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Embryogenesis in Angiosperms: Development of the Suspensor

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INTRODUCTION

The zygote in flowering plants usually divides transversely to form a terminal cell, which gives rise to the embryo proper, and a vacuolated basal cell, which often divides rapidly to form a structure known as the suspensor. Angiosperm suspensors vary widely in size and morphology from a single cell to a massive column of several hundred cells (Maheshwari, 1950; Wardlaw, 1955; Lersten, 1983). In most cases, the suspensor functions early in embryogenesis and then degenerates during later stages of development and is not present in the mature seed. Classically, the suspensor was thought to play a passive role in embryo development by holding the embryo proper in a fixed position within the seed (Maheshwari, 1950). It now appears from extensive structural, biochemical, and physiological studies with a variety of angiosperms that the suspensor plays an active role early in development by promoting continued growth of the embryo proper. In addition, growth of the suspensor during early stages of development may be inhibited by the embryo proper (Marsden and Meinke, 1985). Analysis of reproductive development in angiosperms must therefore include a consideration of developmental interactions that occur between the embryo proper and suspensor.

Although the suspensor appears to play a critical role in zygotic embryogenesis, it usually fails to develop when somatic embryos are produced in culture. The suspensor should therefore be viewed as a specialized structure that functions primarily to facilitate continued development of the embryo proper within the seed. In this review, we present an overview of the structure and function of the angiosperm suspensor and discuss recent attempts to analyze the development of the suspensor through a combination of descriptive, experimental, and genetic approaches. The recent identification of a large collection of Arabidopsis mutants with abnormal suspensors provides a unique opportunity to examine the underlying genetic factors that influence suspensor development.

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SUSPENSOR MORPHOLOGY

Suspensors come in many different shapes and sizes (Maheshwari, 1950; Lersten, 1983; Natesh and Rau, 1984). They may be either unicellular or multicellular, small or large in relation to the early embryo proper, and filamentous, columnar, spherical, or irregular in shape. A few exceptional genera appear to lack an organized suspensor altogether. The boundary between the embryo proper and suspensor is clearly defined in some species and diffuse in others. Cells of the suspensor often contain a variety of structural modifications not found in the embryo proper. Suspensor cells may also be polytene, polyploid, or multinucleate. A few suspensors produce elaborate outgrowths (haustoria) that invade surrounding endosperm or maternal tissues. In light of this impressive diversity, it is difficult to describe the morphology of a typical angiosperm suspensor. Some of the most unusual suspensors have been identified among the legumes, and several examples of suspensor morphology in this family are shown in Figure 1.

Cell division patterns during early embryogenesis in angiosperms have been examined in considerable detail for over a century (Hanstein, 1870; Schnarf, 1929; Johansen, 1950; Crété, 1963). Several conclusions have emerged from these studies: (1) early embryogenesis is often characterized by predictable patterns of cell division, although the real significance of these patterns to plant development remains to be resolved; (2) the zygote is typically a polarized cell that divides to form two cells with different features and developmental fates; (3) the suspensor is usually produced from the basal cell adjacent to the micropylar end of the ovule; and (4) development of the suspensor generally precedes differentiation of the embryo proper. Primitive vascular plants also contain structures that resemble a suspensor (Wardlaw, 1955). The suspensor is therefore a common feature of plant embryogenesis.

Large suspensors are particularly attractive for experimental studies that require physical manipulation and biochemical assays. The massive suspensors of *Phaseolus coccineus* (scarlet runner bean), *Tropaeolum majus* (nasturtium), and the legume *Cytisus laburnum* have therefore been examined in considerable detail. The *Phaseolus* suspensor grows rapidly

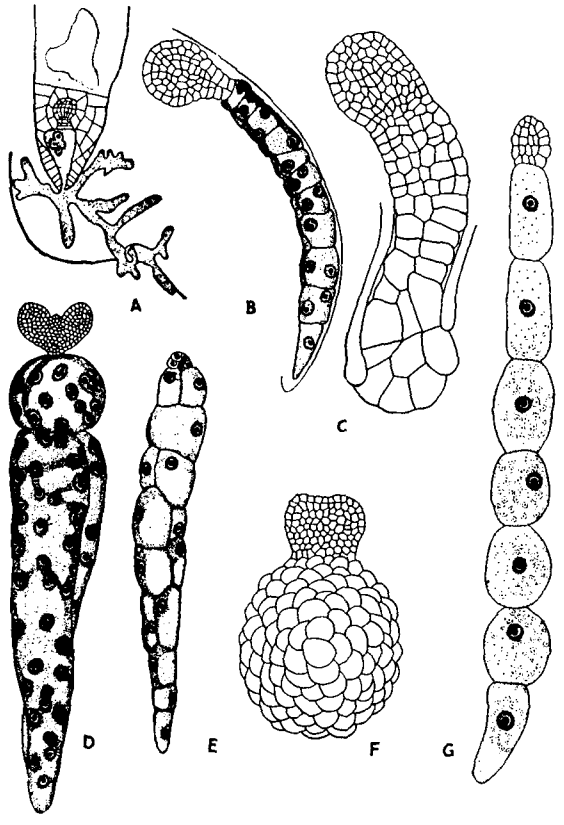

Figure 1. Variation in Development of the Suspensor in Angiosperms.

