Extracellular matrix and pattern in plant embryos: on the lookout for developmental information

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Cells of early plant embryos are totipotent yet follow different predetermined developmental pathways, producing embryos with well-defined autonomously developing tissues. Evidence is accumulating from a few model systems that information maintaining the determined state of a particular cell type is contained in a position-dependent manner in the extracellular matrix. This information can be released to signal back to the cell, thus controlling patterns of gene expression and influencing cell fate.

The ways in which different cell types in an organism originate, and the regulation of their patterns of proliferation, are key processes in developmental biology. Rapid progress is being made in animal systems towards an understanding of some of the underlying mechanisms. We are also witnessing significant progress in understanding how developmental pattern is initiated and maintained in plant embryos. This review begins by summarizing some essential and unique features of plant embryogenesis, and discusses some recent findings concerning the regulation of embryogenesis with particular attention to the fundamental role of extracellular factors.

Features of plant embryogenesis

Structural polarity in the higher plant embryo is established during oogenesis (for detailed reviews see Refs 1–3). In many types of plant embryo, such as *Arabidopsis*, this culminates in a highly asymmetric first division of the polarized zygote to produce two cells with very different developmental fates[1–3]: an apical cell that gives rise to the apical regions of the embryo and part of the root meristem, and a basal cell that produces the suspensor from which the developing embryo is suspended (Fig. 1). The suspensor divides further to produce an apical cell that contributes to the root meristem, although the rest of the suspensor does not contribute cells to the developing embryo. Though this pattern of early cell division is not universal, molecular and biochemical asymmetries are probably present in the oocyte and undivided zygote of higher plants[4]. In *Arabidopsis*, the shoot–root body pattern is laid down during the early embryonic divisions[1]. After the eight-cell stage, the embryo goes through globular and heart stages during which distinct domains are generated. However, organogenesis is developmentally separated from embryogenesis in plants. Floral, leaf and root tissues derive from specialized groups of cells (meristems) in the adult plant. There is no germline in plant development. The meristematic initials are laid down during early embryogenesis and form distinct zones of continuously differentiating cells in the adult plant.

Plant development: guiding and restraining the totipotent state

There are several examples that demonstrate that, unlike most animal cells, certain plant cell types are totipotent[5]. Despite their apparently rigidly determined state in the intact embryo, embryonic plant cells can show a remarkable tendency to dedifferentiate under certain conditions. An early observation of isolated multicellular embryos of the higher plant *Erianthus hiemalis* showed that ablation of the embryo caused redifferentiation of the remaining suspensor into a complete new embryo[6]. The *twin* mutant of *Arabidopsis* produces two whole embryos, one from the apical cell and one from the suspensor cell[7].

A clear example of totipotency is provided by the carrot somatic-embryo system[8], whereby suspension-cultured cells from adult tissue (e.g. hypocotyl) can be induced to dedifferentiate and redifferentiate into complete embryos in the presence of various growth regulators. The totipotency of particular cells in a plant probably underlies certain morphogenetic responses to a changing environment, such as tissue repair in response to injury. The occurrence of totipotency in a wide variety of plant cells as diverse as pollen microspores[9] to differentiated hypocotyl cells[8] suggests that this is a widespread feature of plant cells. While there are clear examples showing that certain plant cell types can become terminally differentiated (e.g. *Zinnia* tracheary cells[10]), the differentiated state of many plant cells is not necessarily fixed. Totipotency and the ability to dedifferentiate shows that cytoplasmic determinants in plant cells do not necessarily produce a permanent fixation of a differentiated state and are not the only factors involved in determining the fate of a cell. This may be related to the presence of cytoplasmic continuity in plants, allowing the movement of macromolecules between cells[11]. This raises questions about the nature of the mechanisms and cues permitting a plant cell to develop along a determined pathway while retaining the potential to dedifferentiate under appropriate conditions.

Position-dependent information and cell-fate determination

Carrot somatic embryos that have been surgically transected at different positions along the longitudinal axis are able to restore the excised tissues by reconfiguring the fates of cells at the surgical site[12]. This regeneration involves redifferentiation of cells in a proximodistal manner with the missing tissues closest to the remaining tissue being replaced first and the more distal tissue last (in this case the root apical meristem). This implies an important role for position-dependent information in the organization of body pattern.

