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REVIEW ARTICLE

Zygotic embryogenesis *versus* somatic embryogenesis

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Abstract

This review will summarize molecular and genetic analyses aimed at identifying the mechanisms underlying the sequence of events during plant zygotic embryogenesis. These events are being studied in parallel with the histological and morphological analyses of somatic embryogenesis. The strength and limitations of somatic embryogenesis as a model system will be discussed briefly. The formation of the zygotic embryo has been described in some detail, but the molecular mechanisms controlling the differentiation of the various cell types are not understood. In recent years plant molecular and genetic studies have led to the identification and characterization of genes controlling the establishment of polarity, tissue differentiation and elaboration of patterns during embryo development. An investigation of the developmental basis of a number of mutant phenotypes has enabled the identification of gene activities promoting (1) asymmetric cell division and polarization leading to heterogeneous partitioning of the cytoplasmic determinants necessary for the initiation of embryogenesis (e.g. *GNOM*), (2) the determination of the apical–basal organization which is established independently of the differentiation of the tissues of the radial pattern elements (e.g. *KNOLLE*, *FACKEL*, *ZWILLE*), (3) the differentiation of meristems (e.g. *SHOOT-MERISTEMLESS*), and (4) the formation of a mature embryo characterized by the accumulation of LEA and storage proteins. The accumulation of these two types of proteins is controlled by ABA-dependent regulatory mechanisms as shown using both ABA-deficient and ABA-insensitive mutants (e.g. *ABA*, *ABI3*). Both types of embryogenesis have been studied by different techniques and common features have been identified between them. In spite of the relative difficulty of identifying the original cells involved in the developmental processes of somatic embryogenesis, common regulatory mechanisms are probably involved in the first stages up to the globular form. Signal molecules, such as growth regulators, have been shown to play a role during development of both types of embryos. The most promising method for identifying regulatory mechanisms responsible for the key events of embryogenesis will come from molecular and genetic analyses. The mutations already identified will shed light on the nature of the genes that affect developmental processes as well as elucidating the role of the various regulatory genes that control plant embryogenesis.

Key words: Development, marker, mutant, somatic embryogenesis, zygotic embryogenesis.

Introduction

In Angiosperms, which represent the most recent evolutionary flourish of higher plants, double fertilization generates the embryo and the endosperm simultaneously, the joint development of which leads to a viable seed.

Since the female gamete is included in the embryo sac embedded in the ovule, studies of the formation of zygotic embryos have, until relatively recently, been carried out mostly using histological approaches. Plant development can be divided in two main steps: (1) embryogenesis *sensu stricto* beginning with the zygote and finishing at the cotyledonary stage and (2) the maturation of a seed followed by germination.

With regard to embryogenesis *sensu stricto*, recent studies on *Arabidopsis thaliana* have highlighted that the development of its embryo, passing through the globular, oblong, heart, torpedo, and cotyledonary stages and eventually to the mature dehydrated embryo, can be

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subdivided into a sequence of 20 different stages representing three major events (Jürgens and Mayer, 1992): (1) the first asymmetric division of the zygote, giving a small apical cell that generates the embryo and a large basal cell which will form the suspensor (Fig. 1), (2) specific pattern formation, which takes place in the globular embryo, (3) the transition to the cotyledonary stage which coincides with the initiation of the root primordium followed, in dicots, by the shoot primordium. At this stage, embryogenesis *sensu stricto* can be considered as completed. Thereafter, at the morphogenetic level, meristem activity is triggered and at the physiological level, the processes of growth, storage accumulation and maturation are initiated. Physiological changes, such as desiccation, and in most cases quiescence, complete the process of seed formation. The strengthening and lignification of the ovule integuments result in the formation of a tough coat which is necessary for seed conservation. At the end of this complex process, the angiosperm seed is particularly well-adapted to withstanding unfavourable environmental conditions.

Seeds may also be generated without fertilization through different pathways collectively referred to as apomixis (for a review, see Koltunow *et al.*, 1995). The term apomixis describes the formation of an embryo in the ovule from somatic cells. In sporophytic apomixis, the embryo arises directly from the nucellus or the integument of the ovule. In gametophytic apomixis, the

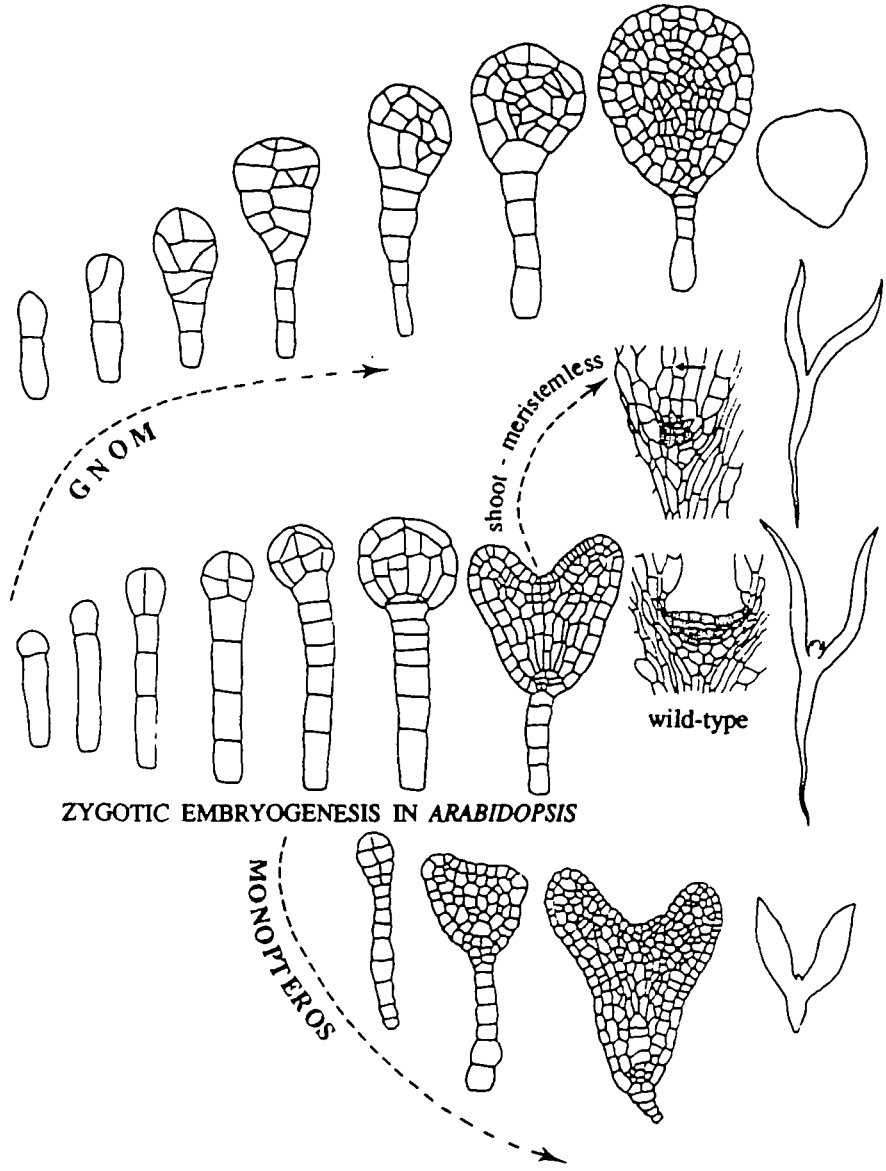

Fig. 1. Zygotic embryo development of wild phenotype, *gnom* mutant (Mayer *et al.*, 1993), *monopteros* mutant (Berleth and Jürgens, 1993), and *shoot-meristemless* mutant patterns (Barton and Poethig, 1993) in *Arabidopsis thaliana*. The arrow indicates the position where cotyledons meet in the absence of shoot apical meristem.

apomictic embryo sac originates either from megaspore mother cells by mitosis or uncompleted meiosis in diplospory, whereas the embryo sac originates from nucellar cells in apospory.

