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Stamen Structure and Function

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INTRODUCTION

Stamens are the male reproductive organs of flowering plants. They consist of an anther, the site of pollen development, and in most species a stalk-like filament, which transmits water and nutrients to the anther and positions it to aid pollen dispersal. Within the anther, male sporogenous cells differentiate and undergo meiosis to produce microspores, which give rise to pollen grains, whereas other cell types contribute to pollen maturation, protection, or release. Pollen development involves an array of extraordinary events, including cell division and differentiation independent of a conventional meristem, the transition from sporophytic to gametophytic generation, and modifications of cell division to produce structures that are unusual in plant development, including coenocytic tissues (the tapetum and the microsporocyte mass), and subsequently free cells (microspores) that give rise to self-contained units for genome dispersal (pollen grains). Reviews of stamen development and gene expression include articles by Bhandari (1984), Scott et al. (1991a), Goldberg et al. (1993), and Irish (1999).

A change in emphasis will be apparent since anther development was reviewed in the first Plant Reproduction special issue of *The Plant Cell* (Goldberg et al., 1993). The earlier review drew largely on studies of tobacco, but this article focuses more tightly on Arabidopsis, which has surpassed other species in the genetic tools publically available, including a sequenced genome, microarrays, well-characterized mutants associated with cloned genes, and knockout collections for reverse genetics. The advances of the last 10 years include an increased understanding of stamen specification, stamen-specific gene expression, internal patterning of the anther, regulation of male meiosis, and anther dehiscence.

Stamen and pollen development are intimately linked. We discuss those aspects of pollen genesis that are essentially controlled by the sporophytic genome (differentiation of the male germline, meiosis, and pollen wall formation), but the biology of the male gametophyte, pollen tube growth, and fertilization are covered by other reviews in this volume (Edlund et al., 2004; Hanson and Bentolila, 2004; McCormick, 2004).

SPECIFICATION OF STAMENS

Flowers of eudicots are organized into four concentric whorls of organs (sepals, petals, stamens, and carpels) that arise sequentially from the floral meristem (Figure 1). The third whorl in Arabidopsis flowers contains six stamens, four medial (long) and two lateral (short). Stamen primordia in Arabidopsis appear during floral stage 5 (defined by Smyth et al., 1990)/anther stage 1 (Sanders et al., 1999), with the long stamens arising first. The stamen primordia differentiate a stalked basal region, which gives rise to the filament, and a wider upper region, which becomes the anther. The anther locules, in which pollen develops, appear as convex protrusions on the adaxial surface (facing the carpels) at floral stage 8/anther stage 4. During this stage, the sporogenous cells, which give rise to pollen, are visible within locules of sectioned anthers. Concentric rings of other cell types associated with pollen development and release differentiate around the sporogenous cells; collectively, these constitute the microsporangium. All anther cell types are present by floral stage 9/anther stage 5. Sporogenous cells develop into microsporocytes (also known as pollen mother cells or male meiocytes), which undergo meiosis to form tetrads of haploid microspores. These are released into the anther locule to begin male gametophyte development. By floral/anther stage 12, the anthers are nearly at their final length and contain tricellular pollen, and the filaments begin to elongate rapidly. At floral/anther stage 13, the flower opens and anther dehiscence occurs to release the pollen. The filaments continue to extend so that the anthers brush past the receptive stigma at floral stage 14.

There are many recent reviews of floral organ specification (Jack, 2001, 2004; Theissen, 2001; Lohmann and Weigel, 2002); therefore, the regulation of stamen identity will be treated only briefly here. The identity of floral organ primordia is controlled by three classes (termed A, B, and C) of homeotic selector genes with overlapping areas of activity; stamens develop in the third whorl, where both B and C genes are expressed. It has been discovered that additional transcription factors, encoded by *SEPALLATA* (*SEP*) genes in Arabidopsis, are required to confer full activity on the homeotic genes in stamens (as well as petals and carpels). The ABC model was based on genetic studies in *Arabidopsis thaliana* and *Antirrhinum majus* (Coen and Meyerowitz, 1991). The B class genes are *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) in Arabidopsis and *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*) in Antirrhinum; the C genes are *AGAMOUS* (*AG*) and *PLENA*, respectively. Mutations in any one of the B or C genes result in homeotic conversion of the third-whorl organs to a different type. Loss of B function causes transformation of stamens to carpels, loss of C function converts stamens to petals, and loss of both transforms stamens to sepals. Single-gene mutations of the *SEP* genes have very subtle phenotypes, but in *sep1 sep2 sep3* triple mutants, all floral organs resemble

