

Male Gametophyte Development

Sheila McCormick

Plant Gene Expression Center, United States Department of Agriculture–Agricultural Research Service and University of California–Berkeley, 800 Buchanan Street, Albany, California 94710

INTRODUCTION

Pollen development offers the opportunity to study cell fate, cell patterning, cell polarity, and cell signaling—in this sense, pollen development can serve as a microcosm for all the interesting questions facing plant biologists today. The events that culminate in the formation and release of the pollen grain from the plant begin with meiosis and involve an intricate and tightly controlled set of structural and molecular changes, requiring gene expression in both the gametophytic and sporophytic tissues of the anther.

The biochemistry of angiosperm pollen development was first reviewed comprehensively 18 years ago (Mascarenhas, 1975), and several subsequent reviews have appeared (e.g., Mascarenhas, 1989, 1990, 1993, this issue; McCormick, 1991; Bedinger, 1992). Although a great deal of progress has been made, it is notable that two of the areas for future research noted by Mascarenhas (1975) remain today: What are the differences in the two cytoplasmic compartments that determine the different cell fates of the generative and vegetative cells? What are the functions of pollen-specific proteins? In this review, I will give an overview of the current state of knowledge of microsporogenesis, minimizing repetition of the topics covered in other recent reviews, and I will try to point out promising avenues of research for the future.

OVERVIEW OF MICROSPOROGENESIS

Male gametogenesis begins with the division of a diploid sporophytic cell, giving rise to the tapetal initial and the sporogenous initial (pollen mother cell). Figure 1 illustrates the subsequent events leading to the formation of a pollen grain. The sporogenous cells undergo meiosis, giving rise to a tetrad of haploid cells. The individual cells of the tetrad are released as free microspores by the action of an enzyme (callase) produced by the tapetum layer of the anther. These uninucleate microspores undergo an asymmetric mitotic division, resulting in a pollen grain containing two cells: a larger vegetative cell and a smaller generative cell that is enclosed entirely within the vegetative cell. The generative cell has a condensed nucleus and a reduced amount of cytoplasm compared to the vegetative cell. In ~70% of plant families (e.g., Solanaceae

and Liliaceae), the pollen grain is released from the anther when it consists of just these two cells; the second mitotic division of the generative cell, to give two sperm cells, occurs while the pollen tube grows through the female pistil. In other plant families (e.g., Cruciferae and Gramineae), this second mitotic division occurs before the pollen is shed from the plant.

From Meiosis to Uninucleate Microspore

In many plants, meiotic divisions are synchronous within an anther, probably due to the cytoplasmic connections that can be seen between pollen mother cells. Mutants can help identify important components of plant meiosis. Many meiotic mutants have been described at the genetic and cytological level (Kaul and Murthy, 1975; see also Chaudhury, 1993, this issue), including mutations that affect entry into meiosis, chromosome synapsis, recombination, and spindle formation. To date, none of the plant meiotic mutant genes has been molecularly characterized, although some genes affecting these processes have been cloned in other organisms (e.g., Komma et al., 1991; Sym et al., 1993) and could potentially be used as heterologous probes to monitor plant meiosis.

Characterization of the distribution of cytoskeletal proteins may help clarify the defects in the mutants. For example, fluorescent antibody probes for cytoskeletal proteins are being used to reexamine maize meiotic mutants. Meiosis in maize, as in many other plants (Brown and Lemmon, 1992), shows cell polarity, with predictable division planes relative to the position in the anther locule. Staiger and Cande (1990) characterized division planes in the maize meiotic mutant *divergent spindle* (*dv*). In this mutant, formation of the metaphase I spindle is defective, and they suggest that the normal product of the *dv* gene plays a role in the formation of the microtubule organizing center at the nuclear envelope. This mutation apparently has no effect on mitotic divisions in the somatic cells of the plant.

In another maize mutant, *ameiotic*, the pollen mother cells cannot enter meiosis (Staiger and Cande, 1992). In almost all plant mitoses, a ring of microtubules, the preprophase band, marks where the new cell wall will insert into the old wall (Wick, 1991). However, a preprophase band is not normally seen in

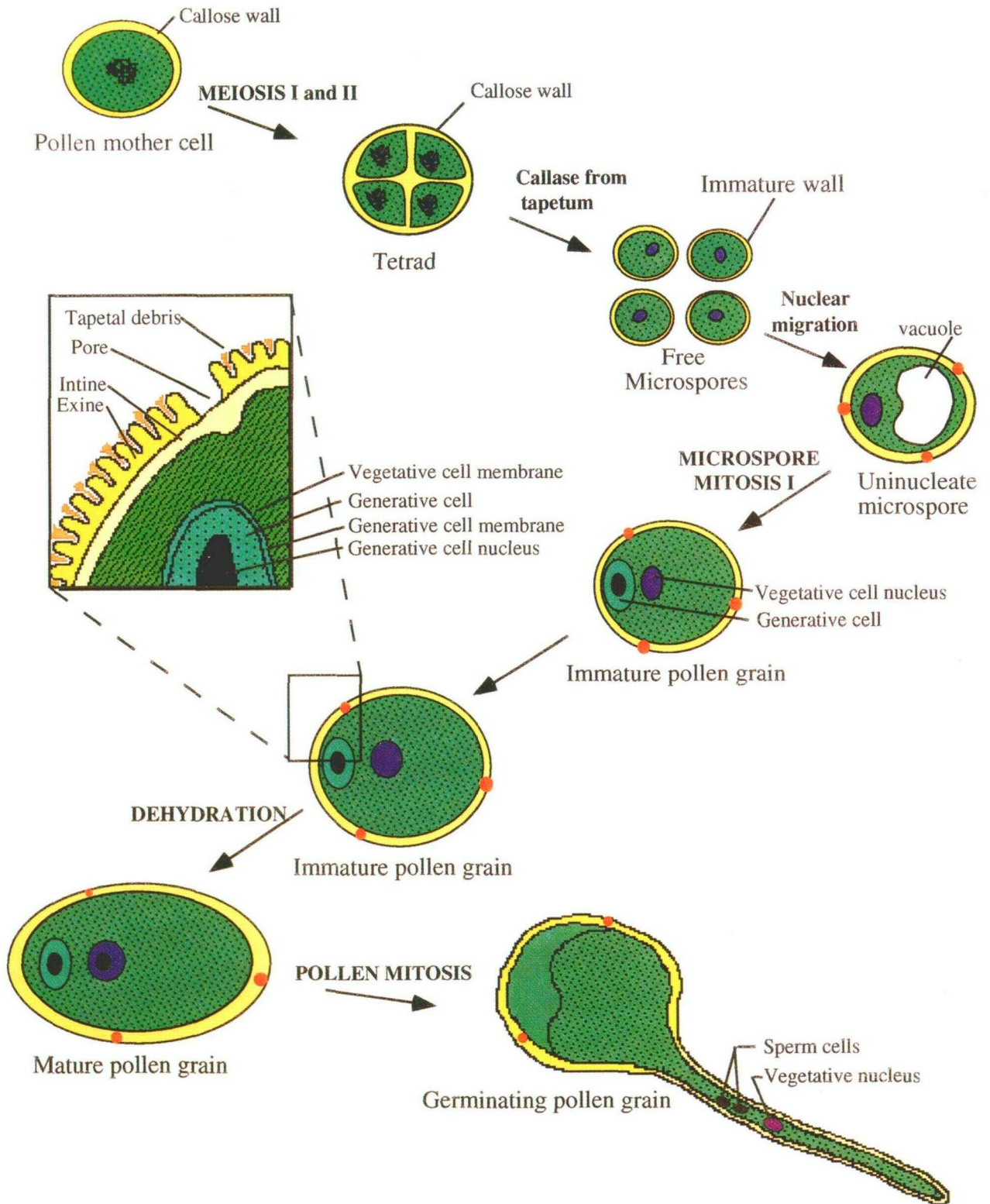


Figure 1. Schematic of Microsporogenesis Typical of Most Angiosperms.

dividing reproductive cells, either when they are dividing meiotically or when they are dividing mitotically. The *ameiotic* mutant replaces meiosis I division with a mitotic-like division, including the presence of the preprophase band (Staiger and Cande, 1992), suggesting that the normal product of the *ameiotic* gene acts to prevent the formation of the preprophase band in reproductive cells.