(A) Basal portion of the ovule in *Sedum acre* showing a suspensor with branched haustoria.

(B) to **(G)** Variation in suspensor morphology in the Leguminosae. The suspensor in each case is oriented below the embryo proper. Figure adapted from Wardlaw (1955) and reprinted from Meinke (1991a).

during early (proembryo) stages of development and ultimately forms a large column with several hundred cells (Figure 1C) at the heart stage (Yeung and Clutter, 1978, 1979). The suspensor then degenerates and becomes compressed as the embryo matures. The elaborate suspensor present in *Tropaeolum* extends through the micropyle and produces large haustoria that penetrate surrounding maternal tissues (Walker, 1947; Nagl and Kühner, 1976; Malik et al., 1977). The suspensor in *Cytisus* is a large spherical structure (Figure 1F) that gradually becomes differentiated from the globular embryo proper (Picciarelli et al., 1984, 1991).

A number of species with smaller suspensors have also been used as models for descriptive studies (Masand and Kapil, 1966). These include *Capsella bursa-pastoris* (Schulz and Jensen, 1969), Arabidopsis (Mansfield and Briarty, 1991), *Stellaria media* (Newcomb and Fowke, 1974), *Pisum sativum* (pea) (Marinos, 1970), *Alisma lanceolatum* (Bohdanowicz, 1987), *Diplotaxis erucoides* (Simoncioli, 1974), *Alyssum maritimum* (Prabhakar and Vijayaraghavan, 1983), and *Ipomoea purpurea* (morning glory) (Ponzi and Pizzolongo, 1972). Development of the suspensor in *Capsella* and Arabidopsis is summarized in Figure 2. Note that the filamentous suspensor contains an enlarged basal cell, which is attached to maternal tissues, and a single file of six to eight additional cells. The suspensor becomes highly differentiated early in development and then degenerates during subsequent cotyledon stages of embryogenesis.

CELLULAR DIFFERENTIATION

Descriptive studies with a variety of angiosperms have clearly supported the view that suspensors play an active role in synthesizing essential growth factors and transporting nutrients to the young embryo proper. The presence of invasive haustoria in members of the Rubiaceae first prompted Lloyd (1902) to suggest that the suspensor might function as an embryonic root to absorb nutrients for the developing embryo. Other common features of suspensor morphology, such as the locations of plasmodesmata and presence of specialized wall ingrowths, provide further evidence that the suspensor functions as a pipeline for transporting nutrients from surrounding maternal tissues to the developing embryo proper. Plasmodesmata are

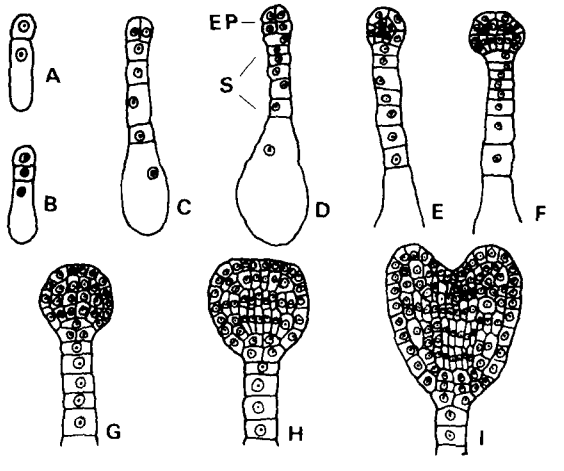

Figure 2. Early Stages of Embryogenesis in *Capsella* and Arabidopsis.

(A) The suspensor (S) develops from the basal cell following division of the zygote.

(B) to **(I)** As the embryo develops, the suspensor becomes a filamentous structure that reaches its maximal size at the heart stage of development **(I)**.

The embryo proper (EP) of Arabidopsis is ~40 μm in diameter at the globular stage **(H)**. Figure adapted from Maheshwari (1950) and reprinted from Meinke (1991a).

frequently found between adjacent cells of the suspensor but only rarely connect the suspensor and embryo proper with other parts of the seed. Extensive wall ingrowths have been found in suspensors from *Phaseolus* (Schnepf and Nagl, 1970; Yeung and Clutter, 1979), *Stellaria* (Newcomb and Fowke, 1974), and several other angiosperms (Raghavan, 1986). These ingrowths are characteristic features of transfer cells (Gunning and Pate, 1974), which facilitate transport of solutes by greatly increasing surface area. Mitochondria commonly found near these ingrowths may play a role in energy-dependent transport of nutrients.

Specialized plastids have been found in suspensors of *Pisum* (Marinos, 1970), *Phaseolus* (Schnepf and Nagl, 1970; Yeung and Clutter, 1979), *Ipomoea*, (Ponzi and Pizzolongo, 1972), *Stellaria* (Newcomb and Fowke, 1974), and *Tropaeolum* (Nagl and Kühner, 1976). These plastids often contain tubular structures that are not present in plastids in the embryo proper. Although the precise nature and function of these unusual plastids remain to be elucidated, they may play a role in the synthesis of compounds required for development of the embryo proper. Smooth endoplasmic reticulum (SER) is another common feature of suspensor cells (Newcomb and Fowke, 1974; Yeung and Clutter, 1979). The presence of extensive SER in *Phaseolus* suspensors is consistent with a metabolic function related to high rates of terpenoid (gibberellin) biosynthesis.

