern plant embryo formation has yet to be realized. One obstacle in achieving this goal is the location of embryos within the plant and their relative inaccessibility to experimental manipulation, particularly at the early stages of embryogenesis. In flowering plants, reproductive processes occur within floral organs (Fig. 1) (*2*). The egg cell is present in the ovule, a multicellular structure that is buried beneath several cell layers of the pistil, the female reproductive organ (*2–4*). Because egg cell formation, fertilization, and embryogenesis occur within the pistil, it has been difficult to dissect the major events that take place during the early stages of higher plant development.

Recently, it has become feasible to isolate plant eggs and fertilize them in vitro in order to investigate the initial events of plant embryogenesis (*5*). In addition, genetic approaches have been used to identify genes required for various embryogenic processes, including pattern formation (*6, 7*). Genetic manipulation of *Arabidopsis thaliana*, by both chemical mutagenesis (*8–12*) and insertional mutagenesis (*13–15*), has identified a large number of mutants that are blocked at different stages of embryogenesis. In this review we outline the major insights that have been derived from studies of *Arabidopsis* embryo mutants, and we summarize gene transcription experiments in other plants that provide new information about the processes regulating higher plant embryogenesis. Both the genetic and molecular approaches suggest that a plant embryo has a modular structure and consists of several regions that form autonomously during embryogenesis.

Authors are in the Department of Biology, University of California, Los Angeles, CA 90024-1606, USA.

*To whom correspondence should be addressed.

Embryos Begin the Diploid Phase of the Higher Plant Life Cycle

The flowering plant life cycle is divided into haploid and diploid generations that are dependent on each other (Fig. 1) (*2, 16–18*). The haploid, or gametophytic, generation begins after meiosis with spores that undergo mitosis and differentiate into either a pollen grain (male gametophyte) or an embryo sac (female gametophyte) (*3, 19–20*). The pollen grain contains two sperm cells, whereas the embryo sac contains a single egg (Fig. 1). Other accessory cells within the haploid male and female gametophytes help facilitate the pollination and fertilization processes (*3, 19, 20*). The male and female gametophytes are derived from specialized spore-forming cells within the reproductive organs of the flower (*3, 4, 21*). By contrast, the diploid, or sporophytic, generation begins after fertilization with the zygote and forms the mature plant with vegetative organs (leaf, stem, root) and flowers that contain the reproductive organs (anther and pistil) (Fig. 1).

Two fertilization events occur in flowering plants (*2, 22*). One sperm unites with the egg cell to produce a zygote and initiate embryogenesis. The other unites with a specialized cell within the embryo sac (central cell) to initiate the differentiation of the endosperm, a triploid tissue that is neither gametophytic nor sporophytic in origin (Fig. 1) (*23*). The endosperm is present during seed development and provides nutrients for either the developing embryo, the germinating seedling, or both (*23*). Fertilization also causes the ovule, containing the embryo and endosperm, to develop into a seed and the ovary to differentiate into a fruit, which facilitates seed dispersal (Fig. 1) (*24*).

Most morphogenetic events in flowering plants occur in the postembryonic sporophyte after seed germination (Fig. 1) (*2*). Vegetative organ systems differentiate continuously from root and shoot meristematic regions that are formed initially during embryogenesis. The reproductive organs of the flower are differentiated from a reprogrammed shoot meristem after the seedling has become a mature plant (Fig. 1) (*25*). Thus, a germline analogous to that found in animals (*1*) is not set aside during plant embryogenesis.

A mature flowering plant embryo contains two primary organ systems—the axis and cotyledon (Fig. 1) (*2*). These organs have distinct developmental fates and are both composed of three basic, or primordial, tissue layers—protoderm, procambium, and ground meristem—which will become the epidermal, vascular, and parenchyma tissues of the young seedling, respectively (*2*). The axis, or hypocotyl-radicle region of the embryo, contains the shoot and root meristems and will give rise to the mature plant after seed germination (Fig. 1). By contrast, the cotyledon is a terminally differentiated organ that accumulates food reserves that are utilized by the seedling for growth and development before it becomes photosynthetically active (Fig. 1). The cotyledon functions primarily during seed germination and senesces shortly after the seedling emerges from the soil. Embryogenesis in higher plants, therefore, serves (i) to specify meristems and the shoot-root plant body pattern, (ii) to differentiate the primary plant tissue types, (iii) to generate a specialized storage organ essential for seed germination and seedling development, and (iv) to enable the sporophyte to lie dormant until conditions are favorable for postembryonic development.

The Shoot-Root Body Plan Is Generated During Early Embryogenesis

How the embryo acquires its three-dimensional shape with specialized organs and tissues, and what gene networks orchestrate embryonic development remain major unresolved problems. From a descriptive point of view, plant embryogenesis can be divided into three general phases in which distinct developmental and physiological events occur: (i) postfertilization-proembryo, (ii) globular-heart transition, and (iii) organ expansion and maturation (*26–28*) (Fig. 2 and Table 1). Although there is considerable variation in how embryos in different plant taxa form (*29*), the overall trends are remarkably similar (*29*). We summarize the *Capsella* and *Arabidopsis* pattern of embryo development (*29–33*) because (i) it is one of the most well-studied forms of plant em-

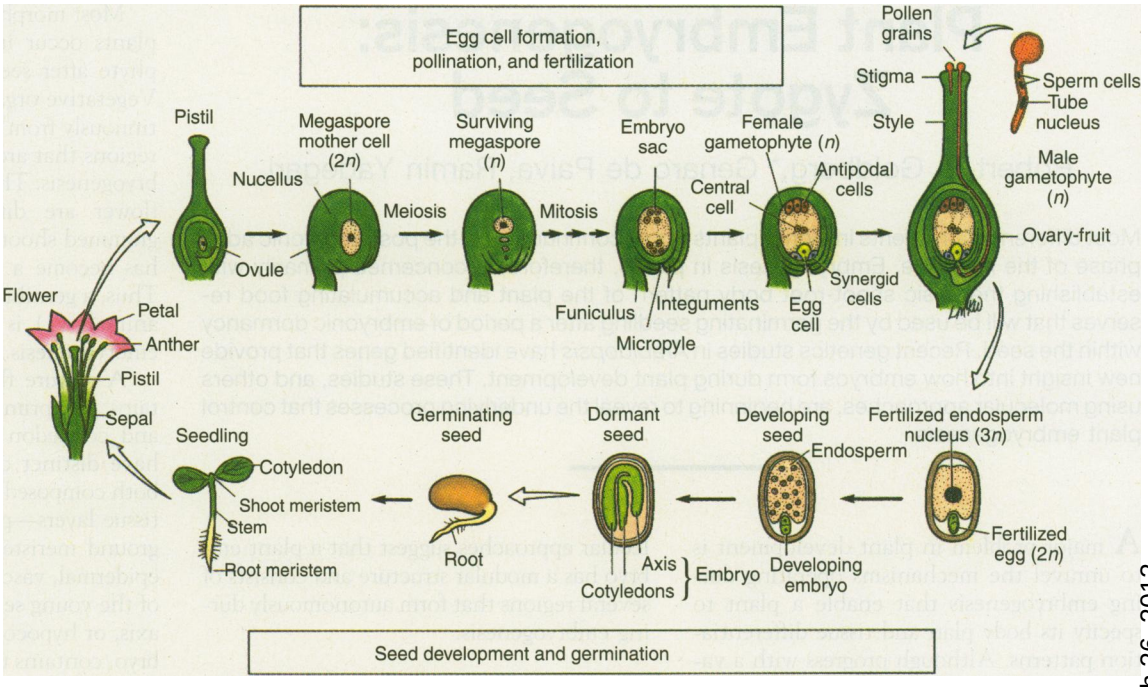

Fig. 1. The life cycle of a flowering plant with emphasis on egg cell formation and seed development. [Adapted from (*16*, *26*).]

bryogenesis, dating back to the classical studies of Hanstein, Schaffner, and Souèges with *Capsella* (*30*–*32*), (ii) it has an invariant division pattern during the early stages, which allows cell lineages to be traced histologically (*33*), and (iii) recent studies with *Arabidopsis* mutants have provided new insights into the processes that control embryo development (6, 9).