Few meiosis-specific plant proteins have been described. Several years ago, Hotta et al. (1985) used lily meiocytes to identify meiotic-specific transcripts that correlated with synapsis during zygotene, although no further molecular characterizations of this system were reported. A protein from lily pollen mother cells, termed pollen mother cell nuclear protein, is similar to the mammalian testis-specific H1 histone (Sasaki et al., 1990). The histonelike proteins are thought to play a role in chromatin packing. Riggs and Hasenkampf (1991) described a meiosis-specific protein from lily, termed meiotin-1, that also shows biochemical features consistent with a histone. Meiotin-1 antibodies recognize proteins in monocots and dicots. Meiosis-specific cDNA clones that show sequence similarity to the small heat shock proteins have been characterized by Bouchard (1990), although the role of these proteins is not known.

Soon after meiosis is completed, synthesis of the pollen cell wall begins. The mature pollen wall is composed of two layers, an outer exine and an inner intine. The intine wall is largely pectocellulosic, whereas the exine wall is composed of sporopollenin, a complex substance that is very resistant to degradation (Bedinger, 1992). The exine is patterned with a complex pattern of spines, netlike ridges, or other projections. The species-specific patterns of wall sculpturing are determined by the sporophyte. For example, no segregation for wall pattern was seen in the pollen of an F₁ hybrid of a cross between *Lycopersicon esculentum* and *L. pennellii* (Quiros, 1975), although independent assortment for genes that control certain features (such as spine density and spine length) was clearly demonstrated.

The blueprint for pollen wall sculpturing patterns is laid down during meiosis, while the tetrads are still encased in the callose wall, and can be seen as projections from the plasma membrane (Takahashi, 1993). How species-specific pollen wall patterning is determined at the cellular level is completely unknown. The callose wall apparently acts as a framework for exine wall deposition because tetrads that are released prematurely from the callose wall have abnormally developed exines and the microspores burst (Worrall et al., 1992).

The positions of apertures (pores where the pollen tube can grow out) are also determined early and may correlate with the position of the meiotic spindle poles (Blackmore and Barnes, 1990). The intine, or inner pollen wall, is first laid down over the sites of these pores and then spreads to encase the entire microspore. The sites of pore formation are marked by stacks of endoplasmic reticulum (ER). Exine deposition is reduced or absent over the apertures.

The microspores grow in circumference soon after their release from the tetrad, and wall synthesis and exine deposition

increase during this stage. The tapetum is largely responsible for the synthesis and deposition of sporopollenin and other wall materials to the developing exine. It is widely assumed that the callose wall that surrounds the tetrads is an impermeable barrier that serves to isolate the developing microspores, possibly to "switch" them to a gametophytic mode of development. However, this view has been questioned (Mascarenhas, 1975; Rodriguez-Garcia and Majewska-Sawka, 1992) in light of the apparent ability of some high molecular weight substances to cross this barrier.

The phenotypes of the wheat mutant *pollen killer* (Loeagering and Sears, 1963) and the tomato mutant *Gamete eliminator* (*Ge*) (Rick, 1966) suggest that the products of meiosis exchange information or otherwise interact in some way. For example, in *Ge* plants that are heterozygous for the two alleles *Ge^c* and *Ge^p*, the pollen grains that are carrying the *Ge^c* allele are aborted, although *Ge^c* pollen grains are not aborted in *Ge^c/Ge^c* homozygotes. This is somewhat reminiscent of the meiotic drive systems in fungi (Raju and Perkins, 1991, and references therein). In these systems, a genetic factor can somehow "kill" the meiotic products that do not contain it or otherwise prevent those meiotic products from achieving transmission to the next generation. The mechanisms for such recognition and "killing" are completely unknown.

From Microspore Mitosis to Pollen Mitosis

Microspore mitosis is an asymmetric division. This division can also be viewed as a determinative division in that the resulting two cells have very different cell fates (Horvitz and Herskowitz, 1992). The vegetative cell nucleus does not undergo another mitosis, and its role is to serve as a "powerhouse" to drive the further development of the pollen grain and the growth of the pollen tube so that the two sperm cells are delivered to the ovary. The generative cell must undergo another mitotic division to give rise to the two sperm cells. The generative and sperm cells contain few mitochondria, sparse ER, and condensed chromatin; these characteristics and a lower nuclear pore density (Wagner et al., 1990) suggest that the generative and sperm cell nuclei are less transcriptionally active than the vegetative cell nucleus.

Numerous studies have shown a close association of the generative cell and sperm cells with the vegetative nucleus that persists (Figure 1) even during pollen tube growth. Serial electron microscopy reconstructions of alfalfa microspores showed that there are more nuclear pores on the surface of the vegetative nucleus that is appressed to the generative cell than on the opposite surface, implying communication between the vegetative nucleus and the generative cell (Shi et al., 1991). To date, no experiments have tested whether specific transcripts or proteins are transported from the vegetative to the generative cell. In situ hybridizations to pollen with several pollen-expressed mRNAs (Hanson et al., 1989; Ursin et al., 1989; Reijnen et al., 1991) suggested that these genes are

expressed in the vegetative cell, but the resolution was not sufficient to exclude expression in the generative cell. By fusing a nuclear targeting signal to the β -glucuronidase (GUS) reporter gene, Twell (1992) showed convincingly that the promoter of a pollen-specific gene, LAT52, directs vegetative cell-specific gene expression.

Is the generative cell metabolically dependent on the vegetative cell? Or are there transcripts expressed specifically in the generative cell? It might be predicted that the generative cell will express genes whose products are required for the second mitotic division, such as cyclin or other cell cycle control genes (Jacobs, 1992). The generative nucleus progresses through S phase and stops in G2 shortly after microspore mitosis, whereas the vegetative nucleus arrests in G1 (Zarsky et al., 1992) and thus would be unlikely to express cyclins.

In support of the partial autonomy of the generative cell, isolated generative cells can be cultured and remain viable for up to 10 days (cited in Yang and Zhou, 1992). A small percentage (3 to 11%) of the cultured generative cells is able to divide mitotically in the absence of the vegetative cell. The low frequency of division might be due to a suboptimal level of some component in the culture medium or might imply that only some of the generative cells were competent for division, perhaps because of their stage in the cell cycle when isolated. Protoplast fusion experiments between pollen protoplasts and isolated generative cell protoplasts have also been used to address potential interactions between the vegetative and generative cells. An additional generative cell introduced into a pollen protoplast showed decondensed chromatin and could not undergo mitosis, although the preexisting generative cell in the protoplast could undergo mitosis (Ueda et al., 1990). It is not clear why the existing generative cell would not be influenced by the proposed "cytoplasmic" factors that cause the introduced generative cell to arrest.