Developmental studies of *Arabidopsis* mutants show that each region of the embryo can develop independently. Thus, in *monopteros* mutants, the central and basal regions of the embryo fail to develop but the

apical region develops normally[13]. Differentiation of cell types can occur in *Arabidopsis* mutants that are defective in various morphogenetic features suggesting that morphogenetic checkpoints are not required for differentiation to proceed. In *raspberry* mutants, development is blocked at the globular stage and the globular to heart-shape transition does not occur[1,14]. While overall morphogenesis is disrupted, preventing the formation of organs, such as the cotyledons and the embryonic axis (hypocotyl and radical containing the shoot and root meristems), position-dependent cell differentiation still occurs. As in the wild type, the three cell layers (epidermis, storage parenchyma and vascular) necessary for the formation of organs are arranged in a radially symmetrical pattern in *raspberry* embryos. These layers can be distinguished by the cell-type expression of specific mRNAs: a lipid-transfer protein mRNA specific for epidermal cells, and storage protein mRNAs specific for storage parenchyma. Thus, positional information specifying tissue types along the radial axis is not dependent on organ development or morphogenesis.

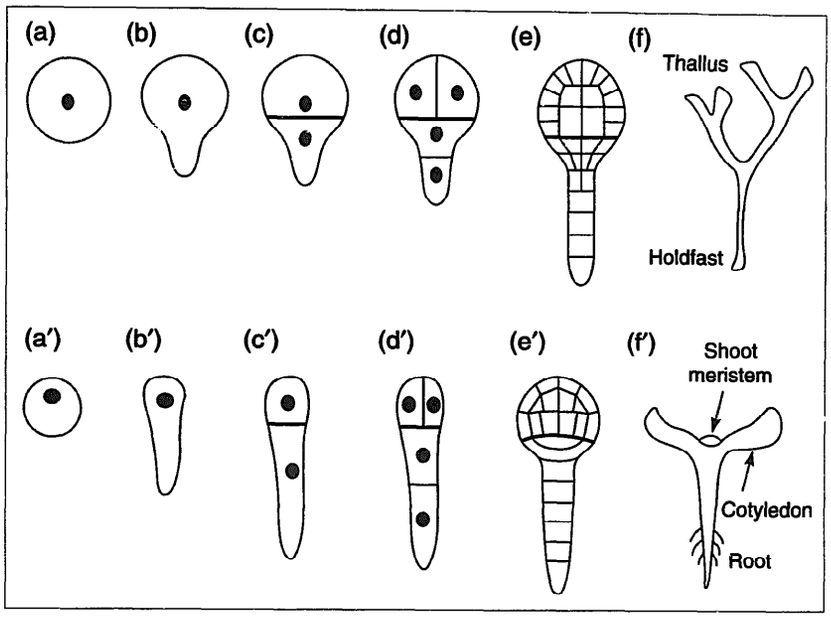

FIGURE 1. Patterns of early embryo cell division of *Fucus* (a–f) and *Arabidopsis* (a'–f').

Mutations in the *Arabidopsis FASS* gene[15] produce an abnormal pattern of cell division in the embryo resulting in abnormal morphogenesis (i.e. strong compression of the body axis and organs). Nevertheless, all tissue types and organs (e.g. leaves, flowers) are present in the seedling and the overall body plan is normal. These mutants showed a normal asymmetric first division and the work suggested that the *fass* mutation did not affect functional cell polarity, which is required for pattern formation. Morphogenesis and pattern formation appear to be independent[15]. While the mechanisms underlying these observations are poorly understood, they indicate strongly that position-dependent information specifying cell types in the embryo does not appear to be dependent on morphogenesis or organ formation.

