Mechanisms of Plant Reproduction: Questions and Approaches

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PLANT REPRODUCTION—AN OVERVIEW

It is a daunting but exciting task to attempt both an orientation to plant development and an overview of the reviews in this special issue on plant reproduction. Our purpose in this article is to provide a guide to the key points of plant development and reproduction, pointing out not only the general themes that are common among angiosperms but also some of the exceptional species and events that illustrate the diversity of types adapted to specific selective forces. We view the elucidation of developmental mechanisms in plant reproduction as both a goal in itself and a prelude to a more comprehensive appreciation of the evolutionary steps that have led to the diversity of existing angiosperm species. Several previous reviews (Walbot, 1985; Goldberg, 1988) discuss more fully the earlier state of the art of many aspects of plant development and reproduction.

Evolution has hit upon at least two distinctly different strategies for being multicellular: the animal strategy, in which the organism—and, within limits, its cells—can move, and the plant strategy, in which both the organism and its cells are largely fixed in place. These two strategies underlie everything the organism does, from the way it responds to predators and obtains nutrition to the way it reproduces. For example, in animals, the entire body plan and the organ systems are generally established during embryo development. As organs grow, they receive new cells from stem cell populations whose derivatives have a limited range of possible pathways of differentiation. By contrast, all postembryonic plant structures emerge from apical meristems that arise early in embryonic development. These meristems serve as reservoirs of cells throughout plant development, replenishing themselves while giving rise to lateral meristems, which in turn develop into organs such as leaves (see Steeves and Sussex, 1989).

Although there is continuous organogenesis from the shoot apical meristem, this cell population matures over time. The meristem produces juvenile leaves initially, followed by adult leaves; in maize, and possibly other plants as well, the same apical meristem earlier gave rise to embryonic leaves (cotyledons). Reproductive development begins with the transition of the apical meristem from producing vegetative structures to producing inflorescence branches, floral bracts, and flowers. Unlike the cells of vegetative apical meristems, floral apical meristem cells actually differentiate into the organs they give rise to. Thus, the production of a flower consumes an apical meristem and prevents a stem from undergoing further vegetative growth.

The differences in plant and animal growth strategies are reflected in the ways they respond to changes in the environment. Animal responses to a variable environment are primarily behavioral and physiological and occur on a short time scale—seconds to hours. Animals also exhibit a limited range of developmental responses to environmental cues. For example, by changing melanin production, some mammals switch their fur color from brown in summer to white in winter, an adaptive response that provides both hunter and hunted with camouflage as the seasons change. In plants, physiology, rather than behavior, provides the means for short-term responses to environmental alterations; the integration of physiological responses then molds subsequent development for longer term acclimation to a variable environment.

The different growth strategies are also reflected in the relative importance of cell lineage relationships in animal and plant development. Animal development is often viewed as a process of progressive restriction of developmental potential within cell lineages, although position-dependent differentiation also occurs frequently. By contrast, the immobility of plant cells gives position a far more important role than lineage in determining plant cell fate. Ablation experiments and mutations provide ample evidence for the unique roles of individual animal cells, both in terms of their final contributions to the animal body and their developmental roles in inductive interactions with other cells. Mutations in plant genes that control developmental events have similarly specific and reproducible phenotypic effects, showing that plant developmental processes are tightly controlled. For most plant cell divisions, the positioning of the division plane does not appear to play a significant role in determining the fate of the daughter cells. However, some divisions are very precise, among them the first zygote division in many plant species and those that give rise to guard cells and root hairs, raising the possibility that in these divisions, the location of the division plane is critical for the proper development of the resulting structure.
As might be expected from their growth strategy, plant cells tend to be totipotent, a property that is rarely seen in animal cells. Totipotency in plant cells can be uncovered by cell culture in completely defined media, for example, by regenerating fertile plants from individual protoplasts from a tobacco leaf via somatic embryogenesis or organogenesis (Steeves and Sussex, 1989). The proportion of cells in the plant somatic body that retains totipotency appears to vary among plants, with monocots, in general, having few cells with this capacity and dicots, particularly solanaceous plants, containing many totipotent cells. The capacity for vegetative reproduction, that is, the ability to organize new meristems from preexisting organs, also varies among plants; among the most dramatic examples of this capacity are ectopic seedling production along the margins of Kalanchoe leaves and de novo root or shoot formation from cuttings.