The vegetative and generative cells differ in the expression at their surfaces of epitopes recognized by monoclonal antibodies. Pennell and Roberts (1990), Pennell et al. (1991), and Van Aelst and Van Went (1992) have shown that certain monoclonal antibodies raised against carbohydrate epitopes of plasma membrane components differentially label the generative and the vegetative cell. It will be interesting to see whether these epitopes are part of glycoproteins and whether the proteins are encoded by messages that are translated in the vegetative or generative cell. That is, does the vegetative cell "mark" the plasma membrane of the generative cell, or does the generative cell "mark" itself? Such generative or sperm cell-specific epitopes might play a role in sperm-egg cell recognition (Blomstedt et al., 1992).

As mentioned above, there is no preprophase band to demarcate where the cell division plane will occur in meiocytes and microspores. Nuclear movement from the center of the cell toward a position near the microspore wall (Figure 1), which is the first step in setting up the asymmetric division, is mediated by components of the cytoskeleton (Brown and Lemmon, 1991), because it can be inhibited with colchicine (Terasaka and Niitsu, 1990). If the asymmetry of this mitotic division is

altered, microspores are "switched" from a gametophytic to a sporophytic mode of development and divide to form callus or microspore embryos that develop into haploid plants (Prakash and Giles, 1987).

Mature ungerminated pollen contains proteins that were synthesized during maturation as well as a store of presynthesized mRNAs that are translated upon germination (reviewed in Mascarenhas, 1989). The presynthesized mRNAs stored in mature pollen might be subject to translational control, as has been shown for maternal messages in animal oocytes (reviewed in Richter, 1991). Translational control in oocytes is at least partially mediated by differences in poly(A) tail length (Bachvarova, 1992); however, whether this mechanism is used for mRNAs synthesized during microsporogenesis has not been examined. If mRNAs that are translated solely in the vegetative cell are present in the uninucleate microspore before the division, are such messages distributed differentially to the two daughter cells? What regulates their distribution and the timing of their translation? No experiment has assessed whether such mRNAs possess localization signals similar to those that have been found in certain animal mRNAs that are differentially localized within a cell (Schwartz et al., 1992).

The second mitotic division occurs either in the developing pollen grain or during pollen tube growth through the style, which implies that hydrated pollen is capable of transcription and translation. The effects of transcriptional and translational inhibitors on the second mitotic division were examined in *Tradescantia* pollen, under conditions in which the generative cells divide \sim 5 hr after germination (Mascarenhas, 1975). During the first 2 hr after germination, both the generative and vegetative nuclei were transcriptionally active, as assayed by the incorporation of radioactive uridine into RNA. Transcriptional inhibitors did not prevent the early stages of pollen germination. However, inhibiting RNA or protein synthesis blocked generative cell mitosis if the treatments were given in the first 1 to 2 hr after germination began but not if given after 3 to 4 hr. This suggests that the RNA or proteins required for cell division are synthesized in the first 2 hr of germination, although whether they are synthesized in the vegetative or generative cells could not be discerned from these experiments. Another indication that hydrated pollen is capable of transcription and translation comes from experiments in which pollen that is bombarded with pollen promoter-reporter gene constructs exhibits reporter gene expression within 30 min of bombardment (McCormick et al., 1991b).

GENES EXPRESSED DURING MICROSPOROGENESIS

By using *in vivo* labeling and two-dimensional PAGE to characterize protein synthesis, Mandaron et al. (1990) showed that the rate of protein synthesis varies during maize microsporogenesis. They showed that the period from tetrad stage to vacuolated microspore (Figure 1) is extremely active in protein synthesis. During starch accumulation, protein synthesis

is low, but just before anther dehiscence, several basic polypeptides are synthesized. Mandaron et al. (1990) suggested that these basic polypeptides may be required during pollen germination. Bedinger and Edgerton (1990) translated mRNAs isolated from staged maize microspore populations and demonstrated that a switch in gene expression occurs around the time of microspore mitosis.

Mascarenhas (1990) separated pollen gene expression into two phases. Transcripts from the "early" genes are first detectable soon after meiosis and are reduced or undetectable in mature pollen. Transcripts of the "late" genes are first detected around the time of microspore mitosis and continue to accumulate as pollen matures. It has been generally surmised that the early genes might encode cytoskeletal proteins and proteins needed for wall synthesis or starch deposition, whereas proteins whose mRNAs accumulate throughout microsporogenesis might be required during pollen maturation or pollen tube growth.

Numerous cDNAs have been characterized that correspond to genes that are activated around the time of the first microspore mitosis (reviewed in McCormick, 1991), but there are many stages of microsporogenesis for which we still lack useful molecular probes. Flow cytometry can be used to separate cells at different stages of microsporogenesis because the cells of different stages exhibit different light scatter parameters (Fuchs and Pauls, 1992). Construction of stage-specific cDNA libraries from staged cells separated by flow cytometry may allow the isolation of genes that are expressed in discrete time windows throughout microsporogenesis.

Two recent reports describe the isolation of cDNAs that probably fall into the "early" class of mRNAs described by Mascarenhas (1990). Message corresponding to the I3 cDNA clone of *Brassica napus* (Roberts et al., 1991) peaks in microspores around the time of microspore mitosis and then declines as pollen matures. The predicted I3 protein is proline rich and has an N-terminal hydrophobic sequence, indicating that it may be secreted to the developing pollen wall. The same researchers (Scott et al., 1991) have also isolated a class of cDNA clones corresponding to genes that are expressed at earlier stages during microspore development than the I3 gene. One such gene (E2) encodes a protein that is similar to phospholipid transfer proteins and is suggested to play a role in sporopollenin deposition (Foster et al., 1992).

Other recent reports describe additional genes that fall into the "late" class of messages. The Bp10 gene of *B. napus* (Albani et al., 1992), the NTP303 gene of tobacco (Weterings et al., 1992), and the LAT51 gene of tomato (McCormick, 1991) are all expressed maximally in mature pollen and encode proteins that show sequence similarity to cucumber ascorbate oxidase (Ohkawa et al., 1989). Sequences that are highly similar to the LAT51 sequence are highly conserved in diverse plant species (S. McCormick, unpublished observations), suggesting a conserved role for the LAT51 homologs. However, it is unlikely that any of these pollen proteins are functional ascorbate oxidases because ascorbate oxidase is a copper-requiring enzyme, and the pollen proteins do not conserve the copper

binding amino acids. Bcp1, a *B. campestris* pollen gene that is expressed after microspore mitosis, encodes a 12-kD alanine-rich protein of unknown function (Theerakulpisut et al., 1991). The Bcp1 protein is present in ungerminated pollen.

Two groups have isolated genes that may play a role in cytoskeletal organization. In mature unhydrated pollen, actin fibrils appear around the plasma membrane, oriented with the cellulose fibrils of the intine and also around the generative cell and the vegetative nucleus. Upon hydration, these actin reservoirs re-form and organize into extended fibrils that are oriented toward the apertures (Tiwari and Polito, 1988; Heslop-Harrison and Heslop-Harrison, 1992). Kim et al. (1993) have isolated a pollen-expressed cDNA (LMP131A) whose sequence is related to an actin-depolymerizing factor; they suggest it may play a role in actin filament formation and cell surface movements. A major tree pollen allergen shows significant sequence similarity to profilin (Valenta et al., 1991), a protein involved in the regulation of actin polymerization.

