Signaling in plant embryogenesis
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Embryogenesis is a critical stage of the sporophytic life cycle during which the basic body plan of the plant is established. Although positional information is implicated to play a major role in determining embryo cell fate, little is known about the nature of positional signals. Recent studies show that the monopterus and hobbit mutations reveal signaling during patterning of the embryonic axis. The LEAFY COTYLEDON1 and PICKLE genes have been implicated to play important roles in controlling embryo development.

Introduction

Embryogenesis in higher plants begins with a double fertilization event in which two sperm nuclei fuse with the egg cell and central cell nuclei, respectively, to initiate embryo and endosperm development. The zygote then undergoes a series of cell divisions and differentiation events to produce the mature embryo [1–4]. As shown in Figure 1, a mature dicot embryo consists of at least five major organs/structures along its apical–basal axis, the shoot apical meristem, the cotyledons, the hypocotyl, the root, and the root apical meristem. Along its radial axis, the embryo comprises three primary tissue systems: the outer protoderm (epidermis); the middle ground meristem; and the inner procambium (vascular tissue).

A great deal is known at a descriptive level about how embryos form. Figure 1 illustrates embryo development in the representative dicot plant, Brassica napus, and shows that the embryonic organ and tissue systems are formed sequentially during embryogenesis [5–7]. First, the zygote undergoes an asymmetric division, producing cells with different fates (Figures 1a and 1b). The apical cell goes on to produce the embryo proper, and the basal cell generates the hypophysis and the suspensor, a transient organ that plays structural and physiological roles in embryo development. Second, periclinal cell divisions within the eight-celled octant stage embryo generate the first embryonic tissue, the protoderm (Figure 1d). Later divisions within globular embryos produce elongated cells in the center of the embryo that define the procambium and the ground meristem (Figure 1f). Third, at the globular stage, division of the uppermost cell of the suspensor defines a lens-shaped cell, the hypophysis, that gives rise to part of the quiescent center and the initials of the central root cap (Figure 1e). Fourth, during the transition from the globular-stage to the heart-stage of embryogenesis, localized cell divisions generate the cotyledons, embryonic storage organs. Fifth, by the torpedo-stage, both the shoot and root apical meristems are visible as organized structures (Figure 1g).

Although progress has been made in establishing a framework for understanding the mechanisms involved in embryo development, many questions remain. Little is known about the molecular processes that induce embryo formation. The morphogenetic events that underlie formation of the embryo also remain to be defined. Because cell fate is largely determined by positional information in plants, signaling and intercellular communication play critical roles in development [8,9]. In this article, I review primarily information published during the past year that provide examples of signaling during plant embryogenesis. Due to space limitations, this review will not be comprehensive but will focus on specific examples.

Induction of embryo development

Although zygotic embryogenesis is induced by the fusion of the sperm and egg nuclei, plant cells can initiate embryo development without fertilization. For example, cultured somatic and male gametic cells can be induced to undergo somatic and microspore embryogenesis, respectively [10]. Non-zygotic embryos can also arise through apomixis, a suite of asexual reproductive processes that occurs in the ovule [11]. The ability to initiate embryogenesis without sex shows that fertilization is not required for embryo development. The recently identified FIE (FERTILIZATION INDEPENDENT ENDOSPERM) and FIS (FERTILIZATION INDEPENDENT SEED) genes have been implicated in the suppression of endosperm development before central cell and sperm nuclei fusion [12,13]. By analogy, it is possible that embryo development is similarly suppressed until fertilization. The specific signals that initiate embryogenesis have not been identified.

Somatic embryos have been studied, in part, to define the signals that initiate embryogenesis. Somatic cells cultured in the presence of auxin are thought to acquire the competence to undergo embryogenesis such that subsequent culturing in the absence of auxin induces embryo formation [14]. Recent studies show that the rate of Arabidopsis somatic embryo formation is increased compared with wild-type when primordia timing, clavata1, and clavata3 mutant seedlings are cultured [15]. Because these mutants have enlarged shoot apical meristems, the authors propose that undifferentiated meristem cells may be more responsive to auxin signaling to acquire embryogenic competence. The enhanced rate of somatic embryo formation, therefore, may reflect the larger number of meristem cells in the mutants. The critical question of what processes underlie embryogenic competence,
Figure 1