Embryogenesis can also arise from isolated somatic or gametic (microspore) cells (de Vries *et al.* 1988; Cordewener *et al.*, 1994), either naturally, as has been observed in *Kalanchoë*, where somatic embryos form spontaneously on the edge of leaves, or *in vitro* after experimental induction. The zygote is intrinsically embryogenic which is the opposite of somatic embryogenesis. The latter requires the induction of embryogenic competence in cells which are not naturally embryogenic. In some cases the process of embryogenesis occurs directly from microspores or somatic explants. Here, the developmental stage is of prime importance to enable the transition from somatic to embryogenic cells. However, the acquisition of embryogenic competence involves an induction phase for which there is no direct counterpart in zygotic embryogenesis (Fig. 2).

For a long time, somatic embryogenesis has been studied in cultures of carrot (*Daucus carota* L.) (Komamine *et al.*, 1990) and alfalfa (*Medicago sativa* L.) (Dudits *et al.*, 1991). Since several authors originally described the latter two systems using their own terminology, De Jong *et al.* (1993) subsequently provided a unified description of the terms employed. *Sus*pension cultures are often described as undifferentiated; 'unorganized' is probably a better term since in many cultures, subcellular populations retain features associated with specific differentiated cell types. The term 'embryogenic cell' would be limited to cells which have achieved the transition from a somatic cell to a stage where no further external stimuli are required to produce a somatic embryo (Komamine *et al.*, 1990). For instance, in carrot, the usual strategy to induce an embryogenic cell *sus*pension consists in exposing explants to a high auxin concentration, then to transfer cells to an auxin-free medium which triggers somatic embryo formation. Cells able to undergo embryo development generally appear as proembryogenic masses (PEM) composed of dense cytoplasmic small cells (Halperin, 1966) (Fig. 2). It is important to note that in most carrot embryogenic cultures, the percentage of cells which are actually embryogenic is rather low, typically 1–2% (de Vries *et al.*, 1988).

In summary, zygotic and somatic embryogenesis are complex phenomena which have been widely described in the literature (for a review, see Meinke, 1995). Despite the fact that these two different types of embryogenesis have been analysed on different model species and are therefore not directly comparable, some common features have been reported. In fact, both types of embryogenesis have been studied either using genetic approaches or by the identification of molecular markers correlated to specific developmental stages. This review will focus on a comparison between zygotic and somatic embryogenesis, without dealing with the use of apomixis in agriculture (Koltunow *et al.*, 1995). The sequence of events will be

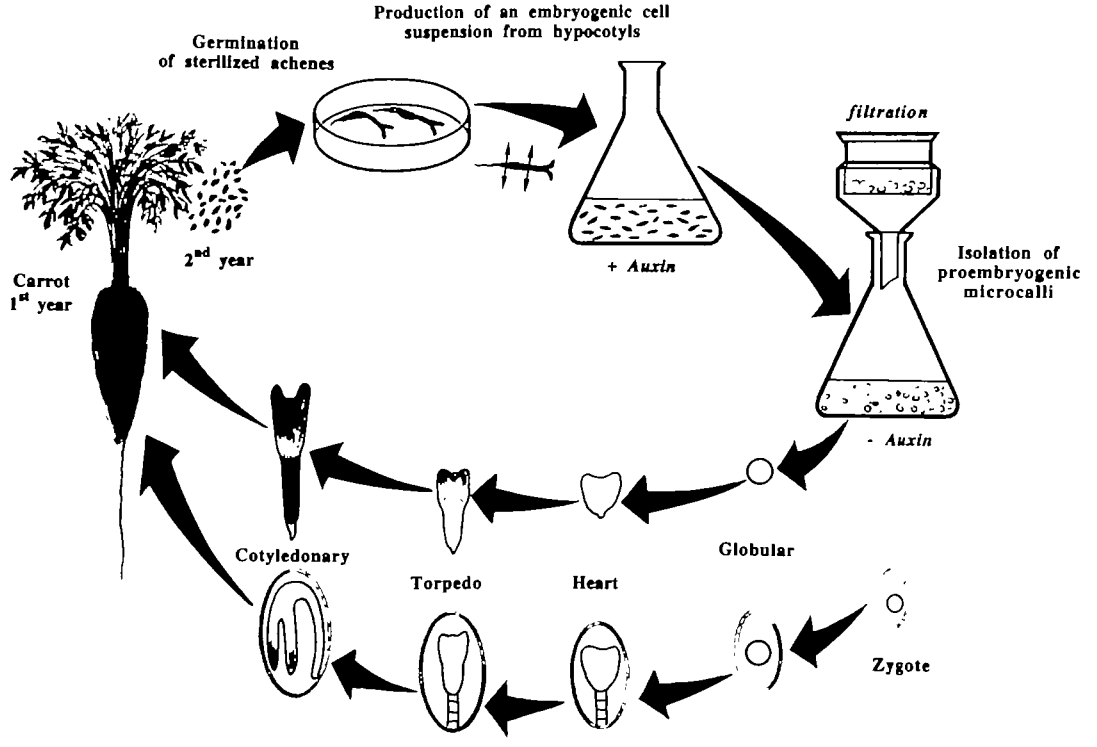

Fig. 2. Cartoon of zygotic and somatic embryogenesis using *Daucus carota* L. as an example.

described in cellular and genetic terms characteristic of zygotic embryo morphogenesis and the physiological maturation which occurs thereafter, focusing on asymmetric cell division, cell polarity and formation of the mature seed in dicots. References to monocots will only be included for features with no counterpart in dicots (e.g. the *viviparous* mutant in maize) (for a review, see Sheridan, 1988).

Polarity and asymmetric cell division

Zygotic embryogenesis

Plant development is initiated inside the female gametophyte (embryo sac). Movement of the two male gametes by siphonogamy into the egg cell and/or the embryo sac nuclei involves complex processes which will not be discussed here. Processes involved in the origin and differentiation of the embryo sac have already been reviewed (Newbigin *et al.*, 1993). The structural organization of the embryo sac at maturity is relatively similar between different plant species and leads to the concept of a female germ unit (FGU), composed of the egg cell, two synergids and the central cell (Dumas and Mogensen, 1993). The polarity of the egg cell is evident from the position of the nucleus at the cytoplasm-rich chalazal pole, while the micropylar pole is highly vacuolated (Russell, 1993). The microtubular cytoskeleton is particularly dense near the nucleus and exhibits neither a specific localization nor a precise orientation. The same observation applies to the actin microfilaments within the cytoplasm.

Early molecular events associated with fertilization are still being investigated, mainly due to the inaccessibility of the female gamete within the embryo sac. Studies aimed at unravelling the cellular mechanisms underlying these processes have been initiated only recently (Dumas and Mogensen, 1993; Russell, 1993). Fertilization in plants might be controlled by mechanisms, at the membrane level, similar to those described in animals. One of the most important events, is the opening of calcium channels, which induces activation of cell division (Kropf, 1992; Goodner and Quatrano, 1993). The ultrastructural organization of the zygote is greatly altered compared to that of the egg cell (Mansfield and Briarty, 1991; Mansfield *et al.*, 1991), both in its cytoplasm distribution and with respect to cell wall changes. The outcome of this organization is a reinforced cell polarity which directly bears upon the first asymmetric mitotic division of the zygote, giving two cells: one cell gives rise to the *sus*pensor and the other to the embryo proper. This mitosis seems to be strictly orientated, but a pre-prophase band showing the position of the future cell wall is not observed. This mechanism is only restored later when the embryo is developing (Webb and Gunning, 1991).