One of the most striking differences between the developmental approaches of plants and animals is the origin of the gametes. Among the initial cell divisions of the animal embryo, specific cells are restricted to the germ line. These germ cells do not participate in the formation of the somatic body of the animal. Indeed, in mouse, and probably other mammals as well, the germ cell lineage migrates from the developing embryo to reside "outside" the embryo on extraembryonic membranes during gastrulation, when the basic body plan is established and organogenesis is initiated. The germ cells later migrate back into the embryo, reaching the immature gonads by crawling single file through the developing gut and kidney. Thus, germ cells are unique among the cells of the mammalian embryo in that they avoid the morphogenetic signals that program the basic somatic body plan of the animal. Even with their unique heritage, however, mammalian germ cells are not totipotent; only a fertilized egg, combining sperm and egg nuclei, can program the development of an embryo.

By contrast, like all other cells of the plant, plant gametes arise from cells that have been through many mitotic divisions and significant environmental exposure throughout somatic and floral meristem function, finalized in the cells of the sexual organs of the flower. As diagrammed in Figure 1, the flowers of the sporophyte, the dominant generation of higher plants, do not produce gametes directly. Instead, meiotic divisions within the sexual organs of the flower give rise to haploid megaspores and microspores, which undergo several mitotic divisions to produce the gametophyte stages of the plant life cycle. A megasporocyte develops within the ovules of the carpel into a female gametophyte, or embryo sac, which typically contains seven cells; one becomes the egg, and the others aid in fertilization or embryo development. The male gametophyte, or pollen grain, arises from a microspore produced within the anther locule and contains three cells at maturity—two sperm

![Figure 1. Life Cycle of a Flowering Plant. From Goldberg (1988) and reproduced with permission of AAAS.](image-url)
cells and a vegetative cell. Upon germination on a receptive stigma, the vegetative cell develops into a pollen tube that grows through the pistil to the ovule, where it delivers the two sperm to the embryo sac (Figure 1).

The mitotic divisions that intervene between meiosis and gametophyte formation serve not only to generate essential accessory cells but also to provide a critical haploinsufficiency test for basic cellular functions. Any spore that contains a deleterious recessive allele of a gene that is required during gametophyte development will fail to produce a functional gametophyte. Thus, although spores may arise from cells that have accumulated many mutations, the existence of the gametophyte generation allows selection against many of the most detrimental mutations, reducing the genetic load in the next sporophyte generation.

In addition to the sporophyte and the gametophyte, angiospermous plants have yet a third life stage, the endosperm. This terminal individual is the product of a second fertilization event, in which the female gametophyte's central cell, which is binucleate in many plant species, fuses with the second sperm cell (Figure 1). The endosperm generally shares all its alleles with the zygote because the central cell and the egg cell are sister cells, as are the two sperm cells. However, its developmental fate is completely different: it divides to become a terminally differentiated mass of tissue that provides nutrition to the growing embryo. The importance of the endosperm to the development of the zygote is underscored by the fact that even in those plants in which embryos develop directly from unfertilized maternal cells in the process known as apomixis, pollination and concomitant endosperm formation are required if the embryo is to develop normally in situ. Endosperm is not, however, absolutely required for normal embryo development. Zygotic embryos removed from the embryo sac and endosperm can develop normally in culture (Liu et al., 1993), and somatic embryogenesis—the development of embryos from cultured somatic cells and microspores of a wide range of angiosperms—occurs in the absence of endosperm.

The most common type of endosperm is triploid (Figure 1), the result of fertilization of a 2n central cell of the embryo sac by a 1n sperm. However, in individual species or higher taxa, other ploidy levels are found. Among the Onagraceae, for example, the endosperm is diploid, whereas in many lilies, 8n (7 + 1) or 16n (15 + 1) ploidy is encountered. What is consistent, however, is that the angiosperm endosperm is always the result of sexual reproduction, with a single sperm fusing with an embryo sac nucleus whose ploidy is characteristic of that species. In fact, recent evidence suggests that endosperm may serve as a “check” that sexual reproduction has occurred. Gene imprinting, the differential expression of alleles transmitted through the sperm versus the embryo sac, appears thus far to be restricted to the endosperm (Kermicle and Alleman, 1990).