Position-dependent information can come in a variety of forms: diffusible morphogens are well known in animal systems (e.g. Ref. 16) and probably have important roles in embryogenesis in animals and in plants. In *Drosophila*, mRNA gradients in the oocyte generate gradients of protein in the developing embryo. The *BCD* mRNA accumulates at the anterior pole and the *NOS* mRNA at the posterior pole[17]. Translation of the *BCD* mRNA produces a transcriptional activator of the *hunchback* gene at the anterior pole. At the posterior pole, the translation product of the *NOS* mRNA represses translation of the *HB* mRNA. The result is a gradient of the *hb* gene product, which itself regulates other transcription factors specifying pattern[17]. Asymmetric determinant distribution can act with intercellular signals as in *Caenorhabditis elegans* embryos where the GLP-1 protein is a membrane receptor involved in inductive interactions between different cell types and is distributed asymmetrically between anterior and posterior blastomeres via translational control of symmetrically distributed mRNA[17].

In the *Arabidopsis* embryo, the normally restricted development of the suspensor is dependent on normal growth of the rest of the embryo[18]. While the mechanisms involved here are unknown, it is probable that either an inhibitor of suspensor development is produced by the embryo proper[18] or that the suspensor responds in a specific way to a morphogenetic gradient established in the embryo. Plant growth regulators, particularly auxins, are implicated as one kind of diffusible messenger controlling embryogenesis in plants. Auxins are produced by meristems in shoot apices and are transported towards more basal regions in plant embryos[19]. In carrot somatic embryos, inhibition of polar auxin transport prevents the transition from the globular to the heart stage[20], while *Arabidopsis pin1-1* mutants that are defective in polar auxin transport fail to develop cotyledons[21].

Embryogenesis and the plant extracellular matrix

Embryos of the multicellular marine alga *Fucus* can be obtained in large numbers, develop autonomously in sea water and, in contrast to higher plant embryos, are directly accessible to experimental manipulation. Zygotes first form a labile axis of polarity in response to external cues, such as unilateral light[22,23]. After several hours, this axis becomes fixed and can no longer be reoriented by changing the direction of the external polarizing vector. There is compelling evidence for an ECM role in fixing the *Fucus*-zygote polar axis according to which fate-determining zygotic asymmetries are established. So far, there is no direct evidence for the involvement of cytoplasmic factors in determining the fate of rhizoid- and thallus-cell types. Maternal mRNA is

present in *Fucus* zygotes[24], but the zygote is initially apolar. However, total mRNA is preferentially accumulated at the thallus pole at around the time of axis fixation[25].

The ECM is also emerging with a leading role in the regulation of position-dependent differentiation in animals[26]. ECM proteins, such as laminin, fibronectin and vitronectin[26–28], have been shown to have an instructive role in development in animals via modulation of adhesion mechanisms involved in morphogenetic movements. Some of these mechanisms may involve direct cell–cell contact (e.g. Ref. 29). ECM proteins can also interact with extracellular signals, such as growth factors, modifying their action by binding and locally altering the growth-factor concentration[26]. Growth factors might be intrinsic components of some ECM proteins. Laminin, for example, contains repeated epidermal-growth-factor-like sequences[30]. ECM proteins bind to cells via cell surface glycoprotein receptors, such as integrins[26–28], which are implicated in a range of signal transduction processes leading to alterations in gene expression[31]. Despite some very obvious structural differences between the plant cell wall and the animal ECM, there are some surprising similarities and there may be some analogous developmental functions for the plant cell wall[32].