In short, the zygote shows some structural and functional characteristics which are intimately linked with the formation of the first embryonic developmental stages. These features can be used as points of reference in order to understand better the initiation of somatic embryogenesis.

The prerequisite of cell polarity and subsequent asymmetric cell division to induce cell differentiation has been established for many animal and plant species (Hyman and Stearns, 1992). The best model available for plants is the zygote of *Fucus*, which consists of a symmetric and apolar cell (Kropf, 1992; Goodner and Quatrano, 1993). The first asymmetric division is generally initiated by a gradient of light, the plane of division always being perpendicular to the light axis. Thus, the sequence of events leading to the establishment of the polarity axis, around which the development takes place, could be investigated (Kropf, 1994). Other stresses independent of light, such as asphyxia or gradients of calcium also induce *Fucus* embryogenesis, allowing comparison with the plant zygote, which is protected from light. In angiosperms, the polarity of both the female gamete and the zygote are essentially constitutive, implying a predetermination of the first division plane. Recent genetic studies of *Arabidopsis thaliana* development have shed further light on this process (Mayer *et al.*, 1993; Weigel, 1993). Genes controlling the formation of zygotic embryos have been identified in *Arabidopsis* (Errampalli *et al.*, 1991; Jürgens *et al.*, 1991; Meinke, 1991), in maize (*Zea mays* L.) (Clark and Sheridan, 1991) and in rice (*Oryza sativa* L.) (Nagato *et al.*, 1989; Kitano *et al.*, 1993). Six apical–basal pattern mutants have been described in *Arabidopsis*, namely *gnom*, *gurke*, *fackel*, *monopteros*, *rootless*, and *shoot-meristemless*. Three mutants showing radial defects (*keule*, *knolle* and *raspberry*) and three showing altered shape (*fass*, *knopf* and *mickey*) have been described (Weigel, 1993; Meinke *et al.*, 1994; Yadegari *et al.*, 1994) (Fig. 3).

Important genes in embryogenesis are often also expressed in vegetative tissues. Many embryo-defective mutants are likely to be altered in basic or so-called 'housekeeping' functions which first become essential during early stages of development. Embryogenesis mutants have thus been attributed to alterations in a splicing factor (Brown and Beggs, 1992), in a metabolic pathway (Schneider *et al.*, 1989), in a secretory pathway (Shevell *et al.*, 1994) or in a homeodomain transcription factor (Long *et al.*, 1996). The first known example of an embryonic lethal mutant with a biochemical defect is the biotin (*bio1*) auxotroph of *Arabidopsis*, which is defective in biotin synthesis and which produces mutant seeds unable to complete normal embryogenesis in the absence of supplemented biotin (Shellhammer and Meinke, 1990). On the basis of such findings, Meinke (1995) predicted that the distinction between housekeeping and regulatory

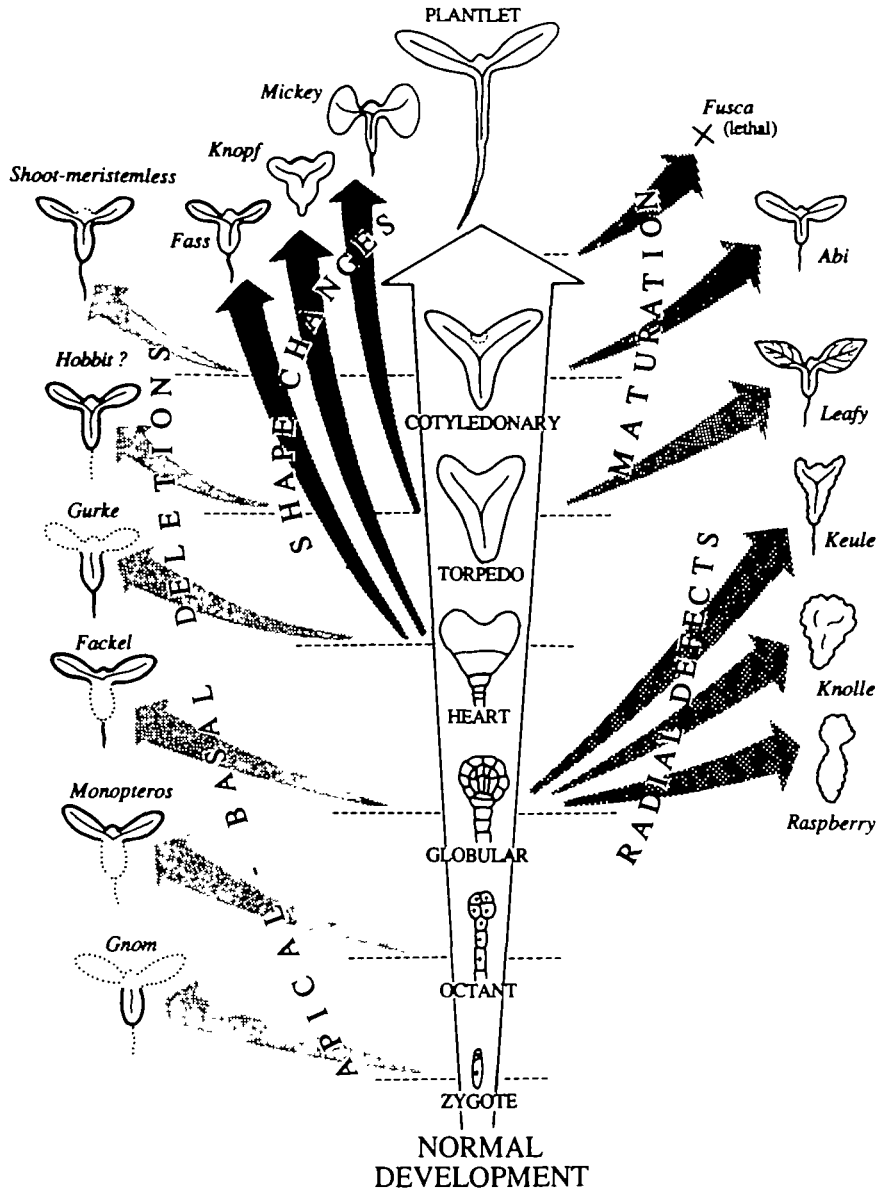

Fig. 3. Main plantlet phenotypes of apical–basal deletions (*gnom, monopteros, fackel, gurke, hobbit, shoot-meristemless*), radial defects (*keule, knolle, raspberry*), shape changes (*fass, knopf, mickey*) and maturation mutants (*fusca, abi*, leafy) in *Arabidopsis thaliana*. Bold line: normal phenotype; plain line: mutant phenotype; dotted line: deletions.

functions in plant embryogenesis will not be easy to establish.

In this respect, one of the previously mentioned mutants, *gnom*, was found to be very informative. Before dividing asymmetrically, the wild-type zygote elongates and microtubules become aligned perpendicular to the axis. The *gnom* zygote expands but does not elongate, producing an enlarged apical cell at the expense of the basal cell. A cytological study of 24 *gnom* mutant alleles revealed that the division of the zygote into a cytoplasm-rich apical part including the nucleus and a vacuolated basal part does not occur (Fig. 1). Therefore, the division plane is inclined to a variable degree when compared to that of the wild-type zygote, which is normally perpendicular to the longitudinal axis. This alteration in the division plane results in phenotypic variability, producing seedlings which are ball-shaped without root and cotyledons or cone-shaped with a well-defined apical–basal pattern (Mayer *et al.*, 1991). Thus, it appears that the *GNOM* gene affects early events in plant morphogenesis: e.g. the position of the cell division plane and the control of the unidirectional cell expansion (Lloyd, 1991). However, the *GNOM* gene was recently cloned and found to encode a protein that showed homology with the Sec7 protein of yeast, a cytosolic protein involved in a secretory pathway (Shevell *et al.*, 1994). The *GNOM* gene might thus affect

the synthesis and secretion of components such as glycoproteins required for proper cell division, cell elongation and cell–cell contact. The *GNOM* gene is expressed in both seedling and adult tissues as well as during embryogenic pattern formation and might, therefore, perform an ubiquitous cellular function rather than one specific to embryogenesis. The analysis of glycosylated proteins in *gnom* mutants and of the various domains of the encoded protein will help to clarify the role of this gene.