Interestingly, in most gymnosperms, the endosperm is haploid and is derived solely from the megagametophyte. Two sperm are present in the pollen tube; they are often dimorphic, with the larger destined to fuse with the egg. Both sperm may be “delivered” to the embryo sac, but despite the proximity of the second sperm and the pre-endosperm cell, fusion does not occur. Thus, a key step in the evolution of the angiosperms was the second nuclear fusion event that ensured that the endosperm is also the product of sexual reproduction. A possible evolutionary intermediate between the standard angiosperm and gymnosperm patterns has been identified in Ephedra, a member of the Gnetales, in which there is a second fertilization event that produces an extra embryo rather than endosperm (Friedman, 1990).

The zygote, meanwhile, develops into an individual in which the structures critical for subsequent development—the shoot and root apical meristems and the cotyledons, storage organs that supply nutrition to the seedling as it germinates and before it has developed its photosynthetic capabilities—are established (Figure 1). Thus, embryonic development involves both the establishment of polarity and cell and tissue differentiation. Because normal embryos can form outside the embryo, that is, via somatic embryogenesis, the events that pattern the embryo are most likely intrinsic to the process of embryogenesis. However, it is possible that maternal or embryo sac cells impart positional information to the developing zygotic embryo or somehow reinforce its intrinsically determined polarity. Similarly, embryogenesis does not require the biophysical constraint of the seed.

In many animals, embryonic development segues immediately into adult development or into a larval stage that is followed seamlessly by the adult stage. Again, however, the immobility of plants generally necessitates a different strategy: in most higher plants, zygotic embryogenesis is followed by a stage of dormancy that lasts as long as necessary, that is, until the seed has moved away from the parent plant and encounters conditions favorable for germination. Thus, plant embryogenesis concludes with the desiccation of the embryo, the hardening of the surrounding maternal ovule tissue into a tough seed coat, and the development of the ovary into a fruit that is specialized for dispersal. The embryos of some plant species, particularly mangroves, actually develop into large seedlings before they break away from their parent. This mode of development, termed vivipary, would be lethal to most plants but is essential for mangrove seedlings, this head start making it possible for them to anchor themselves in muddy areas of their aquatic habitat.

**PLANT REPRODUCTION—THE REVIEWS**

In recent years, many of the events that comprise higher plant reproduction have been studied at the physiological, biochemical, genetic, and molecular levels. With these studies have come new insights into such processes as the transition to flowering, the development of flowers and floral organs, gamete development, fertilization, and embryogenesis. The reviews in this issue, which were invited and edited by Robert B. Goldberg, the founding editor of THE PLANT CELL, and Rebecca Chasan, the news and reviews editor of THE PLANT CELL, discuss the current understanding of the mechanistic basis for many of these processes, point out questions that
remain to be answered and hypotheses that await testing, and propose experimental approaches that are poised to yield further insights, not only into plant reproduction per se but also into plant biology and development in general.

Because lower plants and fungi can be more amenable to certain types of analysis than higher plants, several reviews discuss reproductive strategies and events in lower plants and fungi. Moreover, a complete understanding of plant reproduction will require piecing together the evolutionary events that intervened between life as a single cell capable of somatic or sexual differentiation and the complexities of multicellular life.

Flower Development

The initiation of reproduction generally requires that the plant perceive and respond to the appropriate environmental conditions. What kinds of mechanisms couple changes in environmental conditions to the initiation of flowering? In their review, Bernier et al. point out that the different environmental stimuli that potentially control the flowering transition are perceived by different parts of the plant. Thus, the apical meristem must integrate one or more long-range signals when "deciding" whether to switch from vegetative to reproductive development. The nature of these signals has yet to be determined, but Bernier et al. discuss both physiological studies in Sinapis alba and genetic studies in Arabidopsis that show that a number of different molecules can control the transition to flowering, including carbohydrates, auxins, gibberellins, cytokinins, and calcium.