Asymmetric cleavage of the zygote results in the formation of an embryo with a suspensor and embryo proper that have distinct developmental fates. The zygote in *Arabidopsis* and *Capsella* has an asymmetric distribution of cellular components—the nucleus and most of the cytoplasm are present in the upper portion of the cell, whereas a large vacuole dominates the middle to lower portion (Fig. 2). This spatial asymmetry is derived from the egg cell (*30*). The zygote divides asymmetrically into two distinct-sized daughter cells—a small, upper terminal cell and a large, lower basal cell—which establish a polarized longitudinal axis within the embryo (Fig. 2) (*2*, *30*–*33*). Histological studies over the course of the past 125 years have indicated that the terminal and basal cells give rise to different regions of the mature embryo (*29*–*33*). The small terminal cell gives rise to the embryo proper that will form most of the mature embryo (Fig. 2). Cell lineages derived from the terminal cell and embryo proper will specify the cotyledons, shoot meristem, hypocotyl region of the embryonic axis (*29*–*33*), and part of the radicle, or embryonic root (Fig. 2) (*34*). By contrast, the large basal cell derived from the lower portion of the zygote will divide and form a highly specialized, terminally differentiated embryonic organ called the suspensor (Fig. 2). In *Arabidopsis*, the suspensor contains only 7 to 10 cells (Fig. 2). The suspensor anchors the embryo proper to the surrounding embryo sac and ovule tissue and serves as a conduit for nutrients to be passed from the maternal sporophyte into the developing proembryo (Fig. 2) (*35*). The suspensor senesces after the heart stage and is not a functional part of the embryo in the mature seed. Derivatives of

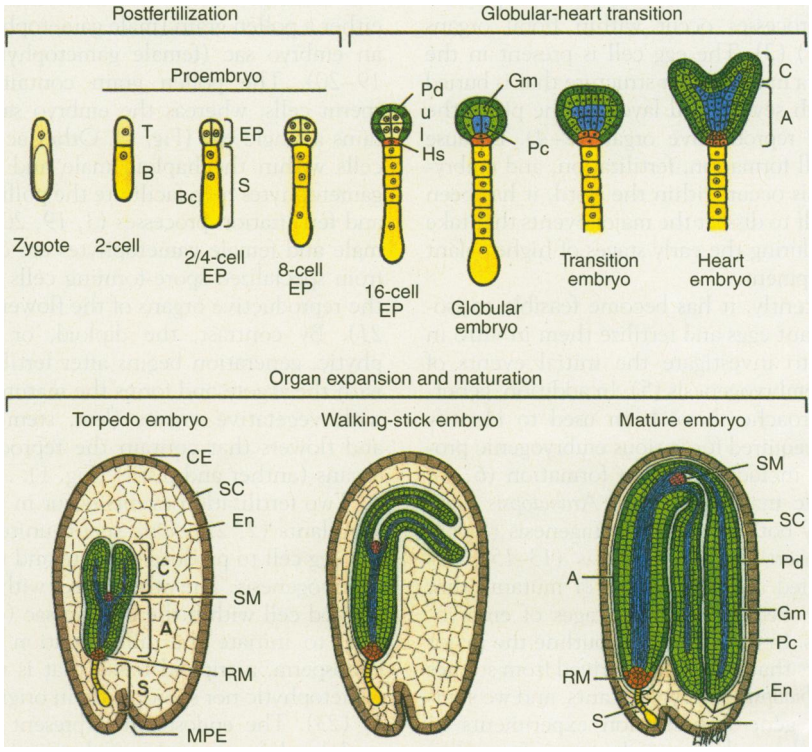

Fig. 2. A generalized overview of plant embryogenesis. Schematic representations of embryonic stages are based on light microscopy studies of *Arabidopsis* (*33*) and *Capsella* (*30*–*32*) embryo development. Torpedo and walking-stick refer to specific stages of embryogenesis in *Arabidopsis* and *Capsella*. Abbreviations: T, terminal cell; B, basal cell; EP, embryo proper; S, suspensor; Bc, suspensor basal cell; Pd, protoderm; u, upper tier; l, lower tier; Hs, hypophysis; Pc, procambium; Gm, ground meristem; C, cotyledon; A, axis; MPE, micropylar end; CE, chalazal end; SC, seed coat; En, endosperm; SM, shoot meristem; and RM, root meristem.

Table 1. Major events of flowering plant embryogenesis.

Postfertilization-proembryo
Terminal and basal cell differentiation
Formation of suspensor and embryo proper
Globular-heart transition
Differentiation of major tissue-type primordia
Establishment of radial (tissue-type) axis
Embryo proper becomes bilaterally symmetrical
Visible appearance of shoot-root (apical-basal) axis
Initiation of cotyledon and axis (hypocotyl-radicle) development
Differentiation of root meristem
Organ expansion and maturation
Enlargement of cotyledons and axis by cell division and expansion
Differentiation of shoot meristem
Formation of lipid and protein bodies
Accumulation of storage proteins and lipids
Vacuolization of cotyledon and axis cells
Cessation of RNA and protein synthesis
Loss of water (dehydration)
Inhibition of precocious germination
Dormancy

the uppermost cell of the suspensor, the hypophysis (Fig. 2), contribute to the formation of the root meristem (*30–34*). Thus, cell lineages derived from the basal cell give rise to the suspensor and part of the radicle region of the embryonic axis (Fig. 2).

Different gene sets must become active in the terminal and basal cells after the division of the zygote. Whether the polarized organization of the egg cell, the zygote, or both control differential gene expression events early in embryogenesis is not known. For example, do prelocalized regulatory factors within the egg cell initiate a cascade of events leading to the lineage-dependent differentiation of terminal and basal cell derivatives? Alternatively, after fertilization does the zygotic genome direct the de novo synthesis of regulatory factors that are distributed asymmetrically to the terminal and basal cells at first cleavage? In either case, these events would lead to the autonomous specification of the terminal and basal cells as a consequence of intrinsic factors rather than extracellular signals (*1*).

Embryonic organs and tissue-types differentiate during the globular-heart transition phase. Two critical events must occur after the embryo proper forms: (i) regions along the longitudinal apical-basal axis must differentiate from each other and generate embryonic organ systems, and (ii) the three primordial tissue layers of the embryo need to be specified. Both of these events take place during the globular-heart transition phase (Fig. 2 and Table 1). The embryo proper has a spherical shape during the proembryo and globular stages (Fig. 2). The first visible cell differentiation events occur at the 16-cell stage when the protoderm, or outer cell layer of the embryo proper, is produced and the hypophysis forms at the top of the suspensor (Fig. 2). Subsequent cell differentiation events within the embryo proper result in the production of an inner procambium tissue layer and a middle layer of ground meristem cells (Fig. 2). The spatial organization of protoderm, ground meristem, and procambium layers establishes a radial axis of differentiated tissues within the globular embryo. By contrast, the presence of the hypophysis at the basal end of the embryo proper establishes an apical-basal polarity within this region and indicates that the globular embryo is not radially symmetrical in the formal sense (Fig. 2).

A dramatic change in the morphology of the embryo proper occurs just after the globular stage. Cotyledons are specified from two lateral domains at the apical end (top), the hypocotyl region of the axis begins to elongate, and the root meristem becomes differentiated from the hypophysis region at the basal end (bottom) (*34*). The embryo proper is now heart-shaped, has a bilateral symmetry, and the body plan and tissue layers of the mature embryo (and postembryonic plant) have been established (Fig. 2). Morphogenetic changes during this period are mediated by differential cell division and expansion rates and by asymmetric cleavages in different cell planes (*2*). No cell migration occurs, in contrast to the migration events that take place in many types of animal embryos (*1*).