Are all "housekeeping" genes expressed in pollen? Immature microspores can partially mount a heat shock response, but mature and germinating pollen exhibits essentially no heat shock response, and pollen germination is extremely sensitive to heat shock. This is apparently due to an inability of pollen to transcribe heat shock genes under stress (Hopf et al., 1992), although the stress conditions per se do not prevent transcription of other genes. Perhaps transcription factors for heat shock proteins are absent in pollen. A family of heat shock transcription factor genes has recently been isolated from tomato (Scharf et al., 1990), so the tissue specificity of these factors can now be tested directly.

A major goal for the future is to determine the function of the proteins corresponding to the mRNAs expressed during microsporogenesis, because although the sequence similarities are suggestive, in no case has function been proven for the anonymous cDNAs. Knowledge of the cellular location of pollen-expressed proteins should help to determine their function. Many of the anther-expressed genes that have been characterized (McCormick, 1991, and references therein) encode proteins with N-terminal hydrophobic sequences; these are thus assumed to be secreted. The ragweed allergens have been localized to the intine layer (Howlett et al., 1973), but the cellular locations of the other potentially secreted proteins are not known. Antibodies to the E2 lipid transfer protein might be expected to label the pollen wall if indeed this protein plays a role in sporopollenin deposition. Antibodies to the LMP131A protein and to the pollen protein that is similar to profilin might be predicted to localize to microtubules or microfilaments if in fact these proteins are important in actin polymerization. Sequence analysis of pollen gene homologs from additional species should also help to identify functionally important domains of the proteins they encode.

Another approach to assign function to anonymous cDNAs is to introduce antisense, overexpression, or dominant negative gene constructs into transgenic plants. If a pollen-expressed gene plays an important and (in the case of antisense experiments) nonredundant role during pollen development or

germination, the pollen of these transgenic plants might exhibit an altered phenotype. Antisense approaches will also be useful to assess the roles of potential microtubule components. When the genes for actin binding proteins were disrupted in *Dictyostelium* (Witke et al., 1992), a phenotype was seen only in double mutants, probably because of the built-in redundancy of cytoskeletal components. A collection of promoter elements that exhibits differing strengths and timing of expression during microsporogenesis will prove invaluable for gene disruption experiments; promoters that show different temporal patterns of activity can be used to direct transcript synthesis at times earlier or later than normal.

We have recently demonstrated that the antisense approach may help assign functions to anonymous genes. The LAT52 gene of tomato encodes a 161-amino acid protein (Twell et al., 1989) that shows 32% amino acid identity to the protein encoded by a pollen-specific gene from maize (Zm13) (Hanson et al., 1989). The LAT52 and Zm13 proteins both show sequence similarity to Kunitz trypsin inhibitor sequences (McCormick et al., 1991a), but neither of these pollen proteins is likely to encode a functional proteinase inhibitor, because they lack essential active site residues. Transgenic tomato plants were regenerated that carry a gene construct composed of the LAT52 promoter driving the LAT52 antisense gene; the plasmid used for transformation also carried a kanamycin resistance gene (McCormick et al., 1991a). Many of the transgenic plants did not transmit the linked kanamycin resistance gene through the male, suggesting that the pollen grains expressing LAT52 antisense transcripts did not function in achieving fertilization. Figure 2 illustrates the striking effect seen with such antisense plants, in which 50% of the pollen is expected to express the antisense transcript. Some of the pollen grains show aberrant tube growth and fail to grow the length of the style (J. Muschietti and S. McCormick, unpublished results). These results clearly demonstrate a functional role for the LAT52 protein during pollen germination, although further work will be needed to define that role.

GENE REGULATION DURING MICROSPOROGENESIS

Several promoters have been shown to direct gene expression in pollen (reviewed in McCormick, 1991). The LAT52, LAT56, and LAT59 genes are coordinately expressed during pollen maturation (Twell et al., 1990, 1991). *cis* regulatory elements in the promoter regions of these genes were defined from deletion analyses of these promoters (using chimeric genes with the GUS reporter gene) in transient assays and/or in transgenic tomato plants (Twell et al., 1991). These experiments indicated that relatively short promoter fragments are sufficient for pollen expression, implying that the *cis* sequences necessary for tissue specificity reside in these minimal promoters. The upstream regions of these promoters control the level of reporter gene expression. A similar deletion analysis

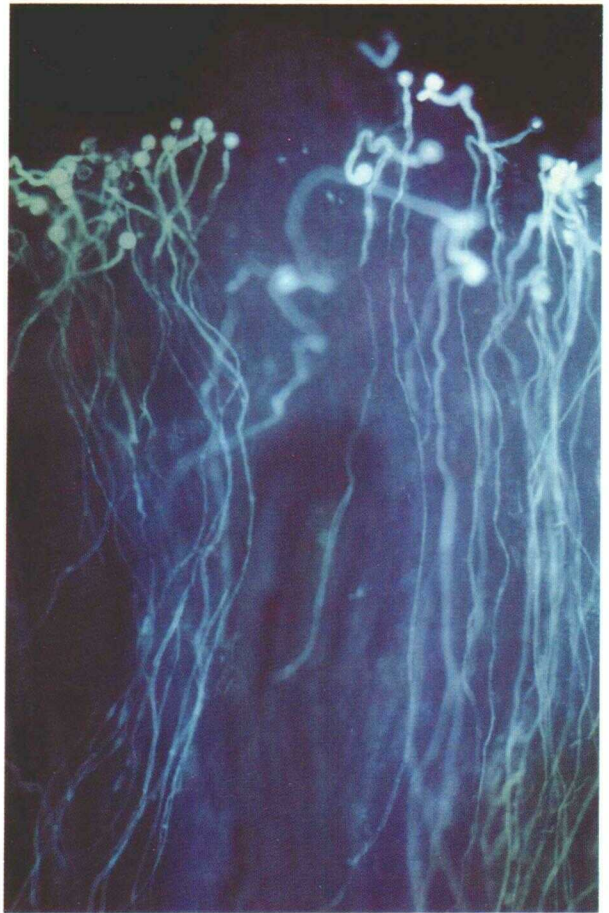


Figure 2. Tomato Pistil Pollinated by a Plant That Is Heterozygous for the LAT52 Promoter-LAT52 Antisense Construct.

The pollinated pistil was stained with decolorized aniline blue 24 hr after pollination.

of the pollen-specific α -tubulin (*TUA1*) promoter of Arabidopsis has recently been reported (Carpenter et al., 1992).

Although they direct pollen expression, the LAT52, LAT59, and *TUA1* promoters are not strictly speaking, pollen specific, because they show low levels of GUS activity in certain other tissues, i.e., roots (LAT59), endosperm (LAT52 and LAT59), and trichomes, tapetum, and receptacles (*TUA1*). The LAT52, LAT59, and *TUA1* promoters apparently have no silencer regions upstream of the pollen specificity region, because 5' deletions of these promoters show no generalized sporophytic expression.

The promoter sequences of the LAT52, LAT56, and LAT59 promoters are rather dissimilar, even though the genes show similar cell specificity and timing of expression (Twell et al., 1990). There is one 12-bp region of identity between the LAT52 and LAT56 promoters (termed the 52/56 box) and a different

10-bp region is conserved between the LAT56 and LAT59 promoters (termed the 56/59 box) (Twell et al., 1991). The 52/56 boxes occur in regions of the promoter that determine the level of activity rather than in regions that determine pollen specificity. Mutational analysis has shown that the 52/56 box and the 56/59 box are important for promoter activity (Twell et al., 1991).