How is it that a meristem influences its derivative cells to adopt particular fates? Why, for example, does a floral meristem produce flowers rather than leaves? Huala and Sussex discuss evidence from experimental manipulations that the determination of the shoot apex and of its derivatives may be separable; the mechanisms that normally couple a change in the meristem's behavior (e.g., the phyllotaxy of its derivatives) to the identity of these derivatives remain to be uncovered. Meristem function requires the integrated behavior of three cell layers, and Huala and Sussex describe studies of chimeric meristems that suggest that signaling among these layers coordinates their growth.

One way to investigate differences in the distinct "states" of the apical meristem is to examine changes in the expression patterns of genes expressed there. A number of genes have been isolated that are expressed in the apical meristem; as Meeks-Wagner discusses, the expression patterns of some of these genes are fixed during the vegetative-to-floral transition, while those of others become modified. Still other genes are expressed only at the floral stage. A major challenge in developmental genetics will be to organize these genes into hierarchies in which we can see the "logic" of the regulatory network that produces their orderly expression.

Among the genes that are expressed only at the floral stage are leafy (lfy) and apetala1 (ap1) of Arabidopsis and their Antirrhinum homologs floricaula and squamosa. Each pair of genes regulates meristem identity; in addition, as Coen and Carpenter discuss, lfy plays a role in partitioning cells in the Arabidopsis floral meristem. Once a meristem is specified as floral, it begins to produce floral organ primordia, whose identities—sepal, petal, stamen, or carpel—are determined by floral organ identity genes expressed in the developing flower. Coen and Carpenter discuss models for how these genes interact to control organ identity, and they point out that the organ and meristem identity genes engage in complex regulatory interactions whose details are just beginning to be worked out.

One of the complications of the analysis of flower development is that some of the genes originally identified as controlling floral organ identity, among them the Arabidopsis genes ap1, ap2, and agamous, are also turning out to function in the actual establishment of the floral meristem, as Okamuro et al. show. For example, ap2 mutations not only cause homeotic changes in floral organ identity but also can result in a partial conversion of the floral meristem to an inflorescence meristem. The phenotypes of mutations in these genes and in lfy and terminal flower, which are also involved in the regulation of meristem identity in Arabidopsis, are enhanced by changes in photoperiod, and Okamuro et al. suggest that one or more of these genes may be regulated by gibberelin, whose levels respond to photoperiod. This may be one physiological mechanism by which environmental changes are relayed to the genes that control flowering.

In addition, the structure of the floral meristem itself may place biophysical constraints on the process of organ formation and may thereby influence organ identity. Hernández and Green (1993) have used a scanning electron microscopic approach to analyze primordium formation in sunflower. From sequential sampling of the same living meristem, which was made possible by the use of replicas, it is clear that organ formation is a multicellular process involving tissue buckling and folding to form ridges and creases. The pattern of formation of these initial structures from an undifferentiated field of cells is constrained—possibly even determined—by the physical properties of the meristem itself; when these properties are altered experimentally, organ identity is altered. Thus, fundamental properties such as phyllotaxy and organ identity may depend on the preexisting physical state of the apex at the time of organ initiation. This physical state is in turn a reflection of previous gene expression and cellular history, i.e., of prior planes of cell division and differential reinforcement in the cell walls.

If the resolution of in situ hybridization could be combined with this direct and carefully timed view of floral organ formation, it should be possible to ask whether the expression of some of the flowering and organ identity genes is influenced by the buckling and folding of the meristem as it initiates the organ primordia. The link between gene expression and cell structure, including the activity of stretch receptors and the state of the cytoskeleton, is a new frontier in plant developmental biology and one that promises to be particularly important if we are to understand how nonmotile plant cells
"know" their positions so as to elaborate the correct spatial and temporal pattern of gene expression.

Genetic analysis of flower development has not been restricted to Arabidopsis and Antirrhinum: Veit et al. discuss maize flowering mutants, and van der Krol and Chua discuss petunia flowering mutants. Homeotic mutations in petunia tend to affect the development of just one whorl of floral organs rather than two adjacent whorls, as is generally the case with the Arabidopsis and Antirrhinum organ identity mutations, hinting at possible differences in the process of organ specification. A large number of maize mutants have been identified that are defective in inflorescence and floret development. In maize, position-dependent, selective cell death plays a major role in setting both the number and function of flowers. All flowers are initially complete (bear both male and female sex organs), but selective cell death results in exclusively male or female flowers (see below); in addition, only one of each pair of female flowers matures. Among the mutants are examples of defective male (tassel) and/or female (ear) inflorescence axes; other mutants have spikelets or florets of altered structure. Veit et al. discuss ways some of the corresponding genes may interact during inflorescence development.