In animals such as *Caenorhabditis elegans*, the first asymmetric cell division is essential to form daughter cells which each differ in their cytoplasmic determinants and consequently follow different developmental fates. The analogy which can be drawn between this animal system and the plant zygote allows the inference that the change in the first asymmetric division of *GNOM* mutants leads to a variability of same type as far as the distribution of the determinants is affected (Mayer *et al.*, 1993).

Somatic embryogenesis

Despite the low percentage of embryogenic cells obtained in a cell *sus*pension, it seems that cell polarity and asymmetric cell division are involved in the initiation of somatic embryogenesis. In alfalfa, stimulation by auxins promotes asymmetric division in embryogenic cultivar-derived protoplasts, while protoplasts of non-embryogenic lines divide symmetrically (Bögre *et al.*, 1990; Dudits *et al.*, 1991). In carrot, the asymmetric division of auxin-induced embryogenic cells gives small daughter-cells from which arise somatic embryos (Komamine *et al.*, 1990). The cell-tracking system developed by Toonen *et al.* (1994) has provided a means, firstly, of identifying single cells which develop in somatic embryos at a frequency of 1% and, secondly, of following the fate of individual cells.

Although auxins, which are known to mediate the transition from somatic to embryogenic cells, are the principal agents used to induce embryogenesis, other stimuli are able to affect cell polarity or the division plane position. In white clover (*Trifolium repens* L.), cytokinin induces a change of the normal anticlinal division plane in favour of oblique periclinal divisions, thereby promoting the formation of embryogenic cells from the epidermis of immature zygotic embryos (Maheswaran and Williams, 1985). A pH shift (Smith and Krikorian, 1990) or an application of electric fields is also thought to affect cell polarity (Dijak *et al.*, 1986). Exogenous growth regulators probably modify the cell polarity by interfering with pH gradients or electrical fields around the cells (Dijak *et al.*, 1986; Smith and Krikorian, 1990). In the case of microspores, an alteration of the division plane is not required because of their high polarization during development. In fact, a symmetric division leads to a heterogeneous distribution of the still unknown cytoplasmic determinants which seem to be as essential as for zygotic embryogenesis (Hause *et al.*, 1993).

Plant cells respond to a variety of environmental and cellular signals, such as hormones and light, which are involved in the control of cell division, elongation, polarity and differentiation. For example, heat shock has been reported to cause the activation of mitogen activated protein (MAP) kinases in animal systems. MAPK is activated by MAP kinase kinase via phosphorylation, enabling MAPK to translocate to the nucleus and phosphorylate transcription factors which may allow cells to enter mitosis (Chen *et al.*, 1992). Several proteins differentially synthesized in microspore-derived embryogenic cultures have been reported in plants, among which two families were shown to belong to the heat shock family (Cordewener *et al.*, 1994). Recent studies provided evidence that, in higher plants, heterotrimeric G-proteins are involved in hormonal and light signal transduction, in defence responses and in the regulation of ion channel activities (Ma, 1994). Multiple MAPK cascades have been highlighted in yeast and animal cells: processes regulated by such pathways include the transduction of growth-stimulating signals in yeast (mating pheromone, pseudohyphal development, invasive growth, sporulation, cell integrity, and response to extracellular osmolarity) and in the *Drosophila* larvae (development of antero-posterior ends) (Ambrosio *et al.*, 1989; Levin and Errede, 1995). Whilst the exact role of phosphorylation in plant morphogenesis remains unknown, it seems likely, based on animal and microbial systems, that G-protein coupled receptors, G-proteins, MAP kinase cascades and other signalling components interact during embryogenesis. Thus, besides studies of embryogenesis mechanisms *per se*, investigations concerning the stimuli which trigger these mechanisms are also required (Tregear *et al.*, 1996).

As has already been underlined by De Jong *et al.* (1993), controlled cell expansion and asymmetric division are important mechanisms in the formation of embryogenic cells. Both are linked with the heterogeneous partitioning of cytoplasmic determinants subsequent to the formation of cell polarity. Thus, a heterogeneous partitioning seems ubiquitous for the initiation of embryogenesis.

Pattern formation

Zygotic embryogenesis

For a long time, the specific pattern formations of angiosperm embryos have occupied plant development biologists. Moreover, pattern formation seems to be sufficiently well-conserved to serve as a reference for phylogenetic classification. Studies on *Arabidopsis* revealed that, following the first asymmetric division, three more steps are determinant: (1) the octant stage composed of two levels

of four cells, (2) the formation of the protoderm and (3) the initiation of primordia (Fig. 1). The small apical cell produced after an asymmetric division neither enlarges during the three following divisions, nor during the subsequent periclinal divisions which give the cells which form the protoderm. The formation of the protoderm, which restricts cell expansion, is essential for the remaining developmental phases. This can be inferred from the development of the *emb101–1* mutant of *Arabidopsis*, in which uncontrolled cell expansion in the embryo leads to the production of enlarged cells which fill the whole seed (see Meinke in De Jong *et al.*, 1993).

Embryo mutations have been analysed as part of a saturating genetic screen. Among the putative 500–1000 essential genes (a number based on statistical extrapolations) required for survival and successful reproduction in *Arabidopsis* (Jürgens *et al.*, 1991), 40 appear to control the formation of embryo axis pattern elements (Mayer *et al.*, 1991). The analysis of mutants has shown that both apical–basal and radial patterns are independently established (Jürgens, 1995). Thus, radially arranged vascular, ground and epidermal tissues are present in mutants disturbed in the apical–basal pattern, such as *gurke* and *fackel* (Mayer *et al.*, 1991). In the same way, the organs of *ton* mutants, which are affected at the molecular level in their cell elongation and division plane alignment, occur at the correct relative positions, thus supporting the idea that polarized cell expansion and division plane alignment are not required for spatial development (Traas *et al.*, 1995). Recent observations on *raspberry* mutants, which fail to undergo the globular-heart transition and do not differentiate the cotyledons and axis, confirmed that tissue differentiation can take place independently of patterning in embryos of higher plants. In fact, embryos remain globular-shaped and the loss of embryonic regions does not affect the formation of tissue layers along the radial axis of globular embryos (Yadegari *et al.*, 1994). In other respects, *knolle* mutants, which lack an epidermal cell layer, show abnormal morphologies suggesting that specification of the radial axis is required prior to the formation of a normal apical–basal pattern (Mayer *et al.*, 1991). Recent cloning and sequencing of the *KNOLLE* gene revealed a similarity of the KNOLLE protein with syntaxins, a protein family involved in vesicular trafficking or in membrane fusion, suggesting that the *KNOLLE* gene might function in cytokinesis (Lukowitz *et al.*, 1996).