Embryogenesis terminates with a dormancy period. A major change in embryonic development occurs during the organ expansion and maturation phase (Fig. 2). A switch occurs during this period from a pattern formation program to a storage product accumulation program in order to prepare the young sporophyte for embryonic dormancy and postembryonic development (Table 1). The cotyledons and axis increase in size dramatically as a result of cell division and expansion events (*33*). Ground meristem cells within both these organs become highly specialized and accumulate large amounts of storage proteins and oils that will be utilized as a food source by the seedling after germination (Fig. 2 and Table 1) (*33*). One differentiation event does occur during this period, however—the shoot meristem forms from cell layers localized in the upper axis region between the two cotyledons (Fig. 2) (*36*). Thus, the differentiation of shoot and root meristems at opposite poles of the embryonic axis does not occur at the same time (*34, 36*). At the end of the organ expansion and maturation period the embryo has reached its maximum size, cells of the embryo and surrounding seed layers have become dehydrated, metabolic activities have ceased, and a period of embryonic dormancy within the seed begins (*26–28, 33*).

Embryogenesis Can Occur Without Surrounding Maternal Tissue

It is unclear what influence, if any, maternal tissue or accessory cells of the female gametophyte have on egg cell formation and subsequent embryonic development (Fig. 1). For example, do either the ovule or cells within the embryo sac (for example, synergids) produce morphogenetic factors that contribute to the establishment of longitudinal asymmetry within the egg? Several arguments suggest that the maternal sporophyte provides only physical support structures and nutrients for the embryo (Fig. 1). First, somatic cells from a variety of vegetative and reproductive tissues can undergo embryogenesis in culture and lead to the production of fertile plants (*37–39*). Somatic embryos undergo developmental events similar to those that occur within the embryo-proper region of zygotic embryos, except that they do not become dormant (Fig. 2 and Table 1). In addition, spatial and temporal gene expression programs appear to be similar in somatic and zygotic embryos (*39, 40*). Second, embryo-like structures leading to plantlets can form directly from the attached leaves of some plants (*41*). Third, zygotes produced by fertilizing egg cells in vitro undergo embryogenesis in culture and give rise to flower-producing plants (*5*). Finally, ultrastructural studies suggest that there is a barrier between the inner ovule cell layer and the embryo sac that prevents the transfer of material directly between these compartments (*42*). Thus, both zygotic and somatic embryogenesis can occur in the absence of surrounding ovule tissue (Fig. 1).

The embryo sac is necessary for zygotic embryogenesis because it contains the egg and associated accessory cells that are required for fertilization and endosperm development (Fig. 1). However, the embryo sac is not essential for embryogenesis per se because (i) somatic embryos produced from sporophytic cells develop normally (*5, 37–40*) and (ii) embryos can be induced to form from microspores that, under normal circumstances, give rise to pollen grains (*43*). These results suggest that normal embryogenic processes do not require factors produced by either the female gametophyte or maternal sporophytic tissue. This conclusion is supported by the fact that the overwhelming majority of mutations that alter embryo development appear to be due to defects in zygotically acting genes (*6–14, 44*). It is possible that somatic cells have the potential to produce putative maternal or gametophytic factors under the proper conditions, or that somatic embryos specify their longitudinal apical-basal and radial tissue–type axes by different mechanisms

than zygotic embryos. However, most of the available data suggest that embryo morphogenesis and cell specification events are directed primarily by the zygotic genome after fertilization occurs. If so, then this would differ significantly from the situation with many animals such as *Drosophila* and sea urchin, in which maternally supplied factors influence the pattern of early embryo development (*1*). More extensive studies with developing female gametophytes, egg cells, and zygotic embryos in the early stages of embryogenesis are needed to clarify this issue.

A Globular Embryo Contains Differentially Transcribed Regions

A large number of genes are expressed during embryogenesis in higher plants (*26*). Although it is not known how many genes are necessary to program morphogenetic and tissue differentiation processes, approximately 15,000 diverse genes are active in the embryos of plants as diverse as soybean and cotton (*26*). Many of these genes are expressed in specific cell types, regions, and organs of the embryo (*26, 40*) and provide useful entry points to unravel the molecular mechanisms that regulate cell- and region-specific differentiation events during plant embryogenesis (*1*).

The axis region of the embryo does not become visibly distinct until the heart stage (Figs. 2 and 3, A and B). Localization studies with a soybean Kunitz trypsin inhibitor mRNA, designated as Kti3 (*45*), indicated, however, that cells destined to become the axis are already specified at the globular stage (*40*). Figure 3C shows that Kti3 mRNA accumulates specifically at the basal, or micropylar, end of a late–globular stage soybean embryo. No detectable Kti3 mRNA is present in other regions of the embryo proper or in the suspensor (Fig. 3C) (*40*). In transition and heart stage embryos, Kti3 mRNA remains distributed asymmetrically at the embryo micropylar end (Fig. 3, D and E) and is localized specifically within the ground meristem cell layer (Fig. 2) of the emerging embryonic axis (Fig. 3D) (*40*). No detectable Kti3 mRNA is present within the newly initiated cotyledons (Fig. 3E). This result differs from that obtained with the carrot EP2 lipid transfer protein mRNA, which is localized uniformly in the outer protoderm cell layer that surrounds the embryo proper at the globular and heart stages (*46*). Taken together, these results indicate that cells along the longitudinal apical-basal axis of the embryo proper are already differentiated from each other at the globular stage and that early in embryogenesis distinct gene sets are expressed in different embryonic regions and cell types.

Transformation studies with tobacco embryos containing chimeric β-*glucuronidase* (*GUS*) reporter genes driven by soybean embryo–specific gene promoters showed that a globular embryo is organized into distinct, nonoverlapping transcriptional regions, or territories (Fig. 3, F to I) (*47, 48*). Blue color resulting from GUS enzyme activity occurs specifically at the micropylar end of a globular stage embryo containing a *Kti3/GUS* reporter gene (Fig. 3, F and G) (*47*). No GUS activity is observed within the suspensor or other embryo-proper regions (Fig. 3G). This result suggests that the preferential localization of Kti3 mRNA at the micropylar end of a soybean globular stage embryo (Fig. 3C) is due to transcriptional regulatory processes. By contrast, GUS activity is visible as a uniform blue belt that surrounds the equator region of a globular embryo containing a soybean *lectin/GUS* reporter gene (Fig. 3H) (*48*). GUS activity is not visible at either the micropylar or the chalazal (apical) ends of the embryo proper (Fig. 3H) (*48*); nor is there