Both petunia and maize contain molecular homologs of Arabidopsis and Antirrhinum genes known to function in flower patterning. van der Krol and Chua present evidence from genetic studies and studies of gene expression that implicate some of the petunia genes in flower development. The existence of maize homologs of the Arabidopsis and Antirrhinum genes suggests that, despite the evolutionary distance and differences in flower structure between monocots and dicots, ancestral pattern formation mechanisms have been preserved.

Although many molecular homologs have been reported, it is important to remember that each species has unique floral characters. Such characters may reflect minor changes in the terminal steps of differentiation or more major alterations in the activity of regulatory genes. Regulatory genes tend to evolve, not so much in structure as in terms of which sets of target genes they regulate. For example, the $R$ regulatory locus of maize, which encodes a putative transcription factor and is required in maize solely for the expression of the anthocyanin structural genes, stimulates anthocyanin accumulation in transgenic tobacco and Arabidopsis, suggesting that the regulation of this pathway is conserved (Lloyd et al., 1992). But in addition, maize $R$ corrects multiple defects in the Arabidopsis transparent testa glabrous mutant, restoring not only anthocyanin pigmentation but also seed coat mucilage and leaf trichomes and suppressing the excess root hairs characteristic of this mutant. These observations suggest that whereas the $R$ gene has a restricted scope of target genes in maize, it is likely that a related Arabidopsis gene has a much more extensive repertoire of target genes, all of them affecting differentiated characters of specific epidermal cell types. Because the details of floral structure provide key taxonomic differences among angiosperm species, we should expect to find many examples of novel combinations of regulatory and target genes contributing to the final diversity of floral form and perhaps a few examples of radical shifts in the regulatory players in the patterning process itself.

Development of Floral Reproductive Organs

Once the organ identity genes have acted to establish the basic pattern of the flower, the organ primordia begin to develop and differentiate into mature floral organs. The reproductive organs, the stamens and carpels, both contain a large number of cell and tissue types, many more than the sterile sepals and petals. The stamens consist of a filament topped by a complex anther structure within which microspores are produced. The development and function of the anther requires the coordinated activity of many different cells and tissues, and a number of questions remain to be answered about anther development, as Goldberg et al. point out. What mechanisms lead to the differentiation of particular anther cell types? Does anther differentiation involve primarily cell-autonomous processes? Or is cell–cell communication an important component of anther differentiation? Genetic ablation experiments, in which highly specific promoters are used to drive cytotoxic gene expression, are beginning to be used to address some of these questions, as is mutant analysis.

The mechanisms that underlie pistil development are also being explored by both molecular and genetic analysis, as Gasser and Robinson-Beers discuss. Pistil development involves not only the development of individual carpels but also, in many plants, their fusion, either before or after primordia initiation. The identification of genes expressed in pistils is beginning to provide information about the kinds of proteins that function in this organ, and genetic studies have yielded mutants that are altered in fusion processes or in the differentiation of specific structures.

Sex Determination

The flowers of most angiosperms are "perfect," that is, they contain both stamens and carpels, but in others, a sex determination process causes the abortion of the primordia for one or the other type of sexual organ. As Dellaporta and Calderon-Urrea discuss, monogy (the production of male and female flowers on a single plant) in maize is under the control of a set of genes known as the tasselseed loci. A number of different mechanisms have been identified that establish the sexuality of dioecious plants, which produce separate male and female individuals. These mechanisms, which Dellaporta and Calderon-Urrea discuss, include sex chromosomes and the measurement of X-to-autosome ratios.

Pigment Biosynthesis in Flowers

Although they are sterile, brightly colored petals often play an indirect role in reproduction, attracting the appropriate birds
or insect pollinators. Petal development therefore involves not only the elaboration of petal structure but also the synthesis and deposition of pigment in the appropriate regions. As Martin and Gerats discuss, pigmentation mutants have been identified in a number of plants, including petunia and Antirrhinum; some mutations lie in structural genes for anthocyanin biosynthesis, whereas others define regulatory loci that control the transcription of the structural genes. Different regulatory genes appear to be active in different regions of the petal, leading to complex pigmentation patterns.