Embryo development in a representative dicot plant. (a) Single-celled zygote of *Brassica napus*. (b) Two-celled embryo comprising the apical cell (ac) that gives rise to most of the embryo proper and the basal cell (bc) that becomes part of the root apical meristem and the suspensor. (c) The O’ line in this octant-stage embryo represents the first transverse division of the embryo proper and separates the apical (a) and central (c) embryonic domains. The basal domain (b) is from the uppermost cell of the suspensor. (d) Periclinal divisions in the octant-stage embryo give rise to the protoderm (p). (e) The hypophysis (h) derives from the uppermost cell of the suspensor in a globular-stage embryo. (f) In this transition-stage embryo, the three major embryonic tissue systems are visible: protoderm (p), ground meristem (gm), and procambium (pc). (g) Five major organs/structures along the apical–basal axis of this early torpedo-stage embryo are cotyledons (cot), shoot apical meristem (sam), hypocotyl (h), root (r), and the root apical meristem (ram). Adapted from West and Harada [1].

However, remains unanswered. It is also not known if auxin plays a role in initiating zygotic embryogenesis.

Recent work has provided insight into signaling that occurs during the induction of somatic embryogenesis [16••]. Cultured carrot cells, destined to undergo somatic embryogenesis, are marked with a cell wall antigen recognized by the antibody, JIM8. These competent cell divide asymmetrically, producing one daughter cell with the antigen and the other without, and the epitope-free cells ultimately form somatic embryos. Thus, embryogenic competence is associated with a cell wall antigen that segregates asymmetrically during cell division. A surprising result is that the daughter cell that does not label with JIM8 requires a soluble signal from JIM8-positive cells, thought to be an arabinogalactan protein, to continue its development into embryos. Thus, signaling between cells of different fates is required for the initiation of somatic embryogenesis, although it is not known if a similar requirement obtains for zygotic embryos.

Two recent studies provide insight into the factors that control embryo formation. First, the *Arabidopsis* LEAFY COTYLEDON1 (*LEC1*) gene that encodes a subunit of the CCAAT box binding transcription factor is a central regulator of both early and late embryogenesis [17••]. The *LEC1* gene is required for the maintenance of suspensor cell fate, specification of cotyledon identity, initiation and maintenance of the maturation phase, and suppression of germination. Analyses of the *lec1* mutant phenotype and of the gene’s expression pattern suggest that *LEC1* functions exclusively during embryogenesis. Ectopic expression of *LEC1* during postembryonic growth is sufficient to induce embryo development in vegetative tissues. Ectopic *LEC1* gene expression inhibits postembryonic development of transgenic plants, causes embryonic cotyledon-like organs to emerge at the position of leaves, and activates embryo-specific genes. Remarkably, as shown in Figure 2, embryo structures occasionally form on the surfaces of leaves without culturing or pretreatments with auxin. These results suggest that *LEC1* plays a critical role in embryo development by directly controlling processes during early and late embryogenesis. *LEC1* may accomplish these diverse functions by establishing an embryonic environment that permits embryo formation to occur.

Mutations of the *Arabidopsis PICKLE* (*PKL*) gene reveal that gibberellic acid signaling may also be important in controlling embryogenesis [18••]. Roots of *pkl* mutant seedlings accumulate lipids normally found in seeds and express embryo-specific genes. Moreover, culturing of excised *pkl* roots on hormone-free media generates somatic embryos. Thus, the *pkl* mutation either induces or fails to suppress embryonic programs in seedling roots. Because the mutant phenotype is suppressed by gibberellic acid, *PKL* is thought to function in a gibberellic acid signaling pathway. The implication is that gibberellic acid signaling may be required to switch root cells from an embryonic to a vegetative fate. Thus, genetic manipulations of *LEC1* and *PKL* gene expression establish a cellular environment that permits embryo formation to occur in the absence of hormone treatments.