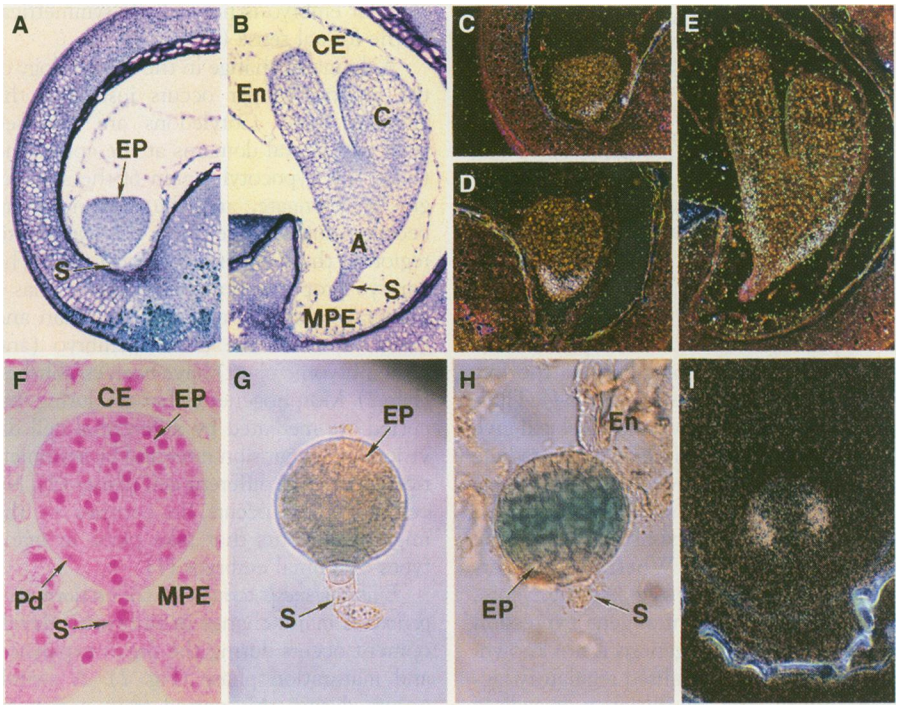

Fig. 3. Differential transcriptional activity during early embryogenesis. (A and B) Bright-field photographs of developing soybean embryos sectioned longitudinally (*40*). (**A**) Late globular stage embryo. (**B**) Heart stage embryo. (C to E) Localization of the major Kunitz trypsin inhibitor mRNA (Kti3) in longitudinal sections of soybean embryos (*40, 45*). In situ hybridization data are similar to those shown in (*40*). Photographs were taken by dark-field microscopy. (**C**) Late globular stage embryo. (**D**) Late globular to transition stage embryo. (**E**) Heart stage embryo. (**F**) Bright-field photograph of a tobacco globular stage embryo sectioned longitudinally. (G and H) Localization of GUS activity within transgenic tobacco globular stage embryos. Photographs of whole-mount embryos were taken by bright-field microscopy. (**G**) Embryo contains a chimeric *Kti3/GUS* gene (*47*). (**H**) Embryo contains a chimeric *lectin/GUS* gene (*48*). (**I**) Localization of GUS mRNA within a transgenic tobacco globular stage embryo similar to that shown in (H) containing a *lectin/GUS* gene (*48*). Abbreviations: EP, embryo proper; S, suspensor; C, cotyledon; A, axis; En, endosperm; CE, chalazal end; MPE, micropylar end; and Pd, protoderm.

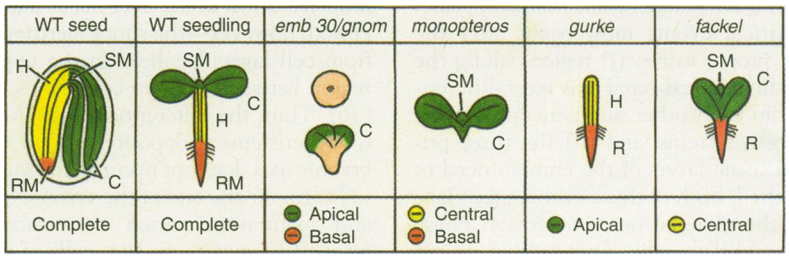

Fig. 4. Schematic representations of *Arabidopsis* pattern mutants [adapted from (*9*)]. The green, yellow, and orange colors delineate the apical, central, and basal regions, respectively. Strong (upper) and weak (lower) *gnom* phenotypes are depicted (*9, 15*). Abbreviations: WT, wild-type; RM, root meristem; SM, shoot meristem; C, cotyledon; h, hypocotyl; and R, root.

detectable GUS activity within the suspensor (Fig. 3H) (*48*). GUS mRNA localization studies with longitudinal globular embryo sections indicated that the *lectin* promoter activity occurs specifically within the ground meristem cell layer of the equator region (Fig. 3I) (*48*).

These experiments indicate that both the longitudinal apical-basal and radial tissue–type axes of a globular embryo are partitioned into discrete transcriptional territories (*1*). The longitudinal axis of the embryo proper contains at least three nonoverlapping transcriptional territories: (i) the chalazal region, (ii) the equator region, and (iii) the micropylar region (Fig. 3, F to H). The suspensor represents an additional transcriptional domain along this axis (Fig. 3, G and H). Each tissue layer of the radial embryo-proper axis also has a distinct transcriptional program (Fig. 3, E, G, and I). Transcriptional activity within these layers, however, appears to be established in a territory-specific manner—that is, ground meristem cells within the equator region activate promoters distinct from those within ground meristem cells of the micropylar region, and vice versa (Fig. 3, E to I) (*47, 48*). These results suggest that a prepattern of different transcriptional regulatory domains has been established in the globular embryo before the morphogenetic events that lead to differentiation of cotyledon and axis regions at the heart stage (Figs. 2 and 3, A and B). Presumably, each transcriptional domain sets in motion a cascade of events leading to the differentiation of specific embryo regions later in embryogenesis.

Table 2. Examples of *Arabidopsis* mutants that have defects in embryo development. Several hundred *Arabidopsis* embryo-defective mutants have been identified by both chemical and T-DNA mutagenesis. Most of these mutants can be obtained from the Arabidopsis Biological Resource Center (arabidopsis+@osu.edu).

Mutant class	References
Pattern mutants	
emb30/gnom	*8–11, 15*
monopteros	*9, 12*
gurke	*9*
fackel	*9*
Cell-type differentiation mutants	
keule	*9*
knolle	*9*
Suspensor transformation mutants	
twin	*73*
sus1	*67*
sus2	*67*
sus3	*67*
raspberry1	*44*
raspberry2	*44*
Meristem differentiation and identity mutants	
shoot meristemless	*36*
embryonic flower	*60*
short-root	*61, 62*
hobbit	*61, 62*
Maturation program mutants	
lec1-1/lec1-2	*44, 76–78*
lec2	*77*
fus3	*79, 80*
abi3	*84, 85*
Seedling lethality mutants	
fus1/cop1	*91, 94*
fus2/det1	*91, 95*
fus6/cop11	*91, 96*
fus7/cop9	*91, 97*

Hormones Affect Embryo Morphogenesis

What physiological events cause the embryo proper to initiate cotyledons and become bilaterally symmetric during the globular-heart transition phase (Fig. 2)? Several experiments implicate a class of plant hormones, the auxins, in this morphogenetic process (*49–52*). Auxins, such as indoleacetic acid (IAA), are involved in a number of plant activities, including photo- and gravitropism, apical dominance, and vascular cell differentiation (*2*). Embryos in plants as diverse as bean and pine synthesize auxins (*49*) and transport them in a polarized, basipetal direction from the shoot meristem to root tip along the embryonic axis (Fig. 2) (*50*). The highest auxin levels occur at the globular stage of embryogenesis (*49*). Agents that inhibit polarized auxin transport either block the transition from the globular to heart stage completely (*51*) or prevent the bilateral initiation of cotyledons at the top of the globular embryo (Fig. 2) (*52*). For example, auxin transport inhibitors cause carrot somatic embryos to remain spherically shaped and develop into giant globular embryos (*51*). By contrast, zygotic embryos of the Indian mustard (*Brassica juncea*), an *Arabidopsis* relative, fail to initiate two laterally positioned cotyledons when treated with auxin transport blockers in culture (*52*). A cotyledon-like organ does form, but as a collar-like ring around the entire upper (apical) region of the embryo (*52*). Treated Indian mustard embryos resemble those of the *Arabidopsis pin1-1* mutant, which has a defect in polarized auxin transport (*52, 53*). These results suggest that auxin asymmetries are established within the embryo-proper region of globular stage embryos (Fig. 3, A and F) and that these asymmetries contribute to the establishment of bilateral symmetry at the heart stage (Figs. 2 and 3, A and B).