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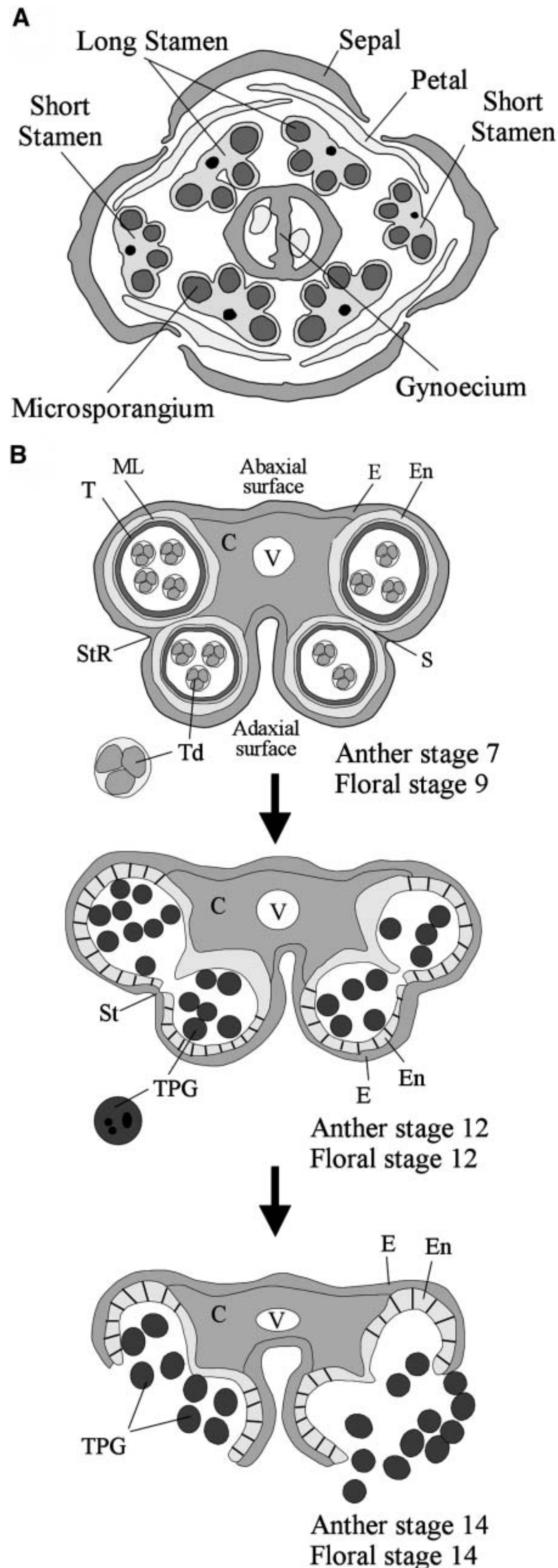

sepals, suggesting that B and C function have been abolished (Pelaz et al., 2000).

The signals that determine the number of floral organ primordia in each whorl are unknown (Irish, 1999). However, the number of stamens can be perturbed by various mechanisms: extra stamens develop in *clavata1* mutants, which form enlarged meristems (Clark et al., 1993), and in *superman* mutants, which have an expanded BC domain (Bowman et al., 1992). By contrast, arrest of stamen primordia is a feature of normal development in some species. In the radially asymmetric flowers of Antirrhinum, five stamens initiate, but the dorsal stamen arrests early in development in response to the activity of the *CYCLOIDEA* gene (Luo et al., 1995). In some species that produce unisexual flowers, such as maize and white campion, stamens in female flowers initiate but later arrest or abort (Tanurdzik and Banks, 2004).

The B class, C class, and SEP proteins all belong to the MADS family of transcription factors, which bind a target DNA sequence (the CArG box) as homodimers or heterodimers. In vitro, the B class proteins DEF and GLO (Antirrhinum) or AP3 and PI (Arabidopsis) bind CArG box sequences only as heterodimers (Jack, 2001; Thiessen, 2001). Yeast two-hybrid assays show that PI/AP3 interacts directly with SEP3 but not AG, and it has been proposed that SEP3 mediates interactions between PI/AP3 and AG dimers so that PI/AP3, AG, and SEP bind DNA in quaternary complexes; this effect would explain the combinatorial action of B and C class genes (along with *SEP* genes) in stamens (Honma and Goto, 2001).