The *TUA1* promoter of Arabidopsis, which like the LAT promoters is activated after pollen mitosis, shares upstream sequences similar to the 52/56 box and to the 56/59 box (at -87 to -79). A -97 deletion of the *TUA1* promoter still showed pollen specificity, whereas deletion to -39 (and removal of the 56/59 box) abolished activity. Clearly, the 56/59 box cannot be generally responsible for pollen expression, because its removal from the LAT59 promoter still allows pollen activity and because the LAT52 promoter has no sequence similar to the 56/59 box. Deletion of the 52/56 box regions in the *TUA1* promoter has no effect on the level of pollen activity.

In summary, there are no obvious shared sequences found in the minimal promoters of the three LAT genes, the *TUA1* gene, or the other characterized pollen promoters (references in McCormick, 1991). The molecular basis of pollen specificity is still unknown. Pollen specificity could be mediated by one *trans*-acting factor that recognizes seemingly different sequences in the minimal promoters, or different pollen genes may be recognized by different *trans*-acting factors but still expressed coordinately. The results to date suggest that additional promoters may need to be included in sequence comparisons before we can determine what sequences are important for pollen specificity. Evolutionary comparisons of promoter sequences in closely and more distantly related plant species may also help in delimiting regions of the promoters that confer tissue specificity.

Once the promoter sequences that mediate pollen-specific gene activity are identified, these can be used to isolate and characterize the protein factors that interact with these DNA sequences. A brute-force biochemical approach toward the isolation of *trans*-acting factors that interact with pollen-specific promoters may prove difficult because of the difficulty in isolating large amounts of pollen. If the gene in question is expressed in the vegetative cell, whose nucleus is very fragile, isolation of nuclei that would contain *trans*-acting factors would be especially difficult. It will be worthwhile, therefore, to explore the use of *in vivo* dimethyl sulfate footprinting (Hammond-Kosack et al., 1993) to determine binding sites for proteins on pollen-specific promoters.

Another avenue toward the isolation of *trans*-acting factors is a genetic approach. A genetic screen for mutations in *trans*-acting factors that regulate pollen gene expression will bypass the limitations of tissue source and nuclei purification. Several pollen-specific promoters are known to function in distantly related plants: the three tomato LAT promoters function in Arabidopsis (Twell et al., 1990) and the maize Zm13 promoter and the *Brassica* Bp4 and Bp10 promoters function in tobacco (Albani et al., 1990, 1992; Guerrero et al., 1990). These results

indicate that the *trans*-acting factors that interact with pollen-specific promoters are evolutionarily conserved. One can therefore take advantage of the useful features of Arabidopsis for mutagenesis and gene isolation.

Mutagenesis of transgenic Arabidopsis that is homozygous for a chimeric pollen promoter-reporter gene such as GUS can theoretically be used to isolate mutations in genes that affect expression from these promoters. For example, a mutation in a gene that is required for activating a pollen promoter might cause flowers to produce 50% blue pollen rather than 100% blue pollen. One caveat to this approach is that if the *trans*-acting factor is required to activate a gene whose product is necessary for pollen development or function, then a mutation in this factor would be lethal to the pollen carrying it, resulting in 50% aborted pollen. However, so long as the *trans*-acting factor is not also required for female gametophyte function, the mutation can be recovered through the female. Mutations in the GUS gene or pollen promoter can easily be distinguished from *trans*-acting factor mutants by outcrosses. Such a screen offers the potential to obtain mutants that act in any step of the signal transduction pathway of pollen gene regulation.

It is possible that some of the pollen-specific cDNAs or proteins that have already been identified correspond to regulatory factors. Many regulatory proteins, including *trans*-acting factors, are likely to be encoded by low-abundance messages. However, Baltz et al. (1992a, 1992b) have described a fairly abundant pollen-specific cDNA clone of sunflower (SF3) that encodes a protein with putative zinc-finger domains of the LIM class, suggesting that it may be a transcription factor. Hodge et al. (1992) used a method they termed "cold-plaque screening" to identify anther-specific genes that correspond to low-abundance messages, and that therefore would probably not be identified in a conventional differential screen. Although this approach is not directed to the isolation of specific DNA binding factors, it is an additional avenue for their isolation. Lastly, it may also be possible to use heterologous systems such as yeast to identify factors that interact with pollen gene promoter elements (Chang and Timberlake, 1993).

GENES CONTROLLING CELL FATE DURING MICROSPOROGENESIS

The cellular reorganizations (nuclear migrations and actin polymerization and depolymerization) discussed previously are certainly under genetic control. It should be feasible to identify mutations that act gametophytically during microsporangium and that alter nuclear division patterns, nuclear migration, or nuclear condensation. Such mutants would be predicted to exhibit 50% normal microspores or pollen and 50% defective microspores or pollen. Several mutations affecting nuclear movement have, for example, been described in *Aspergillus* (reviewed in Doonan, 1992).

Precedent for gametophytically acting genes affecting cellular morphology during microsporogenesis is apparent from the work of Kindiger et al. (1991). They studied microsporogenesis in hypoploid plants (deficient for one chromosome arm) of maize. All of the hypoploid stocks showed normal progression through meiosis, which suggests that the functions required for meiosis are supplied by the microspore mother cell. During microsporogenesis, however, each hypoploid plant showed specific defects in 50% of the products of meiosis, whereas the other 50% of the microspores developed normally. The phenotype of hypoploid plants at anthesis is 50% sterile pollen, which implies that no chromosome arm is dispensable for pollen viability.

Although all hypoploid pollen is inviable, the points of pollen arrest vary depending on which chromosome arm is deleted. For example, a hypoploid plant deficient for the short arm of chromosome 1 produced 50% normal microspores and 50% that arrested as uninucleate microspores, before first microspore mitosis. Plants that were hypoploid for the long arm of chromosome 6 had a normal first microspore mitosis, but the centrally located vegetative nucleus then failed to condense properly, although the generative nucleus migrated normally to the pollen wall. Plants that were hypoploid for a portion of the long arm of chromosome 10 had a normal first microspore mitosis, but the vegetative nucleus then behaved as a generative nucleus, migrating toward the pollen wall as if preparing to undergo a second mitotic division.

Two chromosomal regions apparently important for pollen wall formation in maize were mapped indirectly by Kindiger et al. (1991) in their study of microsporogenesis in hypoploid plants. Both normal and deficient microspores in plants hypoploid for the long arm of chromosome 6 had fragile walls, suggesting that a sporophytically acting (and dose-dependent) gene contributing to wall strength resides on this chromosome arm. In plants hypoploid for the short arm of chromosome 10, only the deficient microspores exhibited fragile walls, suggesting that a gametophytically acting gene affecting pollen wall development maps to this chromosome arm.

Perhaps characterization of mutations that influence pollen development can be complemented by experimental manipulations of microsporogenesis. The major use of *in vitro* culture methods for microspores has been the production of haploid plants. Uninucleate microspores can be induced to form haploid plants, rather than a gametophyte, if the asymmetric division is prevented (Prakash and Giles, 1987; Zaki and Dickinson, 1990). *In vitro* culture of microspores and maturation of pollen grains offers the possibility for other applications, including gametophytic selection for superior sporophytic traits and the possibility of germ line transformation. If a consistently high frequency of *in vitro* development and maturation can be routinely obtained, *in vitro* culture methods will also offer the opportunity to experimentally manipulate single microspores using microinjection (Hemmati-Brivanlou and Melton, 1992), micromanipulation (Ashkin and Dziedzic, 1989), or other methods that have been successful in determining the roles of cell constituents and cell fate in other systems (Horvitz and

Herskowitz, 1992). Are the currently available techniques suitable for such manipulations? Probably yes, at least for some plant species.