Plant Embryos Form from Regions That Develop Autonomously

The longitudinal axis of a mature plant embryo is made up of several regions that are designated as apical, central, and basal (Fig. 4) (*9*). The apical region contains the cotyledons and shoot meristem, the central region consists of the hypocotyl, or upper axis, and the basal region includes the lower axis, or radicle, and the root meristem (Fig. 4). These regions are derived ultimately from the terminal and basal cell lineages and are maintained in the young seedling (Figs. 2 and 4). Can regions along the longitudinal axis develop independently of each other? If territories established within the embryo proper of a globular stage embryo are specified autonomously, then the loss or alteration of cells within a territory should not affect the development of a contiguous region (Figs. 2 and 3). Several experiments suggest that this is actually the case—that is, a plant embryo forms from modules that develop independently of each other (*9–12, 54–59*).

Pattern mutations delete specific embryonic regions. Genetic studies have uncovered *Arabidopsis* mutations that alter the organization of the embryo body plan (Fig. 4 and Table 2) (*9, 10*). Embryo pattern mutations were found that delete (i) the apical region (*gurke*), (ii) the central region (*fackel*), (iii) the central and basal regions (*monopteros*), and (iv) the apical and basal regions (*emb30/gnom*) (Fig. 4) (*9*). Other embryo pattern–forming genes probably exist; however, they have not been identified in the genetic screens carried out thus far (*6, 8–10, 14, 44*). Each mutant gene acts zygotically, indicating that major specifiers of the embryo body plan act after fertilization has occurred (Fig. 2) (*9, 10*). In addition, the loss of a specific region, or combination of regions, does not affect the development of an adjacent neighbor (Fig. 4) (*9, 10*). For example, *gurke* embryos lack an apical region, but have normal central and basal regions (Fig. 4) (*9*). *monopteros* embryos, by contrast, have a normal apical region but are missing the central and basal regions (Fig. 4) (*9*).

Embryo pattern mutations alter the division planes that are established during the postfertilization-proembryo phase of embryogenesis (Fig. 2) (*11, 12*). Thus, deletions of mature embryo regions can be traced back to histological defects at the proembryo and globular stages (Fig. 2) (*11, 12*)—a result that provides functional evidence for the embryo fate maps proposed by the developmental botanists of the late 19th and early 20th centuries (*31, 32*). For example, *emb30/gnom* zygotes do not divide asymmetrically (Fig. 2) (*11*). Two similar-sized daughter cells are produced in the *emb30/gnom* embryo-proper region instead of the unequal-sized terminal and basal cells that are found in wild-type embryos (*11*). Later division events in *emb30/gnom* embryos are also highly variable and abnormal (*11*). By contrast, the *monopteros* division pattern is normal until the 8-cell embryo-

proper stage (Fig. 2) (*12*). During the *monopteros* globular-heart transition phase, lower tier cells of the embryo proper and derivatives of the hypophysis undergo abnormal divisions (*12*) (Fig. 2). *monopteros* upper tier embryo-proper cells, however, develop normally—that is, defects at the basal end of the embryo proper do not affect events that occur at the apical end (Fig. 2) (*12*). The result is a mutant embryo that has cotyledons and a shoot meristem, but is missing the hypocotyl and root regions (Fig. 4) (*12*). These observations indicate that genes which are responsible, in part, for the establishment of the embryo body plan direct territory-specific cell division patterns during early embryogenesis. Abnormal divisions in one embryonic territory do not affect the division pattern of an adjacent territory—that is, cell lineages giving rise to specific regions of the mature embryo develop autonomously.

Agrobacterium T-DNA–tagged *emb30/gnom* alleles have led to the isolation of the *EMB30/GNOM* gene (*15*). This gene encodes a protein that is related to the yeast Sec7 secretory protein, is active throughout the plant life cycle, and is involved in cell division, elongation, and adhesion events required at many stages of sporophytic development, including embryogenesis (*15*). Thus, *EMB30/GNOM* does not appear to establish the embryonic cell division pattern directly, but most likely facilitates a pattern set by other genes. What these pattern-forming genes are and how they interact with downstream genes that mediate events required for the differentiation of autonomous regions along the longitudinal axis remain to be determined.

Mutations affect meristematic zones of the embryo longitudinal axis. *Arabidopsis* mutations have been identified that affect the differentiation of the shoot and root meristems during embryogenesis (Table 2) (*36, 60–62*). These mutations target a specific meristem and have no other effect on embryonic development. For example, *shoot meristemless* fails to differentiate a shoot meristem during embryogenesis and produces seedlings without leaves (*36*). *embryonic flower*, on the other hand, generates a shoot meristem at the top of the embryonic axis (Figs. 2 and 4) (*54*). Remarkably, *embryonic flower* seedlings produce flowers rather than leaves, indicating that the fate of the shoot meristem is altered during embryogenesis—a floral meristem is specified rather than a vegetative shoot meristem (*60*). By contrast, *short root* and *hobbit* affect root meristem development (*61, 62*). These mutations lead to abnormal root development after seed germination, indicating that the root meristem is altered, but not eliminated, during embryogenesis (*61, 62*). Taken together, these results indicate that there are genes which regulate the specification, organization, and fate of meristems that differentiate during embryogenesis. Genes controlling meristem development most likely act downstream of embryo-region specifiers, such as *monopteros* and *gurke* (Fig. 4) (*9, 10, 12*), in the regulatory hierarchy needed to form a plant embryo.

Meristem mutants characterized to date indicate that the shoot and root meristems function autonomously—that is, they do not affect the differentiation of contiguous domains such as the cotyledons or hypocotyl (*36, 60–62*). Meristems, therefore, represent independent submodules within the apical and basal regions of the embryo (Fig. 4). A major question is what effect, if any, do cells adjacent to the shoot and root meristems have on their development? That is, are cell signaling events involved in specifying the root and shoot meristems within a specific embryonic region, or do the meristems differentiate autonomously? The *laterne* mutant provides one answer to this question (*9*). *laterne* seedlings lack cotyledons but produce leaves indicating that a shoot meristem is present (*9*). Thus, cotyledons are not required for the differentiation of the shoot meristem during embryogenesis.

Promoter elements interpret embryo region–specific regulatory networks. One consequence of the modular organization of a plant embryo is that genes which are active throughout the embryo must intersect with several region-specific regulatory networks—that is, the promoters of embryo-specific genes are required to sense and interpret the transcriptional regulatory machinery unique to each autonomous region (Fig. 4). For example, Kti3 mRNA accumulates within the axis region early in soybean embryogenesis, but does not accumulate within the cotyledons until much later (Fig. 3, C to E) (*40*). Thus, discrete promoter elements should exist that are responsible for interacting with transcription factors produced by separate regulatory circuits.

A *Kti3/GUS* gene with 2 kb of 5′ flanking sequence is transcribed in all regions of a mature transgenic tobacco embryo (Fig. 5A) (*54*). Deletion of 0.2 kb from the 5′ end eliminates *Kti3/GUS* transcriptional activity within the embryo radicle region (Fig. 5B) (*54*). Deletion of another 1 kb eliminates *Kti3/GUS* transcription within the cotyledons and shoot meristem, but still permits transcription to occur within the hypocotyl region (Fig. 5C) (*54*). These results indicate that discrete cis-acting domains are required for the transcriptional activation of the *Kti3* gene within the radicle, hypocotyl, and cotyledon-shoot meristem regions of the embryo. Promoter analysis of the soybean *Gy1* storage protein gene (*63*) also uncovered a regulatory domain that directs transcription to the cotyledons and shoot meristem of a transgenic tobacco embryo (Fig. 5D) (*54*). These data, and those of others (*55–59*), indicate that unique transcription factors are active within each embryonic region and that these factors interact with specific promoter elements. The combination of these elements and factors gives rise to the transcriptional pattern of the whole embryo (Fig. 5A). Identification of transcription factors that interact with region-specific DNA elements should provide reverse entry into the independent regulatory networks required for specifying each autonomous region of a plant embryo.