Endopolyploidization of the nucleus often accompanies development of the suspensor (D'Amato, 1984; Raghavan, 1986). The most extensive studies of polytene chromosomes in suspensors have dealt with *Phaseolus* (Nagl, 1974, 1978; Tagliasacchi et al., 1983, 1984; Frediani et al., 1986; Forino et al., 1992). The level of endopolyploidy increases toward the base of the suspensor and reaches 4000 C in *P. vulgaris* (Nagl, 1962) and 8000 C in *P. coccineus* (Brady, 1973). The question of whether preferential DNA amplification occurs during suspensor development remains to be resolved (Brady and Clutter, 1974; Lima-de-Faria et al., 1975; Raghavan, 1986). Puffs and chromosome bands have been observed in some preparations, but their appearance is much less striking than in Drosophila. Large endopolyploid nuclei are also present in the basal cell of *Alisma* (Bohdanowicz, 1987) and in the suspensor of the crucifer *Eruca sativa* (Corsi et al., 1973) and a number of other angiosperms (D'Amato, 1984; Raghavan, 1986). Although the functional significance of polyteny has not been demonstrated, tissue-specific increases in DNA content are consistent with the presence of specialized metabolic activities.

In monocots such as corn and wheat, the suspensor is much smaller than in *Phaseolus*, and many of the specialized features noted above are not present. However, similar modifications are found in surrounding maternal and endosperm cells (Smart and O'Brien, 1983; Schel et al., 1984). This suggests that some functions of the monocot suspensor may be replaced by adjacent tissues. Even in *Phaseolus* and *Capsella*, endosperm cells next to the suspensor often exhibit transfer cell morphology and appear active throughout early stages of development (Schulz and Jensen, 1969; Yeung and Clutter, 1979). It therefore appears that different parts of the seed work together to facilitate nutrient transport. There may even be a correlation between nutritional demands and suspensor morphology, with large suspensors prevalent in seeds with high nutritional demands and limited endosperm at early stages of development.

SUSPENSOR PHYSIOLOGY

In recent years, experimental studies of suspensor function have clearly demonstrated that suspensors are metabolically active, essential for nutrient transport, and important sources of growth regulators during early stages of embryogenesis. The suspensor of *P. coccineus* has been examined in greatest detail because its large size allows experiments to be performed that would be impractical with other angiosperms. In *Phaseolus*, the suspensor is more active than the embryo proper in RNA and protein synthesis during early stages of development (Walbot et al., 1972; Sussex et al., 1973; Clutter et al., 1974). Increased transcriptional activity has been observed directly through autoradiography of polytene chromosomes following exposure to tritiated uridine (Forino et al., 1992). High levels of macromolecular synthesis have also been detected in the large haustorial suspensor of *Tropaeolum* (Bhalla et al., 1981).

Thus, large suspensors with endopolyploid nuclei are active in transcription during early stages of development, when they are most likely to supply essential factors to the developing embryo proper. Whether a similar function is performed by smaller suspensors without polytene chromosomes remains to be determined. The suspensor of *Capsella* contains structural modifications to facilitate transport, but the cells are highly vacuolated and stain less intensely for protein and nucleic acids than adjacent cells of the embryo proper (Schulz and Jensen, 1969). Some suspensors may therefore promote growth of the embryo mainly through facilitated transport of nutrients rather than synthesis of critical growth factors.

Evidence that suspensors stimulate growth of the embryo proper was initially provided by experiments with *Eruca*, in which the growth in vitro of isolated embryos at the early heart stage of development was enhanced by the presence of an attached suspensor (Corsi, 1972). Similar results have been obtained with embryos from another crucifer (*Capsella*) cultured at the globular–heart stage (Monnier, 1984). The role of the suspensor in promoting growth in vitro of *P. coccineus* embryos was first explored by Cionini et al. (1976) and subsequently examined in detail by Yeung and Sussex (1979). An intact suspensor had little effect on embryos cultured at cotyledon stages of development, when cells of the suspensor were starting to degenerate, but clearly enhanced survival of isolated embryos at early heart stages, when the suspensor had reached its maximal size and was probably performing its critical functions. Enhanced survival of cultured embryos was also found when detached suspensors were placed in contact with

the cultured embryo proper, but not when the suspensors were first heat killed (Yeung and Sussex, 1979). It therefore appears from these experiments that the *Phaseolus* suspensor plays an active role in promoting growth of the embryo during the globular–heart transition.

Further evidence documenting the role of the *Phaseolus* suspensor in nutrient transport was provided by experiments in which the movement of ^{14}C-sucrose administered to excised pods and seeds was followed (Yeung, 1980). When labeled solution was introduced directly into the endosperm cavity at the heart stage of development, the highest level of radioactivity was detected in the suspensor and adjacent cells of the embryo proper, not at the other end of the seed where the label was initially applied. Furthermore, the sensitivity of this uptake process to the metabolic inhibitor dinitrophenol was consistent with the model that active transport of label through the suspensor was occurring at this stage of development. Recent studies involving Prussian Blue staining of transport pathways in developing *Phaseolus* seeds (Brady and Combs, 1988) and autoradiography of labeled putrescine administered to developing pods (Nagl, 1990) have provided further evidence that the suspensor is the major route of nutrient uptake for the globular–heart embryo.