**Pattern of the embryonic axis**

Analyses of seedling lethal mutants has led to the hypothesis that the early *Arabidopsis* embryo is divided into three domains — apical, central and basal [19,20•]. As shown in Figure 1, the apical region derives from the upper tier of an octant stage embryo. Although cell fate is generally determined by position, the apical region usually contributes the shoot apical meristem and most of the cotyledons. Part
of the cotyledons, the hypocotyl, the root, and the root apical meristem initials arise from the central domain, a region that comes from the lower tier of an octant stage embryo. The basal region gives rise to the quiescent center of the root apical meristem and the central root cap cells. Basal region cells are descendants of the hypophysis, the uppermost cell of the suspensor. Additional support for the existence of these domains comes from studies showing that they correspond to transcriptional territories in globular stage embryos [21–23]. That is, specific genes, or their promoters, are active in subdomains of either the apical, central, or basal regions.

Recent analyses of genes required for embryonic axis formation suggest that signaling between embryonic domains may be essential for root formation. Seedlings with mutations in the MONOPTEROUS (MP) gene do not possess the hypocotyl, root, root apical meristem, and root cap, suggesting that the gene is required for specification of the central and basal domains [24]. MP encodes a novel protein with structural similarities to DNA binding proteins, opening the possibility that MP is a transcription factor [25•]. Although mp mutants lack embryonic roots, as seedlings they can form adventitious roots. This result suggests a specific role for MP in embryonic root formation. The pattern of MP gene expression and the phenotype of adult mp mutant plants, however, indicate that MP plays a role in establishing vascular tissue organization both in embryos and postembryonic plants [26]. Specifically, the inability of mp mutant embryos to form elongated cells parallel to the axis in their central regions suggests that MP functions primarily in cell axialization required for the formation of vascular cell files. An implication of these results is that communication occurs between the central and basal domains of early Arabidopsis embryos. That is, defects in establishing the cellular organization of the central region of mp mutant embryos may disrupt the signaling required to specify the basal region. These results also suggest that MP may not function specifically in the establishment of embryonic domains. Rather, defects in specification of the central and basal regions may occur as a result of the mutation’s primary defect in organizing provascular cells.

Both the central and basal domains of the early Arabidopsis embryo contribute to root apical meristem formation, and signaling between the two regions may be critical. The HOBBIT (HBT) gene is required for correct formation of the hypophyseal cell of the early Arabidopsis embryo and, therefore, for root apical meristem formation [27••]. Unlike wild-type plants, hbt mutants do not make lateral roots, suggesting that the gene also functions postembryonically. Because the hypophyseal cell is the progenitor of the basal region, HBT may be required for either specification of the basal domain or for specific divisions of the hypophysis. Root apical meristem initials do not form in hbt mutants, though these initials are largely derived from the central region of the early embryo. These results provide an elegant second example of signaling between the central and basal domains in root formation [20•]. In addition to the hypothesis that communication from the central region is required for basal region specification, signaling from the hypophysis, or its derivatives, to the central region may also be required for the formation of root apical meristem initials.

What are the signals required for apical–basal patterning? Many studies of plant development, such as those cited above, indicate that positional information plays the major role in determining cell fate [8,9]. In nearly all cases, however, an understanding of what constitutes positional information is lacking. Studies of embryogenesis in the brown alga, Fucus, implicate at least two types of positional signals. Similar to many higher plants, Fucus zygotes divide initially into cells of different fates, the apical thallus cell and the basal rhizoid. Laser ablation studies of two-celled embryos show that intimate contact of thallus and rhizoid cells with their walls is required to maintain cell identity [28]. Thus, in Fucus, the cell wall is one positional determinant of cell fate. Other ablation studies also suggest that direct cell-to-cell contact and symplastic communication do not play major roles in cell fate determination at later stages of Fucus embryogenesis [29•]. Rather, apoplastic diffusible gradients are implicated as a primary determinant. Although in neither case was the signaling molecule identified, these results provide clues about the positional signals that may operate in higher plants.

Auxin has been implicated as at least one signal involved in apical–basal patterning in higher plants. For example, inhibitors of auxin polar transport have been shown to block embryos at the transition from globular-stage to heart-stage [30] and to prevent cotyledon separation in
developing embryos [31]. Similar phenotypes have been observed with Arabidopsis and tobacco mutants defective in auxin polar transport [32–34]. Two recent studies in wheat and Brassica juncea suggest that auxin may be involved in shoot and root apical meristem formation [35,36]. The mechanisms by which auxin acts during embryogenesis, however, remain to be determined.