Cell Differentiation and Morphogenesis Can Be Uncoupled in Plant Embryos

What is the relation between cell differentiation and morphogenesis in plant embryos? Are processes required for tissue differentiation along the embryo radial axis coupled to those that specify autonomous re-

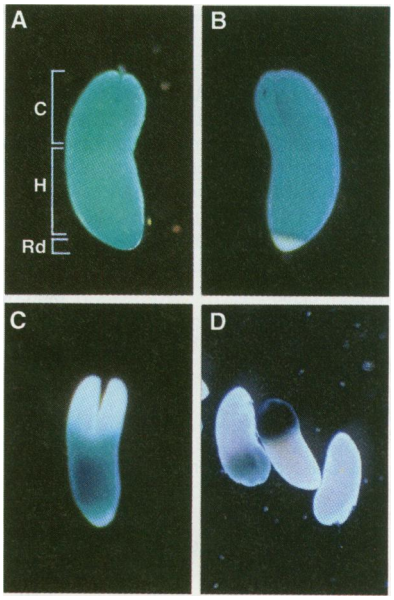

Fig. 5. DNA elements program transcription to specific embryonic regions. Maturation stage embryos were hand-dissected from transgenic tobacco seeds containing the *E. coli GUS* gene fused with different soybean seed protein gene 5′ regions (*54*). GUS activity in whole-mount embryos was localized as outlined in (*47*), and photographs were taken with the use of dark-field microscopy. (A to C) *Kti3* gene upstream regions fused with the *E. coli GUS* gene. (**A**) A 2-kb *Kti3* gene 5′ fragment. (**B**) A 1.7-kb *Kti3* gene 5′ fragment. (**C**) A 0.8-kb *Kti3* gene 5′ *CaMV/GUS* fragment. (**D**) A minimal *CaMV*/GUS gene-promoter cassette (*59*) fused with a 0.36-kb *Gy1* gene 5′ region (−446 to −84) (*63*). The white embryo results from the segregation of a single *Gy1/GUS* gene within this transgenic tobacco line and represents a negative control. Abbreviations: C, cotyledon; H, hypocotyl; and Rd, radicle.

gions of the longitudinal apical-basal axis (Fig. 2)? Studies of *Arabidopsis* embryo pattern mutants suggest that these processes are not necessarily interconnected. For example, a *fackel* embryo does not have a hypocotyl, but epidermal, ground meristem, and vascular tissues differentiate within cotyledon and radicle regions (Fig. 4) (9). Thus, the loss of one embryonic region does not affect the formation of tissue layers within the remaining regions (Fig. 4) (9–12). A more direct question, however, is whether mutant embryos that arrest early in embryonic development and remain globular-shaped differentiate the specialized cell and tissue layers that are found in organ systems of a mature, wild-type embryo.

A maturation stage *Arabidopsis* embryo has specialized epidermal, storage parenchyma, and vascular cell layers within both the cotyledon and axis regions (Fig. 6A). These tissues are derived from the three primary cell layers that are specified along the radial axis of a globular embryo (Fig. 2), and express specific marker genes late in embryogenesis. For example, EP2 lipid transfer protein mRNA accumulates specifically within the epidermal cell layer (Fig. 6B) (46, 64, 65), and 2S2 albumin mRNA accumulates within storage parenchyma cells (Fig. 6C) (64, 66). Neither mRNA is detectable within the vascular layer (Fig. 6, B and C) (46, 64–66). Collectively, the EP2 and 2S2 mRNAs can identify embryo epidermal and storage parenchyma cell layers and, by default, the inner vascular tissue as well (Fig. 6, B and C).

An *Arabidopsis* embryo mutant, designated *raspberry1*, was identified in a screen of T-DNA–mutagenized *Arabidopsis* lines (Table 2) (44). This mutant fails to undergo the globular-heart transition (Fig. 2), has an embryo-proper region that remains globular-shaped throughout embryogenesis, and does not differentiate cotyledons and axis (Fig. 6, D and E) (64). *raspberry1* embryos also have an enlarged suspensor region (Fig. 6, D and E) (64). *raspberry2* (44) and *sus* (67) embryo-defective mutants also have phenotypes similar to that of *raspberry1* (Table 2). Surprisingly, *raspberry1* embryos accumulate EP2 and 2S2 marker mRNAs in their correct spatial context along the radial axis of both the embryo-proper and suspensor regions (Fig. 6, F to I) (64). EP2 mRNA accumulates along the outer perimeter of *raspberry1* embryos (Fig. 6, F and G), whereas 2S2 mRNA accumulates within interior cells (Fig. 6, H and I) (64). By contrast, EP2 and 2S2 mRNAs do not accumulate detectably within the central core of *raspberry1* embryos (Fig. 6, F to I) (64). Similar results were obtained with *raspberry2* embryos (Table 2) (44, 64).

These mRNA localization studies indicate that specialized tissues can differentiate within the embryo-proper region of mutant embryos that remain globular shaped, and that these tissues form in their correct spatial contexts. A similar conclusion was inferred from histological studies of the *sus* mutants (67). Tissue differentiation, therefore, can take place independently of morphogenesis in a higher plant embryo, implying that morphogenetic checkpoints do not occur before cell differentiation events can proceed (68). It does not follow, however, that morphogenesis can occur without proper cell differentiation events. *Arabidopsis* embryo mutants that alter tissue-specification patterns have abnormal morphologies (Table 2) (9). For example, *knolle* embryos lack an epidermal cell layer and have a round, ball-like shape without defined apical and basal regions (Table 2) (9). Similarly, the carrot *ts11* somatic embryo mutant has a defective protoderm cell layer and fails to undergo morphogenesis (38, 39). Addition of either an extracellular chitinase (69) or *Rhizobium* nodulation factors (lipooligosaccharides) (70) can rescue the *ts11* mutant. Lipooligosaccharide nodulation factors have been shown to be signal molecules involved in the differentiation of *Rhizobium*-induced root nodules (70). This suggests that in carrot somatic embryos, the protoderm cell layer may provide signals necessary for embryogenesis to occur (69, 70). Taken together, experiments with mutant embryos that have defective cell layers suggest that specification of the radial axis needs to occur in order for a normal shoot-root axis to form. An important corollary is that cells within the radial axis probably interact with each other (9).