IDENTIFICATION OF GENES INVOLVED IN STAMEN DEVELOPMENT AND FUNCTION

Ten years ago, one of the major unanswered questions about stamen development was the nature of the genes downstream of the B and C class transcription factors (Goldberg et al., 1993). After stamen specification, the B and C class genes as well as *SEP* genes continue to be expressed during stamen development (Bowman et al., 1991; Pelaz et al., 2000; Jack, 2001), so they could be directly responsible for activating many of the genes involved in stamen morphogenesis and function. Among the targets of B class genes in Arabidopsis are *AP3* and *PI* themselves, because both genes are required for the continued expression of each in the developing flower (Lohmann and Weigel, 2002). Two main approaches have been used to identify additional targets of B and C class genes. These involve either transcriptional profiling methods, including subtractive hybridization,

Figure 1. Anther Development.

(A) Scheme of a transverse section through an Arabidopsis floral bud showing the number, position, and orientation of the floral organs (after Hill and Lord, 1989).

(B) Schemes of transverse sections through Arabidopsis anthers at different stages (after Sanders et al., 1999). Floral stages are as described by Smyth et al. (1990); anther stages are as described by Sanders et al. (1999). C, connective; E, epidermis; En, endothecium; ML, middle layer; S, septum; St, stomium; StR, stomium region; T, tapetum; Td, tetrads; TPG, tricellular pollen grains; V, vascular bundle.

differential display of RNAs, and differential screening of cDNA libraries or arrays to find genes with stamen-specific or stamen-preferred expression, or screening for mutations that affect stamens. Both approaches have yielded interesting results, but there is not yet enough information to reconstruct the developmental pathways that start with homeotic gene expression.

Expression Studies

Stamen expression studies have been conducted in many crop and model species, including tomato (Ursin et al., 1989; Chmelnitsky et al., 2003), tobacco (Koltunow et al., 1990), Antirrhinum (Nacken et al., 1991), oilseed rape (Scott et al., 1991b; Shen and Hsu, 1992), maize (Wright et al., 1993), rice (Tsuchiya et al., 1994), lily (Crossley et al., 1995), white campion (Barbacar et al., 1997), *Lotus japonicus* (Endo et al., 2002), and Arabidopsis (Rubinelli et al., 1998; Sablowski and Meyerowitz, 1998; Amagai et al., 2003; Zik and Irish, 2003). Some of these studies have manipulated B and/or C class genes themselves to help identify their targets; these experiments are discussed in more detail below. Proteomics analysis also has been used to compare and identify proteins extracted from rice anthers at different developmental stages (Kerim et al., 2003). The expression studies have identified genes involved in protein, starch, and sucrose metabolism, osmoregulation, cell wall biosynthesis and expansion, sugar transport, lipid transfer, flavonoid synthesis, and cytoskeleton structure. These are consistent with features of stamen and/or pollen development, such as rapid growth, water movements associated with desiccation and dehiscence, stress response, and the accumulation of storage compounds. Many genes of unknown function also were recovered. Few of the genes have obvious developmental or signaling roles. Exceptions to this include several candidate transcription factors in white campion and Arabidopsis (Barbacar et al., 1997; Zik and Irish, 2003) and a subunit of the COP9 signalosome (a repressor of photomorphogenesis in plants) found to be upregulated in rice anthers at the panicle heading stage, suggesting a role in mediating light signals to coordinate flowering with anther dehiscence (Kerim et al., 2003).

In situ hybridization shows that genes identified in the expression studies are specific to a variety of stamen regions and cell types, including microsporocytes, microspores, tapetum, endothecium, connectives, epidermis, and filaments. A common theme is the predominance of tapetum-specific transcripts in early anthers, reflecting the high metabolic activity of this tissue (Scott et al., 1991a; see below).

In some of the experiments referred to above, transcriptional profiling techniques were used in conjunction with mutant or transgenic plants with altered function or expression of B and C class genes to aid the identification of their targets. For example, differential hybridization of an inflorescence-enriched cDNA library was used to isolate mRNAs expressed in wild-type flowers but not *def* mutants of Antirrhinum, which lack B function. This experiment yielded 12 differentially expressed genes, including *tap1*, expressed in the tapetum and encoding a putative secreted protein, and *filamentous flower1* (*fil1*), expressed mainly in stamen filaments and petal bases and encoding a candidate cell wall protein (Nacken et al., 1991). Rubinelli et al. (1998) compared wild-type and *ag* mutant flowers of Arabidopsis, which lack C function, using subtractive hybridization, and after a further screen making use of RNA from *ap3* mutants (which have pistils but not stamens) identified 13 genes that are expressed differentially in stamens. These included genes predicted to encode hydrolytic enzymes and a lipid transferase. Sablowski and Meyerowitz (1998) analyzed gene expression in flowers with no C function and inducible B function, which also were treated with a protein synthesis inhibitor, to discover direct targets of PI/AP3. Three mRNAs were upregulated consistently by induction of B function in *ap3-3 ag3* double mutant flowers in the absence of protein synthesis, one of which, *NAC-LIKE, ACTIVATED BY AP3/PI* (*NAP*), was studied in detail. In situ hybridizations to wild-type flowers showed expression in stamens and petals, both sites of B function, as well as in some organs outside the domain of PI/AP3 activity. Based on ectopic and antisense expression studies, the authors proposed that *NAP* functions in the transition between cell division and expansion, for instance in elongating stamen filaments.