A significant feature that distinguishes plant development from animal development is that plants continue to generate new organs after embryogenesis. However, although plant cells show rigid clonal relationships (Scheres *et al.*, 1994), it has recently been shown that positional control, for the root meristem (van den Berg *et al.*, 1995) and the formation of somatic tissues (Poethig, 1989), is the most important factor in the determination of cell fate). The term 'probability map' has been suggested by Irish and Sussex (1992) to explain the lack of rigid cell lineage. The analysis of the consequences of mutations which affect the embryo apical–basal pattern such as *monopteros* has yielded results which are consistent with this hypothesis. Berleth and Jürgens (1993) reported that the *MONOPTEROS* gene seems to be required for the organization of the basal region of the plant since *monopteros* mutant seedlings lack basal structures. Based on the *gnom-monopteros* double mutant phenotype analysis, it appears that *gnom* is epistatic to *monopteros* (Mayer *et al.*, 1993). The *MONOPTEROS* gene operates later than the *GNOM* gene during development since segmentation is only modified from the octant stage onwards in these mutants. *Monopteros* mutants display four layers of cells instead of two (Fig. 1), each of them dividing as adjacent wild-type layers. Consequently, no root or hypocotyl emerges, since they are normally displayed in the wild-type plant at the previous level of segmentation. Furthermore, the *sus*pensor of *monopteros* mutants shows an aberrant organization, suggesting that the formation of the root primordium requires the participation of the hypophysal cell, namely the upper cell of the suspensor in contact with the embryo (Benfey and Schiefelbein, 1994). Thus, root development depends upon both organized segmentation and on cell–cell interaction. This point is of major importance, since primary root formation is often aberrant in somatic embryogenesis where the two steps described are not so strictly defined.

Somatic embryogenesis

In view of the difficulty of identifying the original cells actually involved in the developmental processes of somatic embryogenesis, few studies concerning the initial stages of development have been carried out. However, some clues are available, indicating that the initial patterns of both somatic and zygotic embryos can be variable. For instance, differences have been reported between the respective pattern types of tobacco zygotic and somatic embryos, the latter being larger in size (Stolarz *et al.*, 1991). Heterogeneity in somatic embryogenesis was reported by Toonen *et al.* (1994) who distinguished three developmental pathways of carrot somatic embryo morphogenesis, through an asymmetrical cell cluster, a symmetrical cell or an aberrantly shaped cell cluster. Whether the differential ability of somatic cells to become embryogenic reflects genetic differences or whether it is due to the presence of a specific responsive cell type is not clear (De Jong *et al.*, 1993). It is supposed that, given the precision of the zygotic embryo pattern formation programme, the first stages of both zygotic and somatic embryos could be similar or at least very close (Van Engelen and De Vries, 1992). A comparison between somatic and zygotic embryogenesis can only be established from the globular stage onwards, from which a

parallel evolution occurs, at least for some species. However, one cannot rule out the existence of either common regulatory mechanisms for the first stages or a flexibility of the embryogenic programme until the early globular stage.

Despite the similarities described between the two types of embryogenesis, two main differences exist, namely the lack of differentiation of endosperm and suspensor tissue in the case of the somatic system. Those are clearly two elements which play a key role in bringing about the successful maturation of the embryo in zygotic embryogenesis.

Suspensor/embryo interactions

Several embryo-lethal mutants exhibiting abnormal growth of the suspensor have been described in *Arabidopsis*. An inhibitory role of the embryo proper on the continued growth of the suspensor and a development of the suspensor throughout the embryogenic pathway when this inhibitory effect is removed were suggested as early as 1985 by Marsden and Meinke. Subsequently, the analysis of the mutant *twin* of *Arabidopsis* (Vernon and Meinke, 1994), which yields viable twin or triplet seedlings, the embryos of which arise from the transformation of cells within the suspensor, revealed that differentiated cells of the suspensor have the potential to duplicate the pathway of embryogenesis. *Twin* mutant embryos also exhibit developmental defects, indicating that the *TWIN* gene is required both for normal development and suppression of the embryogenic potential of the *sus*pensor (Vernon and Meinke, 1994). Polyembryony has also been observed in embryo-defective *sus* mutants (Schwartz *et al.*, 1994), which show an abnormal proliferation of the *sus*pensor cells resulting from a defect in the transition from the globular to the heart stage of the embryo proper. The proliferating suspensor exhibits a structure of embryonary type, accumulating storage proteins and lipid bodies characteristic of late embryogenesis (Schwartz *et al.*, 1994). The characterization of alleles of different *sus* genes (*sus1*, *sus2* and *sus3*) led the authors to suggest two models to explain the role of *SUS* genes. According to the first, the *sus* mutations, by disrupting morphogenesis, and hence the transition from a radial to a bilateral symmetry, might block the transmission of an inhibitory signal to the suspensor. According to the second model, *SUS* genes might produce a signal both promoting normal morphogenesis and maintaining the suspensor cell identity (Schwartz *et al.*, 1994). Recently reported results have shown that an allele of the *SUS2* gene might code for a protein homologous to the yeast PRP8 gene product, a spliceosome assembly factor which commonly functions during plant growth and development (Brown and Beggs, 1992).

During tissue culture, morphogenesis of somatic or androgenetic embryos occurs without the simultaneous development of a normal *sus*pensor. This suggests that either the suspensor does not play a crucial role in embryo development, or embryo culture condition interactions take over from those between the embryo and the suspensor.

Meristem formation

Zygotic embryogenesis

In dicots, the root primordium emerges at the end of embryo pattern formation, namely at the transition stage. The formation and activity of the root meristem seems to be co-ordinated by cell–cell interactions. The coordination is uncoupled by mutations such as the previously described *monopteros*, which controlled the patterning of the basal region, or *hobbit*, which specifically affects the formation of the root meristem (Aeschbacher *et al.*, 1994) (Fig. 3).

Downstream from genes controlling patterning within the apical region, such as *GURKE* (Torres-Ruiz *et al.*, 1996), act genes controlling particular areas, such as *SHOOT-MERISTEMLESS* (*STM*) which affects the formation of the shoot apical meristem (SAM) (Barton and Poethig, 1993; Endrizzi *et al.*, 1996). Furthermore, downstream from *STM*, the *WUSCHEL* (Endrizzi *et al.*, 1996; Laux *et al.*, 1996) and *ZWILLE* (Endrizzi *et al.*, 1996) genes are required in order to control the functional integrity of the SAM. The early phenotype of *gurke* mutants suggests that a medial shoot primordium and two lateral cotyledon primordia might be established simultaneously by partitioning of the apical region. Thus, abnormal growth reflects abnormal organization of the apical region (Torres-Ruiz *et al.*, 1996).

The *SHOOT-MERISTEMLESS* gene controls the initiation of the shoot meristem, but does not interfere with the development of the other parts of the embryo (Barton and Poethig, 1993; Long *et al.*, 1996) (Fig. 1). In *shoot-meristemless* (*stm*) mutants the area where the meristem would normally be forming, namely between the cotyledons, shows a normal organization, but does not proceed to the latter stages. This block appears to be definitive in as much as the tissues of *stm* mutants turn out to be unable to produce adventitious meristems. At the torpedo stage of both wild-type and *stm* mutant embryos, the presumptive shoot apical meristem (SAM) does not yet show the characteristic tunica-corpus organization (Vaughan, 1955; Medford *et al.*, 1992). This organization only becomes apparent at the bending stage of the cotyledons, namely late in embryogenesis after the cotyledons have developed. Despite the fact that *stm* mutants are blocked at the torpedo stage and unable to initiate SAM, they are able to produce cotyledons. Barton and Poethig (1993) suggested that a shoot meristem may not be

required to form cotyledons. This is in contrast to an alternative model for SAM initiation. This model assumes that the entire apical half of the globular stage is the SAM (Kaplan, 1969), and accordingly the first structures produced by SAM are cotyledons. Endrizzi *et al.* (1996) suggested that shoot meristem activity appears to precede its structural definition. This interpretation has been confirmed by the results on *STM* mRNA expression patterns which show that *STM* functions from the early globular stage (Long *et al.*, 1996). Again, this is consistent with the view that the apical region of the globular embryon represents the shoot meristem that initiates cotyledons (Kaplan, 1969).