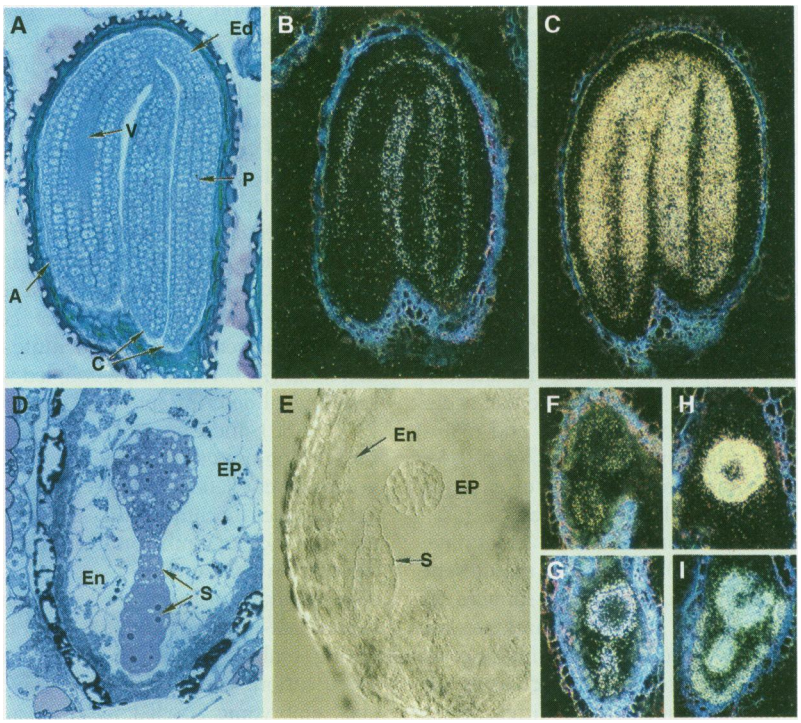

Fig. 6. Localization of cell-specific mRNAs within wild-type and mutant *Arabidopsis* embryos. (**A**) Bright-field photograph of a late-maturation stage *Arabidopsis* embryo sectioned longitudinally (64). (B and C) In situ hybridization of labeled RNA probes with longitudinal sections of *Arabidopsis* maturation stage embryos (64). Photographs taken by dark-field microscopy. (**B**) Localization of *Arabidopsis* EP2 lipid transfer protein mRNA (65). (**C**) Localization of 2S2 albumin mRNA (66). (D and E) *Arabidopsis raspberry1* embryos (44). Embryos were harvested at a stage when wild-type embryos within the same silique were in late maturation [as in (A)]. (**D**) Longitudinal section of a *raspberry1* embryo. The photograph was taken by bright-field microscopy. (**E**) A *raspberry1* embryo photographed with Nomarski interference optics. (F to I). In situ hybridization of labeled RNA probes with *raspberry1* embryo longitudinal sections (64). (**F** and **G**) Localization of EP2 lipid transfer protein mRNA (65). Localization experiments with *raspberry2* embryos, which have larger suspensors than *raspberry1* embryos (44), confirmed that EP2 mRNA is present only in the suspensor outer cell layer (64). (**H** and **I**) Localization of 2S2 albumin mRNA (66). In situ hybridization with serial sections through an entire *raspberry1* embryo confirmed that there is an inner core of cells with no detectable 2S2 mRNA (64). Abbreviations: A, axis; V, vascular tissue; Ed, epidermis; C, cotyledon; P, storage parenchyma; Ep, embryo proper; S, suspensor; and En, endosperm.

Suspensor Cells Have the Potential to Generate an Embryo

One intriguing aspect of the *raspberry1* embryo is its large suspensor (Fig. 6, D and E). *raspberry1* suspensors are indistinguishable from wild-type during the early stages of embryogenesis (Fig. 2) (64). Later in seed development, when neighboring wild-type embryos undergo maturation, cell proliferation events cause the *raspberry1* suspensor

to enlarge at its basal end (Fig. 6, D and E) (64). EP2 and 2S2 mRNAs (46, 65, 66) accumulate in the *raspberry1* suspensor (Fig. 6, F to I) with a spatial pattern similar to that which occurs in mature, wild-type embryos (Fig. 6, B and C) (64). These cell-specific mRNAs do not accumulate detectably in wild-type suspensors, or in *raspberry1* suspensors early in embryogenesis (64). These results indicate that the *raspberry1* suspensor has entered an embryogenic pathway and that an embryo proper–like, radial tissue axis has been specified.

Other *Arabidopsis* embryo mutants have suspensor abnormalities similar to that of *raspberry1*, including *raspberry2*, and *sus* (Table 2) (14, 35, 44, 67, 71). Although the extent of suspensor enlargement varies, all of these mutants have morphological defects in the embryo proper (14, 35, 44, 67, 71). Mutant embryos that resemble wild-type, but arrest at specific embryonic stages, do not have aberrant suspensors (44, 64). Disruptions in embryo-proper morphogenesis, therefore, can induce an embryo proper–like pathway in terminally differentiated suspensor cells, a result first observed by the embryo-proper ablation experiments of Haccius 30 years ago (72). The *Arabidopsis twin* mutant represents a striking example of the embryogenic potential of the suspensor region (73). *twin* causes subtle defects to occur in embryo-proper morphology, generates a second embryo within the seed from proliferating suspensor cells, and results in twin embryos that are connected by a suspensor cell bridge (73).

The nature of mutant genes that affect suspensor development, such as *raspberry1*, *sus*, and *twin*, is not known. These genes are probably not involved in suspensor specification events, because a normal suspensor forms before induction of the embryo-proper pathway in mutant embryos (44, 64, 67, 71, 73). They reveal, however, that interactions occur between the suspensor and embryo-proper regions. One possibility is that the embryo proper transmits specific inhibitory signals to the suspensor that suppress the embryonic pathway (35, 67, 71–73). Alternatively, a balance of growth regulators might be established within the entire embryo that maintains the developmental states of both the embryo-proper and suspensor regions. Disruptions of such signals would cause the suspensor to take on an embryo proper–like fate, a result analogous to embryo induction in differentiated sporophytic or gametophytic cells (37–39).

The Embryo Is Reprogrammed Late in Embryogenesis

How does the embryo prepare for dormancy and postembryonic development (Fig. 1)? Late in embryogenesis a maturation program is induced that is responsible for (i) synthesizing large amounts of storage products, (ii) inducing water loss and a desiccated state, (iii) preventing premature germination, and (iv) establishing a state of dormancy (Fig. 2 and Table 1) (2, 26–28). Several specialized gene sets, such as those encoding storage proteins and late embryo abundant (lea) proteins, are activated transcriptionally during maturation and then repressed before dormancy (26, 74, 75). These gene sets remain transcriptionally quiescent during seed germination when germination-specific and postembryonic gene sets are transcribed (26, 55, 75). Genetic studies with *Arabidopsis* have identified some of the genes that regulate processes required for embryo maturation and dormancy, including the expression of storage protein and lea genes (Table 2).

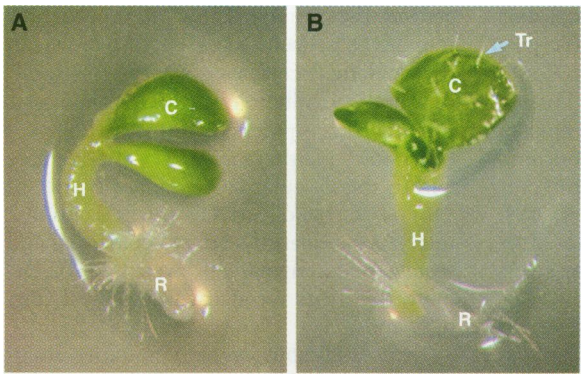

Fig. 7. An *Arabidopsis leafy cotyledon* seedling. An allele of the *lec1* gene (76, 77), designated as *lec1-2*, was identified in a screen of T-DNA–mutagenized *Arabidopsis* lines (44, 78). (A and B) Bright-field photographs of *Arabidopsis* seedlings (78). (**A**) Wild-type seedling. (**B**) *lec1-2* seedling. Abbreviations: C, cotyledon; H, hypocotyl; R, root; and Tr, trichome.

Leafy cotyledon mutations disrupt embryo maturation. A mutant gene class, designated as *leafy cotyledon* (*lec*), has been identified that causes defects in the cotyledon cell differentiation process and in maturation-specific events such as storage product accumulation, desiccation tolerance, and maintenance of dormancy (Table 2) (76–80). *lec* mutations transform cotyledons of embryos and seedlings into leaf-like structures (76–80). Wild-type cotyledons do not have trichomes (Fig. 7A). Trichomes are present only on leaf, stem, and sepal surfaces in wild-type plants and are markers for postembryonic development (81). By contrast, *lec1* cotyledons have trichomes on their adaxial surface which differentiate during embryogenesis from the protoderm cell layer (Fig. 7B) (76–78). *lec1* cotyledons have other leaf-like characteristics including stomata, mesophyll cells, and an absence of protein and lipid storage bodies (77). The axis region of *lec1* embryos also lacks storage organelles, indicating that the wild-type *LEC1* gene functions in both embryonic organs (77).