Zik and Irish (2003) used microarray analysis to identify genes affected by the misexpression of *AP3* and *PI*. Gene expression was compared in flowers from wild-type plants and from mutant and transgenic plants with altered B function. This study identified 47 genes likely to be regulated directly or indirectly by PI/AP3 in petal and/or stamen development. Because the microarray used represented $\sim$25% of the Arabidopsis genome, some 200 genes may be affected by changes in PI/AP3 activity. PI/AP3-responsive genes found to be expressed in stamens, or in stamens and petals, included many genes implicated in the rapid cell expansion that is a feature of petal and stamen growth. Only two candidate transcription factors were identified, suggesting that AP3 and PI act relatively directly in regulating gene expression. Twenty-eight of the 47 genes had one or more candidate CArG boxes in a 1-kb upstream region, indicating that they could be activated directly by PI/AP3, although these sequences do not provide specific targets for particular MADS proteins.

Mutant Analysis

Mutant screens conducted over many decades have revealed a large number of sporophytic genes required for male fertility (Kaul, 1988; Chaudhury, 1993; Dawson et al., 1993; Chaudhury et al., 1994; Taylor et al., 1998; Sanders et al., 1999; Bhatt et al., 2001; Sorensen et al., 2002; Caryl et al., 2003). Pollen development also is affected by male gametophytic mutations (McCormick, 2004) and indirectly by mutations in the mitochondrial genome that result in degeneration of the tapetum (Hanson and Bentolila, 2004). The reported sporophytic mutations disturb a variety of processes in the stamen, such as chromosome pairing or segregation in meiosis, viability of the tapetum, pollen wall formation, filament elongation, and anther dehiscence. Genes required for pollen development that were first identified through mutant analysis include *DETERMINATE, INFERTILE1* (*DIF1*)/*SYN1*, encoding a cohesin required for chromosome segregation (Bai et al., 1999; Bhatt et al., 1999); *MALE STERILITY 2* (*MS2*), encoding a predicted fatty acyl reductase with a potential role in pollen wall formation (Aarts et al., 1997); and *MS1* and *ABORTED MICROSPORES*, both candidate transcription factors expressed

in the tapetum and possibly in microspores (Wilson et al., 2001; Sorensen et al., 2003).

General screens for male sterility yield surprisingly few mutants that affect the differentiation of anther cell types (Sanders et al., 1999). Therefore, we conducted an extinction screen for the loss of expression of a β-glucuronidase reporter gene fused to early tapetum-specific promoters, with the aim of specifically detecting mutations that disrupt microsporangium development (Sorensen et al., 2002). This screen identified three loci involved in patterning of the anther locule. Of these, *EXTRA SPOROGENOUS CELLS* (*EXS*)/*EXCESS MICROSPOROCYTES1* (*EMS1*) has been studied most thoroughly (Canales et al., 2002; Zhao et al., 2002). Because this appears to be a key gene in patterning the stamen, it is discussed in more detail below.

GENETIC CONTROL OF ANTHER DEVELOPMENT

Most plant organs derive from meristems, undifferentiated populations of cells that divide to replenish themselves and also provide founder cells for organ primordia. Anther development is unusual in that the microsporangia arise from single archesporial cells rather than from meristems. Key stages in this process are the establishment of adaxial-abaxial polarity, the specification of cell types, and the formation of the radially symmetrical microsporangia.

Asymmetry first becomes evident in the anther primordium with the formation of the microsporangia, for the two abaxial locules (facing the petals) are larger than the adaxial pair and are further separated by the connective tissue (Figure 1). In Arabidopsis leaves, asymmetry is established by cadastral interactions among genes that promote adaxial or abaxial identity, with the former suppressing the latter (Bowman et al., 2002). Mutations in either set of genes result in either "adaxialized" or "abaxialized" filamentous leaves, and the study of flowers in these mutant lines suggests that anther polarity is regulated similarly (Sawa et al., 1999; Siegfried et al., 1999). For example, double mutants for *fil1* and *yabby1* or *kanadi1* (*kan1*) and *kan2* produce radially symmetrical filamentous structures, the internal structure of which has yet to be determined. As with leaves, *FIL* is expressed abaxially in the connective tissue of the anther, supporting the view that stamens evolved from leaves bearing microsporangia on their upper surfaces.