Several groups have reported success in culturing immature microspores and achieving pollen maturation *in vitro*, although culture of more immature stages often requires anther culture (Pareddy and Petolino, 1992; Stapleton and Bedinger, 1992) rather than free microspore culture. The frequency of functional (as assessed by pollen germination) pollen obtained from *in vitro* maturation varied from plant to plant and depended on the stage of isolation. For example, in an early study, Benito Moreno et al. (1988) demonstrated that ~50% of late uninucleate tobacco microspores could undergo first microspore mitosis and develop into pollen grains; ~20% of the *in vitro*-matured pollen could germinate. In another tobacco study (Ylestra et al., 1992), 25% of the isolated young binucleate pollen grains could germinate after 72 hr of culture, but this frequency could be increased to 60% by the addition of quercetin (a flavonol) to the medium. Flavonol aglycones have a strong stimulatory effect on *in vitro* pollen maturation and on pollen germination, implying that they play a role in normal pollen development. More evidence that flavonols are important for pollen development comes from the finding that anthers deficient for chalcone synthase, a key enzyme in the flavonoid biosynthetic pathway, are male sterile (Mo et al., 1992). Ylestra et al. (1992) suggest that the pollen defect is not due to the absence of flavonoid pigments but to the absence of a hormonal or signaling role of flavonols.

In vitro maturation requires manipulation of medium components and cell density (Benito Moreno et al., 1988) in ways that are presumed to mimic the *in vivo* condition (e.g., lower concentrations of nutrients in later stages). In maize, only spikelet culture yields a high percentage (50 to 70%) of normal, germinable pollen. When isolated maize microspores were cultured, only 5 to 20% of them developed into starch-filled trinucleate grains, and fewer than 1% of these *in vitro*-matured grains germinated (Pareddy and Petolino, 1992). *In vitro* maturation does not completely mimic the *in vivo* situation. *In vivo*-matured pollen desiccates and can be stored for long periods of time, whereas the *in vitro*-matured pollen does not desiccate and therefore cannot be maintained indefinitely. In addition, some protein pattern differences are seen between pollen developing *in vivo* and *in vitro* (Van Herpen et al., 1992). As our understanding of the molecules necessary for completion of microsporogenesis deepens, we should be able to optimize these techniques and adapt them to a wider variety of plant species.

PROSPECTS

Comparative studies suggest that many of the structural and molecular events of microsporogenesis are conserved throughout evolution. A concerted molecular, morphological, and cell biological description in a few plant species should provide

a framework for future studies. In addition to continuing to define the function of anonymous cDNAs, future research should take advantage of the increased understanding of meiosis, cell fate determinants, and cell–cell interactions from work in non-plant organisms, so that similar methods and probes can be used to answer specific questions about the roles of particular molecules or genes in plant microsporogenesis.

ACKNOWLEDGMENTS

I thank all of the members of my lab for discussion, and especially Rima Kulikauskas and Jorge Muschietti for preparation of Figures 1 and 2. The work in my lab is supported by United States Department of Agriculture–Agricultural Research Service Current Research Information System Grant No. 5335-21000-008-00D and by the National Science Foundation Center for Plant Developmental Biology (UC-Berkeley, Grant No. DIR8719933).

REFERENCES

- Albani, D., Robert, L.S., Donaldson, P.A., Altosaar, I., Arnison, P.G., and Fabijanski, S.F.** (1990). Characterization of a pollen-specific gene family from *Brassica napus* which is activated during early microspore development. *Plant Mol. Biol.* **15**, 605–622.
- Albani, D., Sardana, R., Robert, L.S., Altosaar, I., Arnison, P.G., and Fabijanski, S.F.** (1992). A *Brassica napus* gene family which shows sequence similarity to ascorbate oxidase is expressed in developing pollen. Molecular characterization and analysis of promoter activity in transgenic tobacco plants. *Plant J.* **2**, 331–342.
- Ashkin, A., and Dziejczak, J.M.** (1989). Internal cell manipulation using infrared laser traps. *Proc. Natl. Acad. Sci. USA* **86**, 7914–7918.
- Bachvarova, R.F.** (1992). A maternal tail of poly(A): The long and the short of it. *Cell* **69**, 895–897.
- Baltz, R., Domon, C., Pillay, D.T.N., and Steinmetz, A.** (1992a). Characterization of a pollen-specific cDNA from sunflower encoding a zinc finger protein. *Plant J.* **2**, 713–721.
- Baltz, R., Evrard, J.-L., Domon, C., and Steinmetz, A.** (1992b). A LIM motif is present in a pollen-specific protein. *Plant Cell* **4**, 1465–1466.
- Bedinger, P.A.** (1992). The remarkable biology of pollen. *Plant Cell* **4**, 879–887.
- Bedinger, P.A., and Edgerton, M.D.** (1990). Developmental staging of maize microspores reveals a transition in developing microspore proteins. *Plant Physiol.* **92**, 474–479.
- Benito Moreno, R.M., Macke, F., Alwen, A., and Heberle-Bors, E.** (1988). In situ seed production after pollination with in vitro-matured, isolated pollen. *Planta* **176**, 145–148.
- Blackmore, S., and Barnes, S.H.** (1990). Pollen wall development in angiosperms. In *Microspores: Evolution and Ontogeny*, S. Blackmore and R.B. Knox, eds (New York: Academic Press), pp. 173–192.
- Blomstedt, C.K., Xu, H., Singh, M.B., and Knox, R.B.** (1992). The isolation and purification of surface specific proteins of somatic and reproductive protoplasts of lily and rapeseed. *Physiol. Plant.* **85**, 396–402.
- Bouchard, R.A.** (1990). Characterization of expressed meiotic prophase repeat transcript clones of *Lilium*: Meiosis-specific expression, relatedness, and affinities to small heat shock protein genes. *Genome* **33**, 68–79.
- Brown, R.C., and Lemmon, B.E.** (1991). Pollen development in orchid. 3. A novel generative pole microtubule system predicts unequal pollen mitosis. *J. Cell Sci.* **99**, 273–281.
- Brown, R.C., and Lemmon, B.E.** (1992). Control of division plane in normal and griseofulvin-treated microsporocytes of *Magnolia*. *J. Cell Sci.* **103**, 1031–1038.
- Carpenter, J.L., Ploense, S.E., Snustad, D.P., and Silflow, C.D.** (1992). Preferential expression of an α -tubulin gene of *Arabidopsis* in pollen. *Plant Cell* **4**, 557–571.
- Chang, Y.C., and Timberlake, W.E.** (1993). Identification of *Aspergillus brIA* response elements (BREs) by genetic selection in yeast. *Genetics* **133**, 29–38.
- Chaudhury, A.M.** (1993). Nuclear genes controlling male fertility. *Plant Cell* **5**, 1277–1283.
- Doonan, J.H.** (1992). Cell division in *Aspergillus*. *J. Cell Sci.* **103**, 599–611.
- Foster, G.D., Robinson, S.W., Blundell, R.P., Roberts, M.R., Hodge, R., Draper, J., and Scott, R.J.** (1992). A *Brassica napus* mRNA encoding a protein homologous to phospholipid transfer proteins is expressed specifically in the tapetum and developing microspores. *Plant Sci.* **84**, 187–192.
- Fuchs, K., and Pauls, K.P.** (1992). Flow cytometric characterization of microspore development in *Brassica napus*. *Can. J. Bot.* **70**, 802–809.
- Guerrero, F.D., Crossland, L., Smutzer, G.S., Hamilton, D.A., and Mascarenhas, J.P.** (1990). Promoter sequences from a maize pollen-specific gene direct tissue-specific transcription in tobacco. *Mol. Gen. Genet.* **224**, 161–168.
- Hammond-Kosack, M.C.U., Holdsworth, M.J., and Bevan, M.W.** (1993). *In vivo* footprinting of a low molecular weight glutenin gene (LMWG-1D1) in wheat endosperm. *EMBO J.* **12**, 545–554.
- Hanson, D.D., Hamilton, D.A., Travis, J.L., Bashe, D.M., and Mascarenhas, J.P.** (1989). Characterization of a pollen-specific cDNA from *Zea mays* and its expression. *Plant Cell* **1**, 173–179.
- Hemmati-Brivanlou, A., and Melton, D.A.** (1992). A truncated activin receptor inhibits mesoderm induction formation of axial structures in *Xenopus* embryos. *Nature* **359**, 609–614.
- Heslop-Harrison, Y., and Heslop-Harrison, J.** (1992). Germination of monocot angiosperm pollen: Evolution of the actin cytoskeleton and wall during hydration, activation and tube emergence. *Ann. Bot.* **69**, 385–394.
- Hodge, R., Paul, W., Draper, J., and Scott, R.** (1992). Cold-plateau screening: A simple technique for the isolation of low abundance, differentially expressed transcripts from conventional cDNA libraries. *Plant J.* **2**, 257–260.
- Hopf, N., Plesofsky-Vig, N., and Brambl, R.** (1992). The heat shock response of pollen and other tissues of maize. *Plant Mol. Biol.* **19**, 623–630.
- Horvitz, H.R., and Herskowitz, I.** (1992). Mechanisms of asymmetric cell division: Two Bs or not two Bs, that is the question. *Cell* **68**, 237–255.