The possibility that suspensors might provide growth regulators to the developing embryo has been investigated in detail for the past 20 years. Attention was initially placed on gibberellins after high levels of GA_1 were reported in *Phaseolus* suspensors (Alpi et al., 1975, 1979). Cell-free extracts prepared from homogenized suspensors were then used to demonstrate that suspensor cells are capable of synthesizing gibberellins from labeled precursors (Ceccarelli et al., 1979, 1981). Thus, it appears that suspensors not only are rich sources of gibberellin but also are capable of synthesizing this growth regulator at critical stages of development. A greater diversity of gibberellins has recently been identified in *Phaseolus* suspensors by combined gas chromatography and mass spectrometry (Piaggesi et al., 1989). Similar compounds have also been found in the suspensors of *Tropaeolum* (Picciarelli et al., 1984) and *Cytisus* (Picciarelli et al., 1991).

The importance of gibberellins during early embryo development has been demonstrated by in vitro culture and biochemical studies. In *Phaseolus*, gibberellins have been shown to promote the growth in vitro of isolated embryos (Cionini et al., 1976; Yeung and Sussex, 1979), increase translational activity in the embryo proper (Brady and Walthall, 1985; Walthall and Brady, 1986), and enhance transcription in polytene suspensor cells (Forino et al., 1992). Other growth regulators, such as auxins (Przybyllok and Nagl, 1977), cytokinins (Lorenzi et al., 1978), and abscisic acid (Perata et al., 1990), have also been detected in suspensors. These studies provide further evidence that the suspensor is an important source of growth regulators, but the results are not as convincing as with gibberellins. Nevertheless, it appears that suspensors in at least some angiosperms may provide the developing embryo proper with a variety of growth regulators.

DEVELOPMENTAL POTENTIAL OF THE SUSPENSOR

Although the function of the suspensor in supporting growth of the embryo proper has been examined in some detail, the possible role of the embryo proper in regulating development of the suspensor has until recently been largely ignored. Considering the diversity of suspensor morphology, it would seem reasonable to question whether most of these differences in size and shape are determined exclusively by the suspensor itself or whether other parts of the seed may also regulate development of the suspensor. Experimental studies with a variety of angiosperms have provided increasing support for the view that continued growth of the suspensor during early stages of development may be inhibited by the embryo proper (Raghavan, 1976; Marsden and Meinke, 1985). In other words, the developmental potential of a suspensor is often greater than its normal developmental fate.

Support for this model originally came from studies involving irradiation of immature seeds. When applied at the appropriate stage of development, irradiation often destroys cells of the actively dividing embryo proper while leaving the differentiated suspensor relatively unaffected. An interesting pattern emerged from studies with *Nicotiana rustica* (Devreux and Mugnozza, 1962), *Capsella* (Devreux, 1963), *Eranthis hiemalis* (Haccius and Reichert, 1964), and Arabidopsis (Gerlach-Cruse, 1969; Akhundova et al., 1978). Degeneration of the embryo proper in all of these plants was often accompanied by abnormal growth of the suspensor. Many of these suspensors were longer and wider than normal and contained a significant number of additional cells.

The most elaborate suspensors were obtained with *Eranthis*. This plant has an unusual pattern of reproductive development in that "mature" seeds released in the spring contain an undifferentiated embryo proper and a prominent suspensor (Haccius, 1963). The remaining stages of embryo development are completed in the soil during the summer, and a fully differentiated embryo germinates several months later. Haccius (1963) found that in *Eranthis*, cells of the undifferentiated embryo proper were particularly susceptible to acidic solutions; degeneration of the embryo proper in treated seeds was often accompanied not only by renewed growth of the suspensor but also by the formation of a new embryo from the enlarged suspensor. Thus, it appears that suspensors from a variety of angiosperms have an underlying developmental potential that is revealed only when an inhibitory effect of the embryo proper is removed.

ABNORMAL SUSPENSOR MUTANTS OF ARABIDOPSIS

The identification of an embryo-lethal mutant of Arabidopsis that produced aborted seeds with abnormal suspensors

(Marsden and Meinke, 1985) provided further support for the model that continued growth of the suspensor during normal development is inhibited by the embryo proper. Examination of sectioned aborted seeds of this mutant, which was originally isolated following ethyl methanesulfonate (EMS) seed mutagenesis (Meinke and Sussex, 1979), revealed that it produced abnormal suspensors. The embryo proper consistently arrested at the preglobular stage of development, as shown by examining a large number of aborted seeds under a dissecting microscope. Reconstruction of serial sections through several aborted seeds revealed that the embryo proper contained fewer than 20 cells, whereas the suspensor contained as many as 150 cells. Mutant suspensors were both longer and wider than normal, accumulated unusual starch granules late in development, and contained vacuoles with patches of electron-dense material that resembled immature protein bodies.