The *STM* gene has been shown to code for a class I KNOTTED-like homeodomain containing protein. This regulatory gene plays an important role in shoot meristem function and thus represents the first gene involved in a specific regulatory function during plant embryogenesis (Long *et al.*, 1996). While the *STM* gene is required for the initiation of the embryonic shoot meristem, cell differentiation is controlled by the genes *CLAVATA1* (*CLV1*) (Clark *et al.*, 1993) and *CLV3* (Clark *et al.*, 1995). *CLV* and *STM* genes have been shown to play opposite or competitive roles in the regulation of meristem activity (Clark *et al.*, 1996).

The role of growth regulators as signalling molecules during zygotic embryogenesis was highlighted through *Arabidopsis* mutants perturbed in the balance of auxins and cytokinins. The mutant *amp1*, affected in different aspects of plant development (including spatial pattern, multiplication of cotyledons, and initiation of flowering), has a higher level of cytokinin than the wild type, suggesting that cytokinin levels might influence the formation of cotyledon primordia. Hence, the *AMP*-1 gene was assumed either to code for a negative regulator of cytokinin biosynthesis or to be required for the degradation of cytokinins (Chaudhury *et al.*, 1993). The flower mutant *pin1* of *Arabidopsis* shows abnormalities in floral bud formation in that it displays fused cotyledon primordia. Wild-type plants treated with auxin polar transport inhibitors are phenocopies of the *pin1* mutant indicating that the genetic defect of the mutation is related to auxin polar transport in the inflorescence axis (Okada *et al.*, 1991). Moreover, treatment of wild-type plants with CIPB (2-*p*-chlorophenoxy-isobutyric acid), an auxin antagonist which has no effect on polar transport, does not produce aberrant structures. In a similar way, zygotic embryos of *Brassica juncea* cultured *in vitro* and treated with auxin polar transport inhibitors are phenocopies of *pin1* mutants of *Arabidopsis*, showing fused cotyledons. Therefore, auxin polar transport was postulated to be involved in the establishment of bilateral symmetry in globular embryos (Liu *et al.*, 1993*a*, *b*). Similarly, the initiation of cotyledons seems to be associated with auxin polar transport in the late globular embryo (Cooke *et al.*, 1993).

Somatic embryogenesis

The above observations might help to explain the lack of germinating capacity of many somatic embryos. In fact, most somatic embryos display abnormalities which are similar to those found in zygotic embryos, with regard to shape or germination. For instance, somatic embryogenesis of the grapevine cultivar 41B produces aberrant embryos without functional meristems. Histological analysis of these embryos, which are unable to undergo further development, showed that the apical meristem was either only partially organized, or completely lacking, as has been observed for the *shoot-meristemless* mutant. Furthermore, 41B somatic embryos treated with abscisic acid (ABA), then BAP, displayed reduced meristem and vascular system and/or hypertrophic cell distortion (Goebel-Tourand *et al.*, 1993). The *mickey* mutant of *Arabidopsis*, which acts later during development, also exhibits abnormal fuzzy vascular strands (Mayer *et al.*, 1991). These abnormalities are common to somatic embryos from many species. Absent or rudimentary organized apical meristems were reported by Dos Santos *et al.* (1983) in alfalfa by Barwale *et al.* (1986) in soybean and by Gray and Mortensen (1987) in *Vitis longii*. Goebel-Tourand *et al.* (1993) suggest that the developmental process involved might entail a series of interacting processes where the alteration of one factor triggers successive abnormal events.

Whereas somatic embryos from many different species are abnormal, microspore-derived rape embryos obtained on hormone-free medium exhibit fewer abnormalities. A high growth regulator concentration is required to produce somatic cell-derived embryos, and an overwhelming majority of the embryos is aberrant. Is the appearance of abnormal shaped-embryos therefore related to hormonal treatments? Whether this is the case or not, results obtained on the cultivar 41 embryos of grapevine reinforced the idea that hormonal balance is of major importance in controlling embryo development (Goebel-Tourand *et al.*, 1993). The application of different growth regulators such as ABA, BAP or zeatin, either alone or in combination, to cultivar 41B embryos of grapevine, may or may not improve the conversion rate, whilst growth or abnormalities are promoted and frequency of cotyledonary embryos is increased. In other respects, it has previously been seen that the upper cell of the suspensor, namely the hypophysal cell, seems to be required in the formation of the root primordium of zygotic embryos. Although the suspensor has no crucial role in somatic embryogenesis, its lack could generate many root abnormalities.

Once the primary meristems are formed, embryogenesis *sensu stricto* can be considered as completed. The subsequent stages of development are centred on the phenomena associated with maturation and are probably the best studied aspect of development *in planta*.

Maturation and germination

At the end of the developmental phase, the structural organs of embryos are established, but reduced in size. Practically, no storage proteins are accumulated during differentiation and organogenesis, which is followed by a phase involving vastly increased rates of synthesis and deposition of storage proteins, lipids and starch, resulting in cell expansion. Reserves are localized in the endosperm of albuminous seeds or in the cotyledons of non-albuminous seeds. The seed coat is formed during this rapid increase in size and weight. Cell vacuoles exhibit a specialized behaviour during maturation in that they split up and dehydrate to give rise to protein bodies and aleurone grains in which great quantities of protein accumulate. At the end of the maturation phase, seeds enter dormancy, synthesis stops and the water content rapidly decreases (Goldberg *et al.*, 1989). Although proteins are not the only storage products, they are probably the most studied. Storage proteins are generally classified according to their solubility as either albumins, globulins, prolamins or glutelins (for a review, see Shewry *et al.*, 1995). Prolamins and glutelins are predominant in cereals whereas globulins are the major storage proteins in dicots. They are subdivided into two groups, 12S and 7S, a typical 12S type being pea legumin (Gatehouse *et al.*, 1984) and a typical 7S type being pea vicilin (Slightom *et al.*, 1983).

Zygotic embryogenesis

Developmental control. Seed developmental stages are characterized by the accumulation of distinct sets of mRNAs and corresponding proteins in the embryo and endosperm (Hughes and Galau, 1991). The accumulation of storage proteins, considered to be markers of the maturation phase (Galau *et al.*, 1991), is followed by the accumulation of late embryogenesis abundant (LEA) proteins, some of which have been shown to be ABA-inducible and are thought to participate in desiccation tolerance (Dure *et al.*, 1989). The expression of seed storage protein genes is mostly under developmental and genetic controls. It seems likely that most storage proteins are specified by multigene families which have arisen by gene duplication from an ancestral gene. The sequence of the members of these multigene families are evolving independently (Dure and Chlan, 1981). Among the 20 000 RNAs present in developing embryos of angiosperms, some are sequentially expressed and accumulate at different rates. They may be controlled at both the transcriptional or post-transcriptional levels (Goldberg *et al.*, 1989). For example, in species accumulating both globulin types, the synthesis of vicilin always precedes that of legumin and the total deposition of legumin is far higher than that of vicilin (Raynal *et al.*, 1992). This important temporal difference has been shown to occur in many dicots such as pea, grapevine, radish, and soybean.

It is clear that storage proteins are synthesized in differentiated cells, which have lost their mitotic potential. The expression of seed storage proteins is tissue specific since it occurs in embryo and endosperm, but never in mature vegetative tissues (Thomas, 1993). Perez-Grau and Goldberg (1989) showed, by studying the localization of the Kunitz trypsin inhibitor (Kti) mRNA in soybean, that the fate of cells is specified as soon as the globular stage is reached. The Kti3 mRNAs accumulate at the micropylar end of embryos at the late globular and heart stages while it is not detected in other parts of the embryo. Moreover, it is specifically localized within the ground meristem cell layer. These results indicate that the apical–basal and radial patterns of the globular embryo are divided into different transcriptional regions, illustrating that besides being temporally regulated, proteins are also spatially regulated during seed formation (Goldberg *et al.*, 1994).