- Hotta, Y., Tabata, S., Stubbs, L., and Stern, H. (1985). Meiosis-specific transcripts of a DNA component replicated during chromosome pairing: Homology across the phylogenetic spectrum. *Cell* **40**, 785–793.
- Howlett, B.J., Knox, R.B., and Heslop-Harrison, J. (1973). Pollen-wall proteins: Release of the allergen antigen E from intine and exine sites in pollen grains of ragweed and *Cosmos*. *J. Cell Sci.* **13**, 603–619.
- Jacobs, T. (1992). Control of the cell cycle. *Dev. Biol.* **153**, 1–15.
- Kaul, M.L.H., and Murthy, T.G.K. (1985). Mutant genes affecting higher plant meiosis. *Theor. Appl. Genet.* **70**, 449–466.
- Kim, S.-R., Kim, Y., and An, G. (1993). Molecular cloning and characterization of anther-preferential cDNA encoding a putative actin-depolymerizing factor. *Plant Mol. Biol.* **21**, 39–45.
- Kindlger, B., Beckett, J.B., and Coe, E.H., Jr. (1991). Differential effects of specific chromosomal deficiencies on the development of the maize pollen grain. *Genome* **34**, 579–594.
- Komma, D.J., Horne, A.S., and Endow, S.A. (1991). Separation of meiotic and mitotic effects of *claret non-disjunctional* on chromosome segregation in *Drosophila*. *EMBO J.* **10**, 419–424.
- Loefering, W.G., and Sears, E.R. (1963). Distorted inheritance of stem-rust resistance of timstein wheat caused by a pollen-killing gene. *Can. J. Genet. Cytol.* **5**, 67–72.
- Mandaron, P., Niogret, M.F., Mache, R., and Moneger, F. (1990). In vitro protein synthesis in isolated microspores of *Zea mays* at several stages of development. *Theor. Appl. Genet.* **80**, 134–138.
- Mascarenhas, J.P. (1975). The biochemistry of angiosperm pollen development. *Bot. Rev.* **41**, 259–314.
- Mascarenhas, J.P. (1989). The male gametophyte of flowering plants. *Plant Cell* **1**, 657–664.
- Mascarenhas, J.P. (1990). Gene activity during pollen development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**, 317–338.
- Mascarenhas, J.P. (1993). Molecular mechanisms of pollen tube growth and differentiation. *Plant Cell* **5**, 1303–1314.
- McCormick, S. (1991). Molecular analysis of male gametogenesis in plants. *Trends Genet.* **7**, 298–303.
- McCormick, S., Twell, D., Vancanneyt, G., and Yamaguchi, J. (1991a). Molecular analysis of gene regulation and function during male gametophyte development. In *Molecular Biology of Plant Development*, G.I. Jenkins and W. Schuch, eds (London: Company of Biologists), pp. 229–244.
- McCormick, S., Yamaguchi, J., and Twell, D. (1991b). Deletion analysis of pollen-expressed promoters. *In Vitro Cell Dev. Biol.* **27P**, 15–20.
- Mo, Y., Nagel, C., and Taylor, L.P. (1992). Biochemical complementation of chalcone synthase mutants defines a role for flavonols in functional pollen. *Proc. Natl. Acad. Sci. USA* **89**, 7213–7217.
- Ohkawa, J., Okada, N., Shinmyo, A., and Takano, M. (1989). Primary structure of cucumber (*Cucumis sativus*) ascorbate oxidase deduced from cDNA sequence: Homology with blue copper proteins and tissue-specific expression. *Proc. Natl. Acad. Sci. USA* **86**, 1239–1243.
- Pareddy, D.R., and Petolino, J.F. (1992). Maturation of maize pollen in vitro. *Plant Cell Rep.* **11**, 535–539.
- Pennell, R.I., and Roberts, K. (1990). Sexual development in the pea is presaged by altered expression of arabinogalactan protein. *Nature* **344**, 547–549.
- Pennell, R.I., Janniche, L., Kjellbom, P., Scofield, G.N., Peart, J.M., and Roberts, K. (1991). Developmental regulation of a plasma membrane arabinogalactan protein epitope in oilseed rape flowers. *Plant Cell* **3**, 1317–1326.
- Prakash, J., and Giles, K.L. (1987). Induction and growth of androgenetic haploids. In *Pollen: Cytology and Development*, K. L. Giles and J. Prakash, eds., *Int. Rev. Cytol.* **107**, 273–292.
- Quiros, C.F. (1975). Exine pattern of a hybrid between *Lycopersicon esculentum* and *Solanum pennellii*. *J. Hered.* **66**, 45–47.
- Raju, N.B., and Perkins, D.D. (1991). Expression of meiotic drive elements *Spore killer-2* and *Spore killer-3* in asci of *Neurospora tetrasperma*. *Genetics* **129**, 25–37.
- Reijnen, W.H., van Herpen, M.M.A., de Groot, P.F.M., Olmedilla, A., Schrauwen, J.A.M., Weterings, K.A.P., and Wullems, G.J. (1991). Cellular localization of a pollen-specific mRNA by in situ hybridization and confocal laser scanning microscopy. *Sex. Plant. Reprod.* **4**, 254–257.
- Richter, J.D. (1991). Translational control during early development. *BioEssays* **13**, 179–183.
- Rick, C.M. (1966). Abortion of male and female gametes in the tomato determined by allelic interaction. *Genetics* **53**, 85–96.
- Riggs, C.D., and Hasenkampf, C.A. (1991). Antibodies directed against a meiosis-specific, chromatin-associated protein identify conserved meiotic epitopes. *Chromosoma* **101**, 92–98.
- Roberts, M.R., Robson, F., Foster, G.D., Draper, J., and Scott, R.J. (1991). A *Brassica napus* mRNA expressed specifically in developing microspores. *Plant Mol. Biol.* **17**, 295–299.
- Rodriguez-Garcia, M.I., and Majewska-Sawka, A.R. (1992). Is the special callose wall of microsporocytes an impermeable barrier? *J. Exp. Bot.* **43**, 1659–1663.
- Sasaki, Y., Yasuda, H., Ohba, Y., and Harada, H. (1990). Isolation and characterization of a novel nuclear protein from pollen mother cells of lily. *Plant Physiol.* **94**, 1467–1471.
- Scharf, K.-D., Rose, S., Zott, W., Schoff, F., and Nover, L. (1990). Three tomato genes code for heat stress transcription factors with a region of remarkable homology to the DNA-binding domain of the yeast HSF. *EMBO J.* **9**, 4495–4501.
- Schwartz, S.P., Aisenthal, L., Elisha, Z., Oberman, F., and Yisraeli, J.K. (1992). A 69-kDa RNA-binding protein from *Xenopus* oocytes recognizes a common motif in two vegetally localized maternal mRNAs. *Proc. Natl. Acad. Sci. USA* **89**, 11895–11899.
- Scott, R., Dagless, E., Hodge, R., Paul, W., Soufleri, I., and Draper, J. (1991). Patterns of gene expression in developing anthers of *Brassica napus*. *Plant Mol. Biol.* **17**, 195–207.
- Shi, L., Mogensen, H.L., Zhu, T., and Smith, S.E. (1991). Dynamics of nuclear pore density and distribution patterns within developing pollen: Implications for a functional relationship between the vegetative nucleus and the generative cell. *J. Cell Sci.* **99**, 115–120.
- Staiger, C.J., and Cande, W.Z. (1990). Microtubule distribution in *dv*, a maize meiotic mutant defective in the prophase to metaphase transition. *Dev. Biol.* **138**, 231–242.
- Staiger, C.J., and Cande, W.Z. (1992). *Ameiotic*, a gene that controls meiotic chromosome and cytoskeletal behavior in maize. *Dev. Biol.* **154**, 226–230.
- Stapleton, A.E., and Bedinger, P.A. (1992). Immature maize spikelets develop and produce pollen in culture. *Plant Cell Rep.* **11**, 248–252.