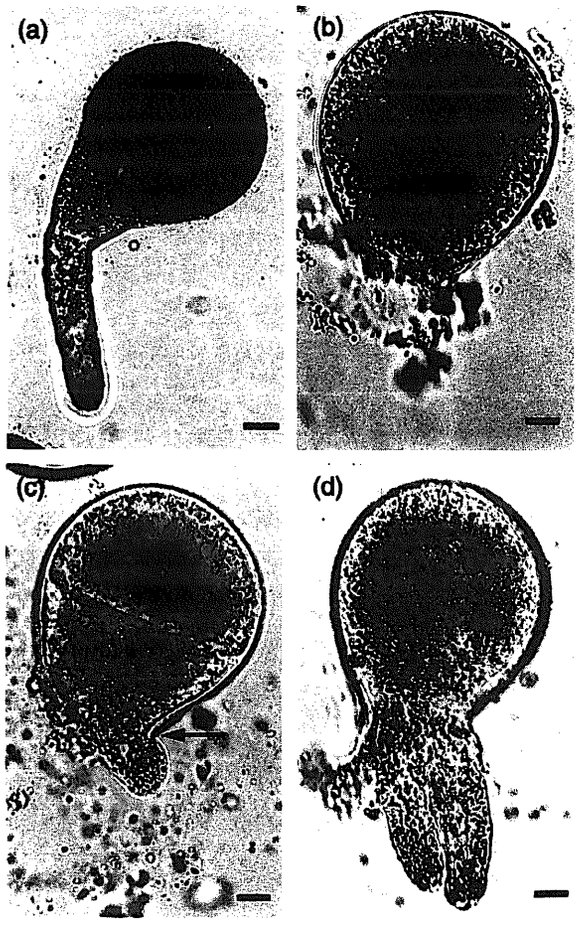

FIGURE 2. Effects of laser ablation of the *Fucus* rhizoid cell and selected portions of the wall of the rhizoid cell on the development of the thallus cell[7]. (a) A normally developing 3 d old embryo. (b) Division of the thallus cell into multicellular thallus tissue after ablation of the rhizoid cell and cell wall at the two-cell stage. (c) Redifferentiation of a thallus cell into a rhizoid cell after ablation of the rhizoid cell at the two-cell stage while leaving a portion of rhizoid wall intact on one side (arrow). (d) Redifferentiation of two thallus cells into rhizoids after ablation of the rhizoid cell contents but leaving the cell wall intact. Scale bar: 15 μm.

A role for the cell wall during development is suggested by the totipotency of protoplasts made from *Fucus*. The early division pattern of *Fucus* resembles that found in *Arabidopsis* – the first asymmetric division produces two differently determined cells: an apical thallus cell that develops into the photosynthetic and reproductive frond tissue, and a basal rhizoid cell that ultimately produces the tissues of the root-like structure (holdfast)[22,23,33] (Fig. 1). Dedifferentiation of either the rhizoid[10] or the thallus cells and repolarization and development into complete new embryos, can be induced by simply removing the protoplast from its surrounding cell wall (F. Berger and C. Brownlee, unpublished).

The presence of the cell wall is required for fixation of the polar axis in *Fucus*[34]. Axis fixation is accompanied by the formation of transmembrane bridges between F-actin, which accumulates at the site of rhizoid germination[22,35], and a vitronectin-like molecule that is localized at the cell wall of the germinating rhizoid[22,36]. There are also reports of the presence of integrins and vinculin in *Fucus* embryos[36]. Vitronectin is proposed to associate with a sulphated fucan (F2) in the cell wall[23,35,36] in a manner analogous to the complex between vitronectin or fibronectin and anionic polysaccharides, such as heparin, in animal cells[23]. The unique association of F2 with the wall of the rhizoid cell in *Fucus*[23] supports this. These components are proposed to form the basis of an axis-stabilizing complex (ASC)[36] having functions analogous to the focal adhesion sites involved in animal cell adhesion and differentiation[37]. It is tempting to postulate a signal-transduction role for these complexes, leading to differential gene expression between the rhizoid and thallus cells and we await further characterization of the ASC structure and function. Certainly at least two intracellular second messengers are locally elevated at these sites. Cytoplasmic Ca^{2+} is locally high at the growing rhizoid tip and at the rhizoid pole of the polarizing *Fucus* zygote before germination[38]. Lower cytoplasmic pH has also been reported at the apex of the growing rhizoid[39]. Vitronectin-like proteins are present in higher plants and are implicated in pollen-tube extension and membrane-wall attachment[40]. Recently, a vitronectin-like protein, PVN1, that has a high binding affinity for heparin, has been isolated from tobacco cells[41]. PVN1 shows little amino acid sequence similarity with animal vitronectin but, like the animal laminin receptor, shows a high degree of similarity with the eukaryotic translational elongation factor 1α. The functional significance of these observations remains to be determined.