It therefore appeared that mutant suspensors not only resumed cell division in the absence of a functional embryo proper but also acquired characteristics normally restricted to the embryo proper. This pattern of development was not an inevitable consequence of embryonic lethality because other mutants arrested at similar stages produced normal suspensors. It was proposed that the mutation disrupted a function essential only for continued development of the embryo proper and that developmental arrest of the embryo proper indirectly relieved an inhibitory effect on the suspensor, resulting in abnormal growth. The fact that the endosperm tissue also continued to develop for several days following arrest of the embryo proper was viewed as further evidence that the mutation specifically blocked development of the embryo proper. Although viable seeds from this mutant are no longer available, the observed pattern of development demonstrated that mutant analysis can be a valuable approach to the study of suspensor structure and function.

Additional mutants with abnormal suspensors have recently been identified in Arabidopsis following EMS seed mutagenesis (Meinke, 1985) and Agrobacterium-mediated seed transformation (Errampalli et al., 1991; Castle and Meinke, 1993; Castle et al., 1993). All of these mutants were initially recovered from mutagenized populations by examining immature seeds under a dissecting microscope and scoring for the presence of defective embryos with enlarged suspensors. Mutants with subtle changes in suspensor morphology were not identified by this method. Table 1 lists 16 mutants with enlarged suspensors that are currently being examined in our laboratories. Mutants differ in seed pigmentation; stage of developmental arrest in the embryo proper; tagging status (i.e., whether the mutation results from T-DNA insertion); and gametophytic gene expression, as revealed by the distribution of aborted seeds in heterozygous siliques. These suspensor mutants are part of a large collection of 250 embryo-defective mutants

Table 1. Overview of Abnormal Suspensor Mutants of Arabidopsis

Mutant[a]	Tagged[b]	Linkage Group	Pigmentation[c]		Percent Mutant Seeds[d]	Percent Top Half[e]	Embryo Shape at Seed Maturity
			Seed	Embryo			
emb18	–	2	1–2	1	20.3	62.8	Globular
emb19	–	–	1–2	1–2	21.5	57.7	Globular–heart
emb76-1	Y	1	1–2	1–2	24.4	50.5	Globular–elongate
emb76-2	N	1	2	1	25.8	49.3	Globular–elongate
emb84	Y	3 + 4[f]	2	1	25.6	42.1	Globular
emb88	Y	1	1	1	27.7	48.4	Globular
emb111	Y	2	2	1	24.0	50.2	Globular–elongate
emb113	U	–	1–2	1–2	ND	ND	Globular–elongate
emb117	N	3	1	1	23.4	46.7	Globular
emb155	N	4	1–2	1	24.6	51.6	Globular–heart
emb158	N	1	1	1	21.8	53.6	Elongate
emb177	Y	1	2	1–2	25.9	49.8	Globular–heart
emb225	N	–	2–3	1–2	25.3	53.6	Small globular
emb243	U	–	1	2	19.3	59.5	Globular
emb244	Y	–	1	1	26.2	ND	Small globular
emb271	U	–	1	1	26.8	ND	Globular

[a] Isolated after EMS seed mutagenesis (*emb18* and *emb19*) or Agrobacterium-mediated seed transformation (*emb76* to *emb271*).
[b] Mutants appear from genetic studies to be tagged with T-DNA (Y), not tagged (N), or unresolved (U) with respect to tagging (Castle et al., 1993).
[c] Mutant seeds and embryos are white (1), pale yellow-green (2), or pale green (3).
[d] Heterozygous plants produce 25% mutant seeds following self-pollination. ND = not determined.
[e] When more than 50% of the mutant seeds are located in the top half of the silique, the mutant allele appears to disrupt pollen development or pollen-tube growth (Meinke, 1982, 1991b).
[f] This line appears to contain a chromosomal translocation (Castle et al., 1993).