- Sym, M., Engebrecht, J., and Roeder, G.S.** (1993). ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* **72**, 365–378.
- Takahasi, M.** (1993). Exine initiation and substructure in pollen of *Caesalpinia japonica* (Leguminosae: Caesalpinioideae). *Am. J. Bot.* **80**, 192–197.
- Terasaka, O., and Niitsu, T.** (1990). Unequal cell division and chromatin differentiation in pollen grain cells. II. Microtubule dynamics associated with the unequal cell division. *Bot. Mag. Tokyo* **103**, 133–142.
- Theerakulpisut, P., Xu, H., Singh, M.B., Pettitt, J.M., and Knox, R.B.** (1991). Isolation and developmental expression of Bcp1, an anther-specific cDNA clone in *Brassica campestris*. *Plant Cell* **3**, 1073–1084.
- Tiwari, S.C., and Polito, V.S.** (1988). Spatial and temporal organization of actin during hydration, activation and germination of pollen in *Pyrus communis* L.: A population study. *Protoplasma* **147**, 5–15.
- Twell, D.** (1992). Use of a nuclear-targeted β -glucuronidase fusion protein to demonstrate vegetative cell-specific gene expression in developing pollen. *Plant J.* **2**, 887–892.
- Twell, D., Wing, R., Yamaguchi, J., and McCormick, S.** (1989). Isolation and expression of an anther-specific gene from tomato. *Mol. Gen. Genet.* **217**, 240–245.
- Twell, D., Yamaguchi, J., and McCormick, S.** (1990). Pollen-specific gene expression in transgenic plants: Coordinate regulation of two different tomato gene promoters during microsporogenesis. *Development* **109**, 705–713.
- Twell, D., Yamaguchi, J., Wing, R.A., Ushiba, J., and McCormick, S.** (1991). Promoter analysis of genes that are coordinately expressed during pollen development reveals pollen-specific enhancer sequences and shared regulatory elements. *Genes Dev.* **5**, 496–507.
- Ueda, K., Miyamoto, Y., and Tanaka, I.** (1990). Fusion studies of pollen protoplasts and generative cell protoplasts in *Lilium longiflorum*. *Plant Sci.* **72**, 259–266.
- Ursin, V.M., Yamaguchi, J., and McCormick, S.** (1989). Gametophytic and sporophytic expression of anther-specific genes in developing tomato anthers. *Plant Cell* **1**, 727–736.
- Valenta, R., Duchene, M., Pettenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D., and Scheiner, O.** (1991). Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* **253**, 557–560.
- Van Aelst, A.C., and Van Went, J.L.** (1992). Ultrastructural immunolocalization of pectins and glycoproteins in *Arabidopsis thaliana* pollen grains. *Protoplasma* **168**, 14–19.
- Van Herpen, M.M.A., de Groot, P.F.M., Schrauwen, J.A.M., van den Heuvel, K.J.P.T., Weterings, K.A.P., and Wullems, G.J.** (1992). In vitro culture of tobacco pollen: Gene expression and protein synthesis. *Sex. Plant. Reprod.* **5**, 304–309.
- Wagner, V.T., Cresti, M., Salvatici, P., and Tiezzi, A.** (1990). Changes in volume, surface area, and frequency of nuclear pores of the vegetative nucleus of tobacco pollen in fresh, hydrated, and activated conditions. *Planta* **181**, 304–309.
- Weterings, K., Reijnen, W., van Aarssen, R., Kortstee, A., Spijkers, J., van Herpen, M., Schrauwen, J., and Wullems, G.** (1992). Characterization of a pollen-specific cDNA clone from *Nicotiana tabacum* expressed during microgametogenesis and germination. *Plant Mol. Biol.* **18**, 1101–1111.
- Wick, S.M.** (1991). Spatial aspects of cytokinesis in plant cells. *Curr. Opin. Cell Biol.* **3**, 253–260.
- Witke, W., Schleicher, M., and Noegel, A.A.** (1992). Redundancy in the microfilament system: Abnormal development of Dictyostelium cells lacking two F-actin cross-linking proteins. *Cell* **68**, 53–62.
- Worrall, D., Hird, D.L., Hodge, R., Paul, W., Draper, J., and Scott, R.** (1992). Premature dissolution of the microsporocyte callose wall causes male sterility in transgenic tobacco. *Plant Cell* **4**, 759–771.
- Yang, H.-Y., and Zhou, C.** (1992). Experimental plant reproductive biology and reproductive cell manipulation in higher plants: Now and the future. *Am. J. Bot.* **79**, 354–363.
- Ylstra, B., Touraev, A., Benito Moreno, R.M., Stoger, E., van Tunen, A.J., Vicente, O., Mol, J.N.M., and Heberle-Bors, E.** (1992). Flavonols stimulate development, germination, and tube growth of tobacco pollen. *Plant Physiol.* **100**, 902–907.
- Zaki, M.A.M., and Dickinson, H.G.** (1990). Structural changes during the first divisions of embryos resulting from anther and free microspore culture in *Brassica napus*. *Protoplasma* **156**, 149–162.
- Zarsky, V., Garrido, D., Rihova, L., Tupy, J., Vicente, O., and Heberle-Bors, E.** (1992). Derepression of the cell cycle by starvation is involved in the induction of tobacco pollen embryogenesis. *Sex. Plant Reprod.* **5**, 189–194.