Another class of ECM-associated molecules now thought to be involved in regulation of cell behaviour and embryogenesis are the diverse proteoglycans – the arabinogalactoproteins (AGPs)[42]. AGPs have been shown to control cell division in liverworts and higher plants[31]. The presence of a cell-surface AGP

correlates with somatic-embryo formation in carrot suspension cultures[43]. Moreover, AGPs secreted from embryonic tissue can promote embryogenesis in carrot somatic embryos and AGPs from non-embryogenic cells can suppress embryogenesis[44]. The recent cloning of a gene encoding an AGP protein[45] will facilitate further studies of functionality.

Releasable cell-wall signals in plants

As with carrot somatic embryos (see above; Ref. 20), position-dependent regeneration of excised cells also occurs in *Fucus* embryos, which can be dissected using laser microsurgery[33]. Ablation of the rhizoid cell in a two-cell embryo, leaving the thallus cell intact in its cell wall, results in a division pattern that is nearly identical to that of a thallus cell in the intact embryo (Fig. 2), in contrast to the dedifferentiation of the rhizoid cell or thallus cell isolated as a protoplast[33]. However, after several cell divisions, expansion of the thallus tissue and contact of a daughter thallus cell with the residual wall of the rhizoid cell invariably results in redifferentiation of the daughter thallus cell into a rhizoid cell (Fig. 2). The interpretation of this result is that the wall of the rhizoid cell contains position-dependent information that can switch the fate of a thallus cell in close proximity. Because the redifferentiating thallus cell also possesses a cell wall, the fate-determining factor(s) in the wall of the rhizoid cell must be able to diffuse across the wall of the thallus cell, but must be sufficiently long-lived to be retained by the isolated wall of the rhizoid cell. In the isolated protoplast, dedifferentiation is presumably a response to the absence of any cell-wall-associated fate-determining factors.

These findings are consistent with the operation of a further class of extracellular molecules that control certain aspects of plant morphogenesis. These extracellular molecules are oligosaccharides that can be released from the cell wall and that have regulatory properties (oligosaccharins). Oligogalacturonide oligosaccharins are known to be involved in phytopathogen defence along with oligoglucoside oligosaccharins[46]. Thin-cell-layer (TCL) explants of tobacco floral stem tissue can be induced to form flowers, roots or vegetative shoots depending on the composition of the culture medium. Oligogalacturonides from sycamore [generated by endopolygalacturonase (EPG) treatment of cell walls] can induce flower formation and inhibit root formation in TCLs[46]. Oligogalacturonides can also induce rapid plasma-membrane-associated signal transduction events in plant cells[47]. It has been postulated that endogenous EPG activity might be regulated developmentally and be tissue specific, with particular developmental functions[46].

A striking example of the role played by cell-wall proteins in the generation of developmental oligosaccharide signals comes from work with temperature-sensitive mutants (*ts11*) of carrot embryos. An endochitinase secreted by wild-type embryos could rescue *ts11* mutants from developmental arrest at the non-permissive temperature[48]. The substrate for chitinase is characterized by polymers of *N*-acetylglucosamine. Of the *N*-acetylglucosamine-containing oligosaccharides tested for activity, only the lipo-oligosaccharide root-nodulation factor, nod, produced by the symbiotic bacterium *Rhizobium*, was able to release mutants from developmental arrest[49]. Developmentally active analogues may be produced autonomously by chitinase activity in the cell wall in plant embryos[50].

Future prospects

Embryos of *Fucus* can be obtained in sufficient numbers to allow biochemical extraction of wall components. An endogenous factor has now been extracted from *Fucus* embryo cell walls, which, when reapplied to fresh two-cell embryos, causes fate-switching of thallus cells, i.e. thallus-to-rhizoid redifferentiation (F. Berger and C. Brownlee, unpublished). This releasable factor is probably the same as that involved in cell-wall-induced thallus-to-rhizoid redifferentiation in