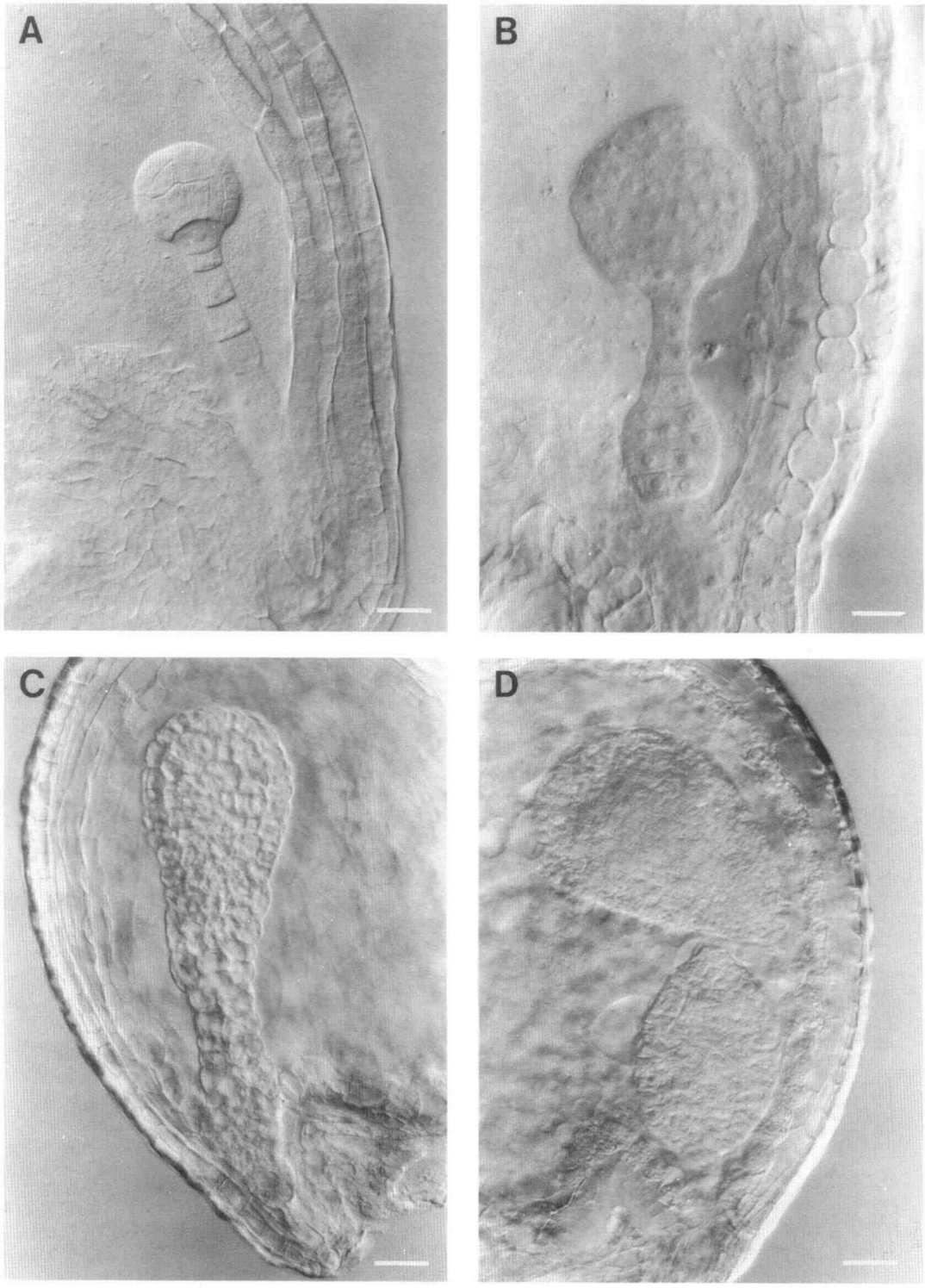

Figure 3. Light Micrographs (Nomarski Optics) of Wild-Type and Mutant Arabidopsis Suspensors.

(A) Wild-type suspensor. Bar = 15 μm.
(B) Mutant suspensor from *emb117* aborted seed. Bar = 15 μm.
(C) Mutant suspensor from *emb76-1* aborted seed. Bar = 30 μm.
(D) Mutant suspensor from *emb158* aborted seed. Bar = 30 μm.
Seeds were removed from immature siliques and cleared in Hoyer's solution. Wild-type suspensors contain a single file of cells; mutant suspensors contain additional cells and exhibit a variety of developmental abnormalities.

isolated and characterized by the Meinke laboratory (Meinke, 1985; Castle et al., 1993).

Further analysis of this collection using light microscopy with Nomarski optics has revealed that minor defects in suspensor morphology are more common than originally expected. It is therefore difficult to divide mutants into separate groups based strictly on suspensor morphology. Instead there appears to be a continuum of mutants ranging from those that rarely produce defective suspensors to others that often produce highly abnormal suspensors. Examples of mutant and wild-type suspensors viewed with Nomarski optics are shown in Figure 3. One useful feature of Arabidopsis is that large numbers of developing seeds can be readily cleared and examined with Nomarski optics for defects in suspensor morphology. Shapes of abnormal suspensors can therefore be determined without examining sectioned material. Traditional light and electron microscopy are nevertheless required to obtain details on cellular morphology. Examples of plastic sections through aborted seeds from mutants with particularly large suspensors are shown in Figure 4. Note that some mutant seeds from *emb158* (Figures 3D and 4A) appear to contain two embryos. Similar abnormalities are occasionally found upon dissection of aborted seeds. The secondary embryo in this case is actually an enlarged suspensor that superficially resembles an embryo proper but fails to continue development or produce a viable seedling in culture.

Suspensor mutants often have vigorous suspensors with densely cytoplasmic cells at a stage of development when normal seeds in the same silique contain vacuolated or degenerated suspensors. Mutant suspensors have therefore delayed their programmed cell degeneration and replaced it with another program that more closely resembles that of the embryo proper. This is consistent with the model that the developmental potential of the suspensor often exceeds its normal developmental fate.

Different mutants also have characteristic patterns of abnormal development. Some mutants typically produce columnar suspensors that blend into an elongated embryo proper. Others are more likely to produce suspensors with enlarged basal portions that connect to the embryo proper through a thin junction. Some mutants accumulate excessive amounts of starch, starting with the suspensor and spreading to the embryo proper. Mutants may also differ with respect to the stage of development when aberrations are first detected and whether abnormalities appear first in the embryo proper or suspensor. Any model proposed to explain the abnormal suspensor phenotype must account for these different patterns of development.