Expression of storage protein genes has been analysed in monocots by characterizing their *cis*-regulatory sequences and the associated *trans*-acting DNA-binding proteins. This has led to the identification of sequences which control gene expression. These regulatory elements represent targets for specific DNA-binding transcription factors. For example, analysis of the amino acid sequence of the protein encoded by the *OPAQUE 2* (*O2*) gene, which was cloned by transposon tagging, revealed that it contains a basic domain and a leucine zipper (bZIP) which binds to the promoter of 22 kDa zein genes with a high specificity. Thus, the O2 protein functions as a transactivator of the 22 kDa zein promoter and hence, regulates the expression of the 22 kDa zein protein of maize seeds (Schmidt *et al.*, 1994). Histochemical analysis showed that the protein O2 is expressed in aleurone and endosperm cells of developing maize kernels (Varagona *et al.*, 1991), as is the bZIP transcriptional activator RITA-1 recently identified in developing rice seeds (Izawa *et al.*, 1994). Since, RITA-1 exhibits the same temporal and spatial expression pattern as O2, it has been suggested that it regulates the expression of genes expressed during seed development, namely in controlling the regulation of starch synthesis (Izawa *et al.*, 1994).

Maturation involves physiological processes which ensure embryo dormancy, including the accumulation of ABA (which is involved in senescence), environmental stress, growth inhibition and the maintenance of quiescence. The application of exogenous ABA has been shown to prevent precocious germination prior to desiccation and to promote embryo maturation (Thomas, 1993). Hence, ABA is an essential regulator of the process, which has been shown to peak in abundance during late embryogenesis, modulating gene expression, at least at the transcriptional level, during seed development (for a

review, see Giraudat *et al.*, 1994). Studies on gene expression patterns, in the presence or absence of exogenous ABA, showed that over 150 genes from a range of species are ABA-inducible (Giraudat *et al.*, 1994).

Genetic control. To date, maturation has been analysed most extensively at a physiological level; recently however, a genetic approach has revealed the influence of late embryogenesis specific genes. Subsequently, the genetic control of late embryogenesis in *Arabidopsis thaliana* and *Zea mays* has been studied by the characterization of mutants that either cannot synthesize ABA or fail to respond to the hormone.

The availability of ABA-deficient (biosynthetic) mutants in *Arabidopsis* (the *aba* mutants) and maize (the *Vp2*, *Vp5*, *Vp7*, *Vp9* mutants) has shed light on the ABA-biosynthetic pathway as well as the role of ABA on the regulation of seed gene expression. The maize *Viviparous* mutants, which exhibit precocious germination on the mother plant, and the *Arabidopsis aba* mutants, which remain non-dormant, do not display the peak of ABA at the end of the maturation phase and contain reduced levels of ABA (Neill *et al.*, 1986; Rock and Zeevaart, 1991). In *Arabidopsis* and maize, the accumulation of storage proteins is sometimes reduced, and at other times not, thus showing that variations in ABA levels are not the only developmental signal controlling the expression of the storage protein genes (Pang *et al.*, 1988; Paiva and Kriz, 1994). Although the accumulation of various LEA mRNAs is reduced in seeds of the ABA-biosynthetic mutants, the expression of ABA-inducible genes is not necessarily correlated with the level of ABA (Pang *et al.*, 1988; Finkelstein, 1993). These observations reveal that developmental variations in ABA content may control seed dormancy, but they are apparently not the main factor which regulates the expression of storage and LEA protein genes, although ABA-dependent regulatory pathways appear to be involved (Hughes and Galau, 1991).

Mutants impaired in their responsiveness to ABA are distinct from ABA-biosynthetic mutants in that they do not have reduced levels of endogenous ABA. Moreover, their phenotypes cannot be reversed by an exogenous supply of ABA. The *abi3* mutation of *Arabidopsis* (Koornneef *et al.*, 1984) and the *vp1* mutation of maize (Robertson, 1955) lead to precocious germination, thus bypassing dormancy. Mutant embryos exhibit reduced sensitivity to growth inhibition by exogenous ABA in culture. The *Abi3* and *vp1* mutations result in reduced *in vivo* accumulation of various endogenous mRNAs characteristic of developmental stages occurring late in seed development. The mRNAs in question were shown to include several globulin storage proteins, such as cruciferin and napin in *Arabidopsis*, as well as LEA mRNAs (Nambara *et al.*, 1992; Paiva and Kriz, 1994). The same mRNAs show slightly reduced abundance in both the *aba* and the *vp* mutants, indicating that these genes are under the control of developmental factors other than ABA levels alone and that the *ABI3* and *VP1* proteins are essential for the regulation of the expression of these genes (Pla *et al.*, 1991; Finkelstein, 1993).

Other data indicate that ABI3 and VP1 interact with regulatory pathways distinct from ABA-signalling. In fact, *vp1* mutants are defective in anthocyanin accumulation (this is not the case for the ABA-deficient *vp* mutants), resulting from their failure to express the *C1* regulatory gene, known to be activated by VP1 or exogenous ABA (Hattori *et al.*, 1992). Also, accumulation of lipids is inhibited in *abi3* mutants (not in *aba* mutants) which fail to break down their chlorophyll and to acquire desiccation tolerance (Finkelstein and Somerville, 1990). The molecular cloning of the *VP1* and *ABI3* genes (McCarty *et al.*, 1991; Giraudat *et al.*, 1992) support the idea that these genes code for proteins which are transcriptional activators. The primary structures of the VP1 and ABI3 proteins display a similar arrangement of domains with distinct biochemical characteristics. No significant sequence similarities to other known proteins were found, and no typical motifs associated with DNA-binding were detected. However, several regions display features previously identified in transcriptional activation domains. As transcriptional activators, the ABI3 and VP1 proteins could control levels of gene expression during seed development by interacting with various transcription factors related to distinct regulatory pathways. Recently, Parcy *et al.* (1994) showed that the *ABI3* locus encodes a protein which shares sequence similarities with the 2C class of serine/threonine protein phosphatase identified in rat and yeast; however, the role of the ABI3 protein remains to be elucidated.

A genetic approach has revealed the influence of late embryogenesis specific genes involved in maturation. For instance, a mutant identified in *Arabidopsis*, named *leafy cotyledon* (*lec*), causes defects in the differentiation process of cotyledons and in maturation-specific events such as storage product accumulation, desiccation tolerance and the maintenance of quiescence (Meinke *et al.*, 1994; West *et al.*, 1994). *Lec* mutations result in the transformation of the cotyledons of embryos and seedlings into leaf-like structures, characterized by trichome, stomata and mesophyll cell differentiation and a lack of protein and lipid storage bodies. The axis region also lacks storage organelles, indicating that the wild-type *LEC1* gene functions in both regions of the embryo. Moreover, *lec* embryos germinate precociously, implying that embryonic and post-germinative programmes occur simultaneously.

Genes, required for post-embryonic development are also active in late embryogenesis (Goldberg *et al.*, 1994). The *fusca* mutants of *Arabidopsis*, which accumulate anthocyanins in their cotyledons in late embryogenesis, fail to develop into mature flowering-plants after germina-

tion. *Fusca* mutants show normal embryogenesis, with the exception of *fus3* mutants which exhibit a leafy-like phenotype, but their lethal nature indicates that *FUSCA* genes are essential for critical developmental processes and that anthocyanin accumulation is only a secondary effect (Castle and Meinke, 1994). In fact, several *FUSCA* genes have been shown to be alleles of *CONSTITUTIVE PHOTOMORPHOGENIC (COP)/DEETIOLATED (DET)* genes which function in light-regulated development during seed germination (Chory *et al.*, 1989; Deng *et al.*, 1992; Wei and Deng, 1992). The products of the *COP/DET* loci appear to suppress light-regulated gene activities in the dark and stimulate these genes in the light by means of a light-mediated signal transduction pathway. Since the mutant *cop/det* genes were detected as *fusca* embryo mutants, the wild-type *COP/DET* alleles must be active during maturation. Thus, regulatory genes expressed at the end of embryogenesis prepare the plant for life after germination (Golberg *et al.*, 1994).