**Gametophyte Development**

Once the sex organs have matured, gametogenesis ensues. Microsporogenesis begins with the meiotic division of a diploid pollen mother cell in the anther; the haploid microspores then divide mitotically to produce the three-celled pollen grain. How these events are controlled is not yet known; as McCormick discusses, conserved genes have been identified that are expressed at specific stages of microsporogenesis but whose functions remain obscure. Antisense approaches may help define the role of some of these genes in microsporogenesis. Methods have been developed for culturing microspores in vitro, which may make additional kinds of experimental manipulation possible.

A large number of nuclear genes that act sporophytically to control male fertility have been identified through genetic analysis of a number of different plant species, as Chaudhury shows. These include genes required for stamen development; for microsporogenesis, including both premeiotic and postmeiotic events; for anther function (i.e., pollen release); and for pollen function. Gametophytically acting genes are also likely to be necessary for normal pollen development, although few such genes have yet been identified.

Studies of cytoplasmic male sterility (CMS) may also yield insights into microsporogenesis. Levings describes the T type of maize CMS, in which certain anther tissues become abnormal soon after meiosis. cms-T appears to be caused by a mitochondrial gene that encodes an inner membrane protein, URF-13, of unknown function. The susceptibility of cms-T maize to a fungal pathotoxin raises the possibility that anther tissues contain a molecule similar to this toxin; alternatively, because of the heavy demands on anther cell mitochondria during microsporogenesis, these cells may be particularly sensitive to an URF13-associated attenuation of mitochondrial function.

By contrast with microspores, which develop within anther locules but are not connected with the parent sporophyte, megaspores develop deep within parent sporophyte tissues. The embryo sac is a highly asymmetric structure, and as Reiser and Fischer point out, associated maternal tissues may control its development and give it polarity. Genetic analysis has yielded sporophytic mutants defective in both ovule and embryo sac development, but no sporophytic mutants specifically defective in embryo sac structure have been identified. Further mutant analysis may begin to answer some fundamental questions about embryo sac development, such as the extent to which it relies on gametophytically expressed genes. Interestingly, in maize, at least, there are far more male-sterile than female-sterile mutants; moreover, many deletion mutations can be transmitted through the egg but not the sperm. These genetic results suggest that microsporogenesis may overlap less with sporophyte development than does megasporogenesis (for discussion, see Coe et al., 1988).

**Pollination**

To reach the embryo sac, the pollen grain vegetative cell, which encloses the sperm cells, must grow through the pistil. As Mascarenhas shows, pollen tubes grow by tip growth, unlike most other plant cells, and are highly active in the synthesis of wall components, which appear to be brought to the growing tip by an actin-based motor system. How the pollen tube finds a path through the style and locates the ovule is among the major unsolved problems that Mascarenhas discusses; there is some evidence that both positive and negative chemical signals influence the directional growth of pollen tubes.

Pollen tube growth does not occur indiscriminately: a pistil will support the growth of pollen tubes from only the same species or a closely related species. In addition, many plant species have a genetic self-incompatibility (SI) mechanism that promotes outcrossing by arresting "self" pollen tubes, as determined by the genotype at the S locus. The two types of SI, gametophytic and sporophytic, differ in whether the haploid pollen genotype or the diploid pollen parent genotype, respectively, determines the success of pollination on a stigma of a particular genotype.

In solanaceous plants, which have gametophytic SI, the S locus encodes a glycoprotein with RNase activity. As Newbigin et al. show, this protein is expressed in the stigma, but it is not known how—or even whether—RNase activity is involved in the arrest of incompatible pollen tubes. An S locus-encoded pollen component must exist, but this molecule remains to be identified. The S locus of crucifers, many of which display sporophytic SI, encodes two proteins, a secreted glycoprotein (that is unlike that encoded by the S locus of solanaceous plants) and a receptor-type kinase. Nasrallah and Nasrallah present evidence that self-recognition may involve the activation of the kinase, possibly by binding of the S locus-encoded glycoprotein.