FUTURE DIRECTIONS

The descriptive and experimental studies outlined in this review have clearly demonstrated that the angiosperm suspensor is a variable and dynamic structure with important functions during plant embryogenesis. What general conclusions can be drawn from these studies, and what questions remain to be answered? The most obvious conclusion is that in flowering plants both the suspensor and endosperm tissue have evolved specialized features (structural, molecular, and physiological modifications) to support development of the embryo proper. The suspensor is thus a terminally differentiated structure that interacts with other parts of the developing seed but does not directly contribute cells to subsequent generations. In light of this supporting role, it is not surprising that different species have evolved elaborate modifications in suspensor structure (presence of haustoria, extensive wall ingrowths, and variations in general morphology) and suspensor physiology (synthesis of growth regulators and changes in chromosome structure). Experiments with model systems such as *Phaseolus* and Arabidopsis may therefore provide insights into different strategies employed by angiosperms to support development of the embryo proper.

Despite recent advances in our appreciation of suspensor structure and function, the molecular basis of interactions between the suspensor and other parts of the developing seed remains to be elucidated (Meinke, 1991a). Molecular analysis of T-DNA insertional mutants of Arabidopsis with abnormal suspensors may help to reveal the mechanism used by some angiosperms to limit continued growth and development of the suspensor. Plant sequences adjacent to T-DNA inserts in several of these mutants have recently been cloned (L. Castle, B. Schwartz and D.W. Meinke, unpublished data), and other mutants with similar phenotypes are being examined (R.B. Goldberg and J.J. Harada, unpublished data). These mutants should provide a direct test of the model that continued growth of the suspensor in Arabidopsis is inhibited by the embryo proper. In light of the established developmental potential of the suspensor, we expect that mutant suspensors may in some cases acquire characteristics of the embryo proper. The presence of structures resembling immature protein bodies in abnormal suspensors of Arabidopsis (Marsden and Meinke, 1985) has suggested that storage protein synthesis may be activated in some mutants and that cell differentiation may continue in the absence of morphogenesis (Patton and Meinke, 1990).

The question of what causes the suspensor to degenerate during later stages of development may be more difficult to address from a genetic perspective. Although the process of suspensor degeneration has been examined in some detail in *Phaseolus* (Nagl, 1976, 1977; Gärtner and Nagl, 1980) and *Tropaeolum* (Singh et al., 1980), molecular signals responsible for initiating this developmental program remain to be identified. Some of the suspensor mutants identified in Arabidopsis may be directly altered in this process, but distinguishing these regulatory mutants from others with more general defects may be difficult. Additional screens could be performed in the future to identify mutants with subtle but potentially interesting defects in suspensor development. Existing