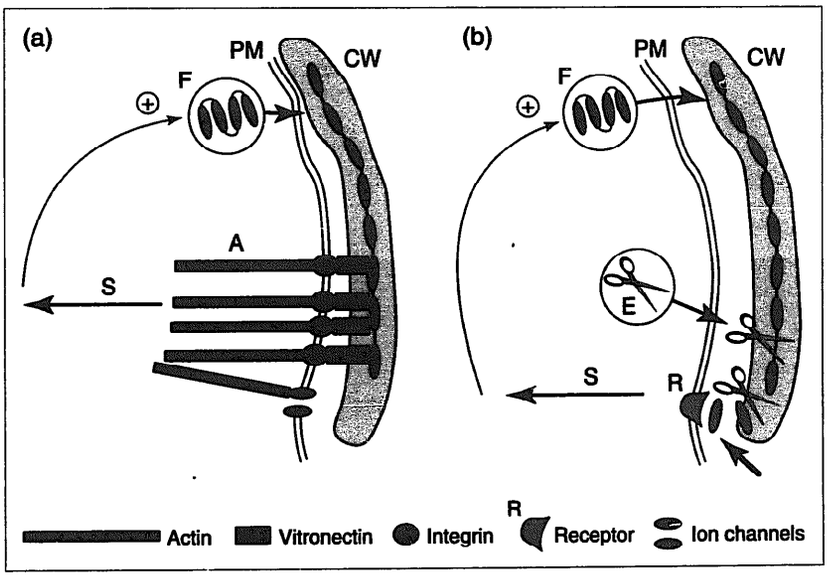

FIGURE 3. Two possible mechanisms by which position-dependent fate-determining information can be stored externally to the cell in developing plant embryos. (a) Shows the formation of localized focal adhesion complexes between the cell wall (CW), the plasma membrane (PM) and the cytoplasm. A position-dependent factor (F) is inserted into the wall matrix, where it can act as an anchorage for a vitronectin-like molecule, initiating (1) links across the plasma membrane with F-actin components of the cytoskeleton and (2) activation of signal transduction pathways (S) in the plasma membrane (e.g. ion channels) and the cytoplasm, which result in altered gene expression. The feedback loop is reinforced by continuous production and localized insertion of factor F. (b) In this model, a fate-determining factor (F) is locally inserted and bound into the wall matrix to be cleaved subsequently or released by the activity endogenous wall-modifying enzymes (E) involved in cell growth. Interaction with a plasma-membrane receptor (R) initiates signal transduction, leading to differentiation and production of more cell-type specific factors.

the microsurgery experiments described above[33]. This factor has not yet been characterized but at least two possibilities exist for its mode of action (Fig. 3). The factor may be a cell-wall-bound component of the ASC which, when preferentially inserted into the rhizoid wall during zygote polarization, might establish focal adhesions (Fig. 3). Signal transduction to the nucleus via focal-adhesion-associated tyrosine kinase[37] could activate rhizoid-specific genes, including those involved in production and insertion of cell-wall factors. Alternatively, secreted enzymes might cleave fragments from cell-wall polysaccharides or release diffusible signals from the wall matrix that could activate a plasma-membrane-receptor signal-transduction pathway. Characterization of the rhizoid inducer will allow us to distinguish between the two possibilities presented above.

Whatever the mechanism involved, it is clear that cells in the *Fucus* embryo insert information into their walls that can signal back to the cells to determine and maintain their fate (Fig. 3). It remains to be seen whether or not this mechanism is widespread among plants. If diffusible oligosaccharide or AGP-related signals are involved in determining cell fate, then their putative receptors need to be identified. While genetic analysis of the *Fucus* system is not yet possible, conventional molecular techniques can be applied to this system[51] and should enable analysis of the alterations in gene expression in response to fate-determining factors. Genetic and molecular approaches using *Arabidopsis* and carrot somatic embryos will allow progress towards characterization of the molecular machinery underlying the processes outlined here. The recent demonstration[52] of an *Arabidopsis* embryogenic mutant *emb30* defective in a gene with similarity to the yeast secretory control gene *SEC7* is an example of such progress.

The separation of fate-determining factors into the extracellular matrix and the totipotency of the protoplast may be one mechanism underlying morphogenic plasticity of plants. The concept that the extracellular matrix can regulate the development of plant embryos is relatively new. The likelihood that there are position-dependent releasable factors in the wall acting in a positive-feedback manner to maintain cell fate may allow us to address the problem of how order is established among developmentally unruly plant cells.

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