The selective pressure for outcrossing in flowers that could otherwise be self-pollinated presumably maintains both CMS and self-incompatibility in wild plant populations. However, some plants have mechanisms that actually encourage self-pollination. These include cleistogamy (in which pollination takes place while the flower is still closed), a process that can occur even in self-incompatible species, and, in maize, a compensatory gametophytic advantage (GA) system that favors germination and growth of pollen with the same GA factors as the silks. With multiple GA loci, the definition of self can be precise, and the advantage of self-pollen can be 100-fold or more.
Fertilization

Once the pollen tube reaches the embryo sac, double fertilization—of the egg cell by one sperm cell and of the central cell by the other sperm cell—can take place. Double fertilization is sometimes nonrandom, as Dumas and Mogensen point out in their review of maize fertilization, possibly because the two sperm of a pair bear different recognition determinants. Recently developed methods for isolating pure sperm and egg cells should help answer this and other questions about fertilization, such as why sperm and egg cells are naturally fusigenic.

The location of the embryo sac has made it difficult to study egg cell development, but as Russell shows, ultrastructural analyses have begun to paint a portrait of the cellularization events that result in the production of the mature egg cell. The pollen tube deposits the sperm cells into the synergids, and they then move toward the appropriate fusion partner. There are some indications that actin bundles in the embryo sac may facilitate the movement of the sperm cells to the incompletely cellularized regions of the egg and central cells, where fusion appears to take place. The synergid cytoplasm probably aids fertilization in some way, possibly because, as Russell points out, its vacuole contains large amounts of calcium.

Embryogenesis

Localized maternally derived molecules within the egg cell may define asymmetries in the early embryo. As West and Harada discuss, the first zygote division often creates daughter cells that differ in cytoplasmic constitution and that have different fates, one becoming the embryo and one the suspensor, a multicellular structure that connects the embryo to maternal tissues. In some plants, this first division is predictable and asymmetric; in other plants, it is random or symmetric. No maternal mutations have been described that alter the polarity of the early plant embryo, but a number of zygotic mutants have been described in which the early patterning events that define specific domains along the apical–basal axis are abnormal. In other mutants, later morphogenetic processes are disrupted but tissue differentiation still takes place.

The suspensor has traditionally been viewed as unimportant for embryo development per se, but Yeung and Meinke show that mounting evidence indicates that the suspensor cells are metabolically active and supply both nutrients and growth regulators to young embryos. In turn, the embryo appears to dictate the fate of suspensor cells: destruction of the embryo is often accompanied by the abnormal growth of the suspensor. Some insights into the interactions between embryo and suspensor should come from the analysis of mutants that have both arrested embryos and abnormal suspensors.

Like the suspensor, the endosperm does not contribute directly to the embryo but plays an essential, apparently nutritive role. The endosperm nucleus develops into a simple, terminally differentiated tissue with few cell types. Why its development is so different from that of the diploid zygote nucleus, with which it shares all of its alleles, is a major unsolved question, as Lopes and Larkins point out. There are a number of different patterns of endosperm development, but no matter how it develops, endosperm tissue is ephemeral, being assimilated into the developing embryo or germinating seedling. Among the components of endosperm are starch, storage proteins, oils, and carbohydrates; much still needs to be learned about the regulation of the synthesis of many of these compounds. The reliance of much of the world's population on endosperm as a food source lends great importance to the goal of manipulating endosperm composition.

The later stages of plant embryonic development are devoted to preparing for dormancy and the germination event that will follow. Thus, late in development, the embryo synthesizes storage protein and lipids at high levels. Abscisic acid, whose levels increase early in the maturation phase, is thought to activate the transcription of genes for seed storage proteins and proteins that may protect the embryo during desiccation. Promoter analyses of many of these genes, discussed by Thomas, have uncovered a number of conserved sequence elements, some of which confer ABA responsiveness or localized gene expression within the seed.

The capacity to support zygotic embryogenesis is not limited to the egg cell or even to the megaspore mother cell: in many plants, embryos arise readily from unfertilized ovule cells in the process known as apomixis, which is discussed by Koitunow. Apomictic embryos can derive from embryo sacs formed by a megaspore mother cell that fails to undergo meiosis or by a non-megaspore mother cell of the nucellus; adventitious embryos can also be formed directly from ovule cells that have not formed embryo sacs. Apomixis appears to be controlled by just a few genes, none of which has yet been isolated. These genes may be ectopically expressed alleles of genes that normally initiate or regulate sexual maturation.