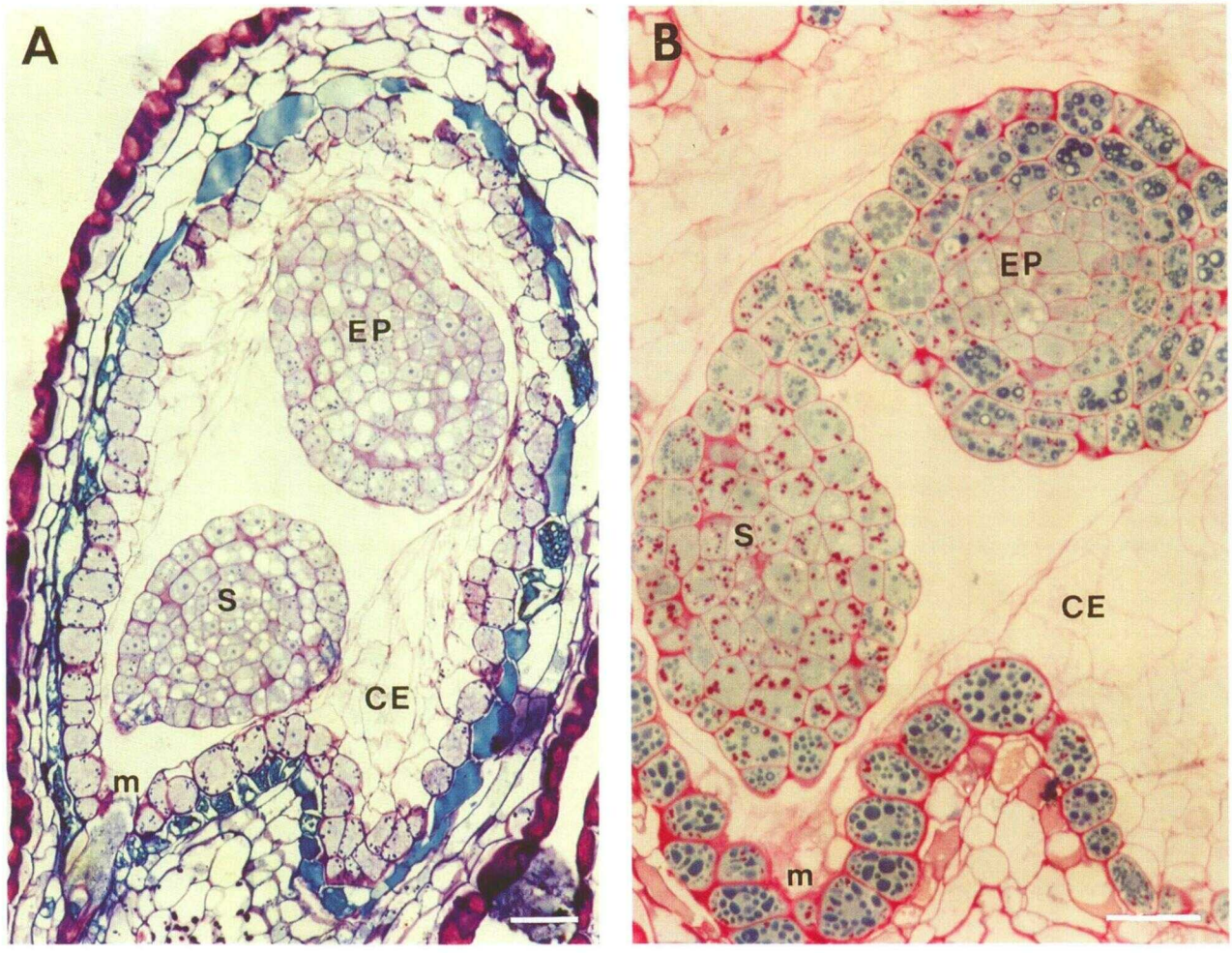

Figure 4. Light Micrographs of Aborted Seeds from Suspensor Mutants of Arabidopsis.

(A) *Mutant seed from emb158 with an arrested embryo proper* (EP) *and elongated suspensor* (S) that resembles a second embryo. The suspensor and embryo proper were connected by a thin filament in subsequent sections. Note the similarity in appearance between the embryo proper and suspensor. Bar = 30 μm.

(B) Mutant seed from *emb177* with an arrested embryo proper (EP) and enlarged suspensor (S). Note the starch grains (red) in cells of the suspensor and putative protein bodies (dark blue) in cells of both the embryo proper and suspensor. Bar = 30 μm.

Mutant seeds were removed from immature siliques, fixed in formaldehyde and glutaraldehyde, embedded in Historesin (glycol methacrylate), cut with glass knives into 2-μm sections, stained with the periodic acid-Schiff procedure, and counterstained with toluidine blue O **(A)** or amido black 10B **(B)** as described previously (Yeung, 1984; Yeung and Law, 1987). Both seeds contain cellular endosperm (CE) tissue. Attachment of the suspensor to the micropylar (m) end of the ovule was visible in subsequent sections.

mutants of Arabidopsis with defects in hormone response or cell differentiation could also be examined for changes in suspensor morphology.

An alternative approach might be to examine patterns of gene expression in the suspensor by constructing stage-specific cDNA libraries. This would be a demanding but feasible task with large suspensors such as those of *P. coccineus*. In situ hybridization could be used to examine the expression of genes involved in hormone biosynthesis and metabolic pathways in the suspensor once appropriate probes become available. If tissue-specific promoters can be identified that activate genes preferentially in cells of the embryo proper and suspensor, a variety of cell ablation studies similar to those performed with developing anthers (Mariani et al., 1990) could be attempted to analyze the role of the suspensor in seed development. The *Phaseolus* suspensor also provides a rare opportunity to examine factors controlling cell cycle and endopolyploidization in plants. As details on cell cycle control become available from studies with model systems, it might be appropriate to study the effects of overexpression or loss of function of putative regulatory genes in transgenic *Phaseolus* suspensors. Analysis of polytene chromosomes in *Phaseolus* hybrids is another

promising approach to studying the relationship between gene amplification and plant development (Pomper et al., 1992).

Factors responsible for establishing the developmental fates of basal and apical cells following division of the zygote also remain to be identified. Differential gene expression may indeed play an important role, but localized distribution of cytoplasmic factors and surface components may be even more critical in establishing polarity within the zygote and determining fates of descendant cells. The recent demonstration that a plasma membrane arabinogalactan protein is differentially localized during embryogenesis in *Brassica* (Pennell et al., 1991) is consistent with the view that important developmental signals may originate from the cell surface. The relationship between surface glycoproteins, intracellular hormone concentrations, and localized suppression of cell division in plant morphogenesis and phylogeny has recently been reviewed (Basile and Basile, 1993) and may provide a model for developmental interactions between the embryo proper and suspensor. Chemical gradients may also play an important role during early stages of embryogenesis. Gradients in osmotic potential and element concentration have already been documented in developing seeds (Ryczkowski, 1960; Ryczkowski and Reczynski, 1988). Interactions between these gradients and the early embryo may help to establish cell differentiation.

Analysis of somatic embryos has suggested that the chemical and physical environment of the seed can influence the differentiation process (see Zimmerman, 1993, this issue). Suspensors are often not present in somatic embryos, and when structures that superficially resemble a suspensor are found, they typically lack specialized features characteristic of normal suspensors (Yeung, 1993). Thus, structural modifications related to nutrient transport are not required when somatic embryos are produced in a rich nutrient environment. Results of two recent studies further support the view that nutritional status can influence development of the suspensor. In parthenogenic embryos of the genus *Poaceae*, failure of endosperm development and subsequent changes in nutritional status are associated with the formation of large suspensors (Matzk, 1991), and in cultured ovules from crosses in the genus *Beta*, suspensors lose starch upon culture and then develop into a callus mass, perhaps in response to alternative pathways of nutrient supply (Bruun, 1991). The formation of the suspensor should therefore be examined within the context of the total environment of the seed. As collections of mutants defective in seed development are analyzed further at the molecular level, we should begin to identify a wide range of genes that influence not only the development of the suspensor but also the development of many other parts of the angiosperm seed.

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