Specification of organ identity

Signaling within the shoot apex appears to be important in the specification of organ identity. Mutations of Arabidopsis EXTRA COTYLEDON1 (XTC1), XTC2, and ALTERED MERISTEM PROGRAMMING1 (AMP1) genes cause the transformation of the first seedling leaves into ‘extra cotyledons’ [37•]. These ‘extra cotyledons’ have characteristics of embryonic cotyledons, such as reduced trichome numbers and the presence of lipid and protein bodies. The effects of these mutations are similar to those observed when immature Brassica napus embryos are germinated prematurely [38]. In precociously germinated Brassica napus embryos, leaf primordia that would normally develop into leaves are converted into cotyledon-like organs that lack trichomes and express embryo-specific genes [39]. The extra cotyledon phenotypes of the Arabidopsis xtc and amp mutants and the cultured Brassica napus embryos are attributed to their precocious activation of their shoot apical meristems during embryogenesis. Premature development of leaf primordia in an embryonic environment is thought to be sufficient to alter the identity of these organs, suggesting that these primordia respond to embryonic signals. The converse situation is observed with lec1 mutants whose cotyledons have trichomes, normally a leaf trait. In lec1 mutant embryos, premature activation of the postgermination program is thought to interfere with the specification of cotyledon identity [40]. These results emphasize that an embryonic environment has profound effects on plant development.

Conclusions

In recent years, progress has been made in unraveling some of the complexities of embryogenesis in higher plants. I have discussed primarily two areas that provide examples of signaling during embryogenesis. One conclusion to be drawn from recent work is that specific genes appear able to create a cellular environment that enhances embryo formation. The effects of the pkl mutation and the ectopic expression of LEC1 are similar in that both induce embryonic characteristics in the organs of postembryonic plants. Although the specific mechanisms by which these genetic alterations enhance embryo formation is not known, it is possible that the creation of an embryonic environment may be critical. How the induction of embryo formation by LEC1 and PKL relates to somatic and zygotic embryogenesis remains to be determined. It is interesting to note, however, that somatic embryo formation is much more efficient when immature zygotic embryos are used as a starting material rather than seedlings [15]. A second conclusion is that signaling between different embryonic domains may be required for precise patterning of the embryo’s apical–basal axis. These studies provide elegant examples of signaling in plant embryogenesis, although more work is needed to define, at a mechanistic level, the nature of the signals that constitute positional information.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


The cloning of LEAFY COTYLEDON1, a major regulator of Arabidopsis embryogenesis, and the consequences of ectopically expressing the gene postembryonically is described. LEC1 is a homolog of a subunit of the CCAAT box binding transcription factor. Results show that ectopically expressed LEC1 induces embryonic programs and embryo formation in vegetative tissues.

A novel mutation, pickle, that induces embryonic characteristics in the roots of Arabidopsis seedlings is described. Somatic embryo formation is induced when mutant plk roots are cultured. The ability of gibberelic acid to suppress the mutation suggests that PKL functions in a gibberelic acid signaling pathway that controls the transition of root cells from an embryonic to a vegetative fate.


A useful review that presents the authors' current hypotheses about the patterning of the embryonic axis.


This paper describes the cloning of the Arabidopsis MONOPTEROUS gene, a gene required for specification of the embryonic central and basal domains. The corresponding protein possesses motifs characteristic of DNA binding proteins. Analyses of MP RNA accumulation provide support for the conclusions that MP functions in the early organization of vascular tissue in embryonic and postembryonic plants.


This paper presents a detailed analysis of the effects of the hobbit mutation on root development. HBT is required for root apical meristem formation in embryos and postembryonic plants. Results suggest that HBT functions in the formation of the hypophyseal cell in early embryos and, therefore, in the specification of the basal domain.


Ablation studies in the brown alga Fucus suggest that cell walls and gradients of diffusible apoplastic signals are involved in pattern formation during embryo development.


32. Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y: Requirement of the auxin polar transport system in the formation of the hypophyseal cell in early embryos and postembryonic plants. Results suggest that these changes in organ identity result from the precocious activation of the shoot apical meristems of these mutants during embryogenesis.


Three mutations, extra cotyleden1 and 2 and altered meristem programing1, are shown to cause the first leaves of Arabidopsis seedlings to be transformed into cotyledon-like organs. The authors suggest that these changes in organ identity result from the precocious activation of the shoot apical meristems of these mutants during embryogenesis.