Plant embryos can arise not only from fertilized eggs or unfertilized ovule cells but also directly from undifferentiated somatic cells. The development of somatic embryos is similar to that of zygotic embryos throughout most of embryogenesis, as Zimmerman shows, although they differ in both the initiation of embryogenesis and in the events of late embryogenesis. Because somatic embryos can be prepared in large quantities, they have served as useful starting materials for the isolation of embryo-specific genes, some of which have proven to be molecular markers for particular embryonic tissues. Somatic embryos may also be used for genetic analysis, and Zimmerman points out that conditional mutants may be particularly informative.

Fruit Development

The development of the embryo is coordinated not only with that of the endosperm but also with that of the fruit, which is formed by the ovary. Even before fertilization, as Gillaspy et
al. show, the growing pollen tube stimulates fruit growth; the formation of an embryo and endosperm are normally necessary for continued fruit growth, although, as Gillaspy et al. discuss, hormone treatment can induce fruit to form in the absence of fertilization. Thus, the signals that initiate fruit development may do so by influencing phytohormone levels or transport. The developing embryo also regulates both the division and expansion of fruit cells, again by modulating phytohormone levels. Photoassimilate import and isoprenoid levels also control early fruit development. Gillaspy et al. point out that many aspects of fruit development make this a valuable plant organ in which to study the mechanisms that regulate plant cell proliferation and differentiation.

**Reproduction in Lower Plants and Fungi**

Reproductive and morphogenetic processes are often more accessible in lower plants and fungi than in higher plants. For example, zygotes of the alga *Fucus* undergo a process of polarity establishment and fixation before they begin to divide. Because these zygotes are large and form apart from the parent plant, the establishment of polarity can be studied experimentally in a way that is not possible for the embryo sac or zygote of flowering plants. Goodner and Quatrano show that distinct processes are involved in the induction of polarity, the fixation of polarity, and the expression of polarity. The possibility that these events involve *Fucus* homologs of gene products known to function in asymmetric divisions in yeast is beginning to be explored.

Another lower plant, the moss *Physcomitrella patens*, has great potential for the study of plant development and reproduction, as Cove and Knight show. The gametophyte, which is the dominant stage of the life cycle, contains relatively few cell types but can give rise to gametes or apogamous progeny by a number of different pathways. Mutants have been identified that are defective in hormone levels or response and, as a result, are blocked at a particular stage of gametophyte development. New transformation techniques should allow the isolation of the corresponding genes by complementation.

As Timberlake shows, the fungus *Aspergillus nidulans* provides the opportunity to study conidiation, a morphogenetic process that is induced by environmental signals. Genetic, biochemical, and molecular data support a model in which a central regulatory gene, which encodes a transcriptional activator, is subject to translational control. Translation of this gene product results in the activation of another regulator gene, which activates both structural and regulatory genes, including itself. Thus, once initiated, the conidiation pathway rapidly becomes fixed.

Fungi have also been used to study the initiation of sexual development. As Bölker and Kahmann discuss, sexual development is initiated when compatible mating types secrete pheromone signaling molecules that cause cell cycle arrest, morphological changes, and changes in gene expression. The pheromones secreted by many different fungi have been purified and found to be oligopeptides that are often highly hydrophobic. Some of the components that transduce pheromone–receptor binding to cellular effectors have been identified in fission and budding yeast.

**CONCLUSION**

The reviews in this special issue provide an overview of the current understanding of many of the events of plant reproduction, that of angiosperms in particular. True comprehension of the biochemical and cellular mechanisms that underlie the intricacies of plant development will come only as we gain a more detailed understanding of the cell biology, biochemistry, and physiology of plant cells in general. Among the most important questions are: How do plant cells communicate with one another? What kinds of signal transduction pathways do plant cells employ? How are asymmetries established in fields of plant cells, or within single plant cells? Studies of plant development may in their turn shed light on some of the most basic questions in plant cell biology. We do not know what our understanding of plant reproductive development will be 5 years from now, but we can confidently predict that although some questions will have been answered, many more that we cannot even begin to formulate today will have been posed.

**REFERENCES**