In addition to the leaf-like cotyledon transformation, *lec1* embryos (i) germinate precociously about 5% of the time, (ii) have leaf primordia emerging from their shoot apex, and (iii) fail to survive desiccation (76–78). Other *lec* mutants, such as *lec2* and *fus3*, have phenotypic characteristics that overlap those of *lec1* (77–80). For example, *fus3* and *lec1* embryos are almost indistinguishable from each other (77). *lec2* embryos, by contrast, have leaf-like cotyledons, but are desiccation tolerant, do not germinate precociously, and have normal levels of storage bodies in their axis region (77). This indicates that leafy cotyledons can occur without corresponding defects in desiccation and dormancy, and that wild-type *LEC* genes are activated independently in each embryonic organ system (77). A corollary is that gene networks that function in desiccation and dormancy are independent of those responsible for cotyledon cell specialization.

Molecular studies with *lec* embryos have indicated that the transcription of maturation-specific genes, such as those encoding storage proteins, is greatly reduced (80). Conversely, the transcription of germination-specific genes, such as isocitrate lyase, is activated (78). These data, coupled with the histological descriptions of *lec* embryos (Fig. 7) (76–80), indicate that *LEC* genes function during maturation to activate genes involved in cotyledon cell specialization, storage product accumulation, induction and maintenance of embryonic dormancy, and desiccation tolerance (76–80). *LEC* genes also simultaneously suppress the manifestation of leaf-like characteristics in cotyledons during embryogenesis, including trichome specification. In the absence of *LEC* products, embryonic cotyledons enter a default state and express leaf-like characteristics (76, 77), many of which develop normally in postgermination, wild-type cotyledons (82).

Abscisic acid maintains embryonic dormancy. The plant hormone abscisic acid (ABA) is involved in several plant processes, including senescence, responses to environmental stresses, growth inhibition, and maintenance of a dormant state (83). Exogenous ABA prevents seed germination as well as the precocious germination of embryos in culture. In addition, *Arabidopsis* mutants that either cannot synthesize ABA or fail to respond to ABA germinate preco-

ciously (84, 85). These data indicate that ABA prevents germination while seeds are still dormant or present within siliques.

Three mutant *Arabidopsis* loci, designated as *abi1*, *abi2*, and *abi3*, have been identified that result in ABA insensitivity and allow seed germination to occur in the presence of ABA (84). In addition to precocious germination, *abi3* embryos are also desiccation intolerant and defective in the synthesis of maturation-specific mRNAs, such as those encoding storage proteins and lea proteins (85, 86). This indicates that the wild-type *ABI3* gene is a positive regulator of gene networks leading to storage product accumulation, desiccation, and dormancy (85, 86). The *ABI3* gene encodes a transcription factor (87) related to the corn viviparous-1 protein, which can activate the transcription of chimeric *GUS* reporter genes containing embryo maturation–specific gene promoters (88). Thus, *ABI3* mediates its effect on embryo maturation at the transcriptional level. Because *aba* embryos, which fail to synthesize ABA, are normal with respect to most maturation-specific processes (84–86), ABA probably does not regulate the *ABI3* gene (84, 86). Rather, *ABI3* probably operates through an ABA-independent pathway that is involved in establishing desiccation and dormancy states late in embryogenesis (85, 86). Failure to achieve these developmental states results in ABA insensitivity—that is, ABA responsiveness is a consequence of *ABI3* gene activity (85, 86). *lec* embryos are sensitive to ABA and fail to germinate if ABA is present (77, 78). Thus, *LEC* and *ABI3* genes are part of independent regulatory networks that control embryo maturation–specific events.

In contrast to *abi3* embryos, *abi1* and *abi2* embryos carry out normal maturation-specific events, including the activation of storage protein and lea genes (84–86). *ABI1* and *ABI2* loci, therefore, are involved only in maintaining embryonic dormancy. The *ABI1* gene encodes a Ca^{2+}-dependent phosphatase with similarity to serine-threonine phosphatases involved in signal transduction processes (89). In response to ABA, the ABI1 phosphatase might counteract phosphorylation events required for the initiation of root meristem cell division, resulting in a dormant embryonic state (89).

Regulatory loci required for postembryonic development are active late in embryogenesis. A large number of *Arabidopsis fusca* mutants have been identified that accumulate anthocyanins, or red pigments, on their cotyledons late in embryogenesis (10, 90–92). With the exception of *fus3* (Table 2), embryogenesis is normal in *fusca* mutants (10, 90–92). After germination, however, *fusca* seedlings fail to develop into mature flowering plants (10, 90–92). Several *fusca* genes have been shown to be alleles of *constitutive photomorphogenic (cop)/deetiolated (det)* genes that function in light-regulated development during seed germination (Table 2) (91–97). The products of *COP/DET* loci appear to suppress light-regulated gene activities in the dark and activate these activities in the presence of light by way of a light-mediated signal transduction pathway (91–97). Because defective *cop/det* genes are detected as *fusca* embryo mutants, their wild-type *COP/DET* alleles are active during maturation. Thus, regulatory genes expressed at the end of embryogenesis prepare the plant for life after the seed.

Conclusion

Plant embryogenesis provides a vital bridge between the gametophytic generation and postembryonic differentiation events that occur continuously in the shoot and root meristems of the sporophytic plant. As such, plant embryos must establish the polarized sporophytic plant body plan and enable the young plant to survive harsh environmental conditions and a period of below-ground growth from seeds. Plant embryos are simpler than their animal counterparts, yet they must carry out the same developmental tasks—that is, form a three-dimensional organism with specialized regions, compartments, and cell-types from a single-celled zygote. These events occur early in plant embryogenesis and are poorly understood. Genetic studies in *Arabidopsis* have begun to reveal genes that are necessary for embryogenic events such as pattern formation, cell differentiation, and organ development.

The precise molecular mechanisms responsible for specifying different cell lineages early in plant embryogenesis are not known. A major void in our knowledge concerns the events that occur within the egg cell and in the early embryo after fertilization. In this respect it is crucial to obtain molecular markers in order to follow the specification events that take place during early embryogenesis and gain entry into regulatory networks that are activated in different embryonic regions after fertilization. Although a large amount of progress has been made in recent years in understanding how a plant embryo forms, there is still a long way to go. The next few years should be a very exciting time to study plant embryos.

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laboratories of R. B. Goldberg [University of California, Los Angeles (UCLA)], J. J. Harada (UC Davis), R. L. Fischer (UC Berkeley), J. L. Zimmerman (University of Maryland, Baltimore), and A. Koltunow (University of Adelaide, Australia). A total of 5822 T-DNA–mutagenized *Arabidopsis* lines (*13*) were screened for mutants defective in embryogenesis (*6*, *8*, *14*). Sixty-six heterozygous lines were identified that segregated seeds in a ratio of 3 wild-type to 1 mutant within their siliques, indicating that they contained embryo-defective alleles that were inherited as simple Mendelian recessive genes. The *raspberry1*, *raspberry2*, and *lec1-2* genes were uncovered in three different heterozygous lines.

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98. We express our gratitude to J. Harada, R. Fischer, J. Okamuro, D. Jofuku, L. Zimmerman, A. Koltunow, T. Laux, L. Perez-Grau, and G. Drews for many critical and stimulating discussions of plant embryogenesis. We thank B. Timberlake, R. Chasan, and E. Davidson for insightful comments on this manuscript. We gratefully acknowledge K. Brill, B. Phan, and T. Zeyen for diligent typing of this manuscript and M. Kowalczyk for the colorful art work. We dedicate this article to Professor E. H. Davidson, who has provided intellectual leadership and direction to the field of development biology over the past 25 years.