Somatic embryogenesis

Somatic embryos develop through stages similar to those reported for zygotic embryos, except that they do not become dormant. Furthermore, the integuments and endosperm, which are required, respectively, for conservation and germination, are not formed.

Early studies on spatial gene expression suggested that the somatic and zygotic embryos show similar developmental programmes. For instance, the Kti3 mRNA localization pattern of somatic embryos of soybean at maturation is similar to that of zygotic embryos (Perez-Grau and Goldberg, 1989). More recently, Dahmer *et al.* (1992) reported that somatic embryos of soybean induced with ANA were able to express the 11S storage globulin at the same level as that observed in zygotic embryos matured in culture, but to a lower level than that seen in mature seeds. However, the 7S embryo-specific marker protein detected in zygotic embryos was shown not to be expressed in somatic embryos. Conversely, soybean somatic embryos induced with 2,4-D and arrested relatively earlier in their development than ANA-induced embryos, do not accumulate either the mature 7S nor the 11S storage globulins. The synthesis of 7S and 11S precursor polypeptides is similar in both types of soybean somatic embryos. Zygotic and somatic embryos of rape synthesize the same 12S storage protein, but with different timings and extents of accumulation. In fact, zygotic embryos contain the 12S storage protein at the cotyledonary stage while in somatic embryos this protein is detected at the globular and heart stages, but at a lower level (Crouch, 1982). In the same way, studies of storage protein synthesis in somatic embryos of cotton at the early globular stage confirms that protein synthesis and accumulation patterns mimic those reported for zygotic systems, but at much earlier stages and to a lower degree (Shoemaker *et al.*, 1987). Somatic embryos of alfalfa express seed storage proteins. In zygotic embryos, 7S, 11S and 2S proteins are abundant during maturation at the same time; the 7S appears first, followed by the 11S and 2S proteins. In somatic embryos, the 7S protein (which is the first storage protein synthesized, followed by the 11S and much later by the 2S) remains predominant throughout development (Krochko *et al.*, 1992). These results indicate that although they exhibit differences in their synthesis kinetics and accumulation rates, somatic embryos are able to synthesize the specific storage proteins of their zygotic counterparts.

From the above, it can be seen that the synthesis of storage proteins occurs as soon as embryogenesis is initiated and independently of any maturation background. However, in a standard developmental medium, carrot somatic embryos do not accumulate storage proteins (Dodeman, 1995). In fact, the synthesis and deposition of storage and LEA proteins require the application of a stress or exogenous ABA, for their induction, as well as desiccation and quiescence. It should also be borne in mind that the previously described cases involved exalbuminous seeds whereas the carrot achene is an albuminous seed.

Endosperm/embryo interactions

The reserves of the endosperm are mobilized and enable the development of the organs of the still heterotrophic seedling. Therefore, a normal endosperm and surrounding maternal tissue is required for embryo development. These different tissues arise from cells of different genetic origin, different function and different ploidy level. Embryo development may be arrested if there is abnormal endosperm development, showing that interactions exist between the two. However, interactions between endosperm and embryo remain one of the more complex and less studied aspects of seed development (Lopes and Larkins, 1993). In many cases, it remains unclear whether an abnormal embryo results from a mutation of an embryo-specific gene, of the embryo–endosperm complex, or of a housekeeping gene.

Morphogenesis of somatic embryos in culture tissues occurs without the simultaneous development of an endosperm. Thus, as already mentioned above for the suspensor, either the endosperm is not required for embryo development, or the culture medium conditions take over from interactions between the embryo and the endosperm.

Conclusion

Plant embryogenesis and development is a particularly complex process. Plantlets can be obtained through the normal zygotic pathway or from somatic or androgenetic

cells. Even at the zygotic level the mechanisms underlying the genesis of an embryo are as yet poorly understood.

The sequential and interactive action of many genes is clearly involved in the establishment of the embryonic axis and/or morphogenesis. The recent characterization of regulatory genes identified through chemical and insertional mutagenesis has provided the first glimpses of the developmental pathways involved in zygotic embryogenesis. However, the precise role of these genes remains to be determined in most cases. Similarly, little is known of how regulatory genes function to specify major events. However, a few of them have been cloned and revealed to be 'housekeeping' genes (e.g. *GNOM*, *ABI*). Only the *SHOOT-MERISTEMLESS* gene seems to be involved in a specific regulatory function of plant embryogenesis (Long *et al.*, 1996). More generally, the regulatory mechanisms which co-ordinate the asymmetrical division and subsequent determination are still unknown.

The use of the new techniques, such as transposon tagging or promoter trapping followed by partial sequencing will allow identification of regulatory genes and their encoded products (Höfte *et al.*, 1993; Topping *et al.*, 1994). However, further advances in the understanding of the control of embryogenesis will require information on genes expressed in early embryogenesis (prior to the heart stage). Thus, both approaches, zygotic and somatic, should provide complementary information.

One of the major obstacles to understanding in detail the events which govern early embryo formation is the localization of the embryos within the plant and their relative inaccessibility to experimental manipulation, particularly at the early stages of embryogenesis (Goldberg *et al.*, 1994). Despite differences, in the acquisition of embryogenic potential and the difficulty in identifying the initial cell, somatic embryogenesis is an alternative approach which circumvents this problem in some respects and which therefore provides a means of studying gene expression programmes which regulate early embryogenic development. It appears that cell polarity followed by asymmetric cell division leading to daughter cells differing in their cytoplasmic determinants are universal to initiate cell differentiation in plant embryogenesis (Bouget *et al.*, 1996).

New potential is offered by progress made in the isolation of plant egg cells and their fertilization *in vitro* as a means of investigating the initial events of plant embryogenesis (Dumas and Mogensen, 1993; Kranz and Lörz, 1994). Sensitive techniques, such as DDRT-PCR (differential display reverse transcribed polymerase chain reaction), will enable the study of gene expression during the first divisions of the embryo (Bauer *et al.*, 1993).

Both somatic embryos and cultured zygotic embryos are currently used to answer questions concerning mechanisms of gene action and the role of growth regulators such as auxins in embryogenesis. An important question to be addressed is whether both types of embryogenesis are regulated by the same basic cellular mechanims, based on cell expansion and asymmetric cell division, as was suggested by De Jong *et al.* (1993).

MADS box genes, which encode transcription factor proteins, have been shown to control floral development in the same manner as the homeobox genes which regulate animal development. The only gene so far discovered which is currently known to be involved in plant embryogenesis is the *SHOOT-MERISTEMLESS* gene, which codes for a class-I knotted-like homeodomain-containing protein (Doebley, 1993; Theissen and Staedler, 1995). Therefore, the possibility that the construction of the embryo might involve analogous control mechanisms requires further investigation. Besides the functional hierarchies suggested by Lindsey and Topping (1993), it can also be asked whether there are any spatial hierarchies in embryogenic gene expression.

It emerges from this survey that embryogenesis is a complex and difficult process. Nevertheless, the range of approaches being employed, notably at the genetic, biochemical and molecular levels will provide important insights into the mechanisms underlying embryogenesis. With regard to somatic embryogenesis, there are two moot points to be addressed: can somatic embryogenesis be controlled and, if this were the case, would somatic embryogenesis be under the same regulatory mechanisms as zygotic embryogenesis? Clearly, molecular markers are required in order to follow specific events in embryo development, with the ultimate goal of unravelling the regulatory networks which operate.

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