The floral meristem of Arabidopsis, like the shoot apical meristem, is composed of three "histogenic layers" of cells with separate lineages: L1 (epidermis), L2 (subepidermis), and L3 (core). Stamen primordia are initiated by periclinal divisions in the floral meristem, usually within the L2 (Jenik and Irish, 2000). The L2 gives rise to most of the cell types of the anther, including the sporogenous cells (discussed in detail below). The L3 contributes to the vasculature and sometimes to the connectives. With the growth of the anther primordium, cells of the L2 undergo a complex series of divisions leading to the formation of the four radially symmetrical microsporangia (Figure 2) and conducting tissue that will eventually become linked to the filament. The founder cells of the four microsporangia are single L2 archesporial cells, each of which divides periclinally to form a primary parietal cell (PPC) subjacent to the L1 and a primary sporogenous cell (PSC) facing inward (Canales et al., 2002).

There has been significant progress in the last few years toward identifying genes involved in the first steps of microsporangium differentiation. In Arabidopsis, the candidate transcription factor NOZZLE/SPOROCYTELESS (NZZ/SPL) (Schiefthaler et al., 1999; Yang et al., 1999) is required for archesporial specification, because *nzz/spl* mutant anthers fail to form archesporial cells. In maize, *male sterile converted anther1* (*msca1*) mutants produce archesporial cells, but these do not divide into a PSC and a PPC, and no microsporangia are formed (Chaubal et al., 2003). Intriguingly, the *msca1* mutation also appears to convert the entire anther to another structure, because stomata form ectopically on the epidermis, although the filament maintains its identity.

The number of cells that can acquire archesporial fate in the anther appears to be regulated by a similar mechanism to that used in the shoot apical meristem to control numbers of pluripotent cells. In the shoot apical meristem, cell number is determined by the Leu-rich repeat (LRR) receptor kinase *CLAVATA1* (*CLV1*), its signaling partner *CLV2*, and its ligand *CLV3* (Clark, 2001). In the anther, the number of cells acquiring archesporial fate is restricted to one per premicrosporangial domain by EXS/EMS1, a putative Leu-rich repeat receptor kinase (Canales et al., 2002; Zhao et al., 2002). In *exs/ems1* mutants, which are affected only in male development, multiple L2 cells in the anther enter archesporial development (Figure 2A), with the result that an excess of PSCs is formed after the first periclinal division. The ligand for EXS/EMS1 is unknown, but mutants in the *TAPETAL DETERMINANT1* (*TPD1*) gene have phenotypes similar to those of *exs/ems1* lines (Yang et al., 2003b), suggesting that TPD1, a novel putative secreted protein, is involved at some point in this signaling pathway, whether or not as a ligand for EXS/EMS1. However, *TPD1* expression differs from that of EXS/EMS1, and although still consonant with a role in archesporial cell fate determination, this finding suggests that *TPD1* plays a wider role in microsporangial development. An *EXS/EMS1* ortholog, *MULTIPLE SPOROCYTE1* (*MSP1*), has been reported in rice (Nonomura et al., 2003), and in common with *multiple archesporial cells1* (*mac1*) mutants in maize (which also share phenotypic features with *exs/ems1* mutants [Sheridan et al., 1999]), *msp1* lines show defects in both male and female development.

The developmental fates of the two products of archesporial cell division in Arabidopsis are very different. The PSC undergoes a small number of divisions to generate the meiocytes, whereas the PPC divides periclinally to form an endothecial cell subjacent to the L1 and a secondary parietal cell (SPC). The SPC again divides periclinally to generate a middle layer cell next to the endothecium and a tapetal cell adjacent to the sporogenous cells. Mutants have been described with defects in anther wall layer development—for example, *ms23* and *ms32* in maize, in which the prospective tapetal layer undergoes an extra periclinal division but neither layer differentiates as tapetum (Chaubal et al., 2000)—but no clear picture is emerging regarding how cell fates are specified. Interestingly, this process is affected in *exs/ems1* anthers, for the tapetal and, frequently, middle layer cells are absent (Canales et al., 2002; Zhao et al., 2002). Whether EXS/

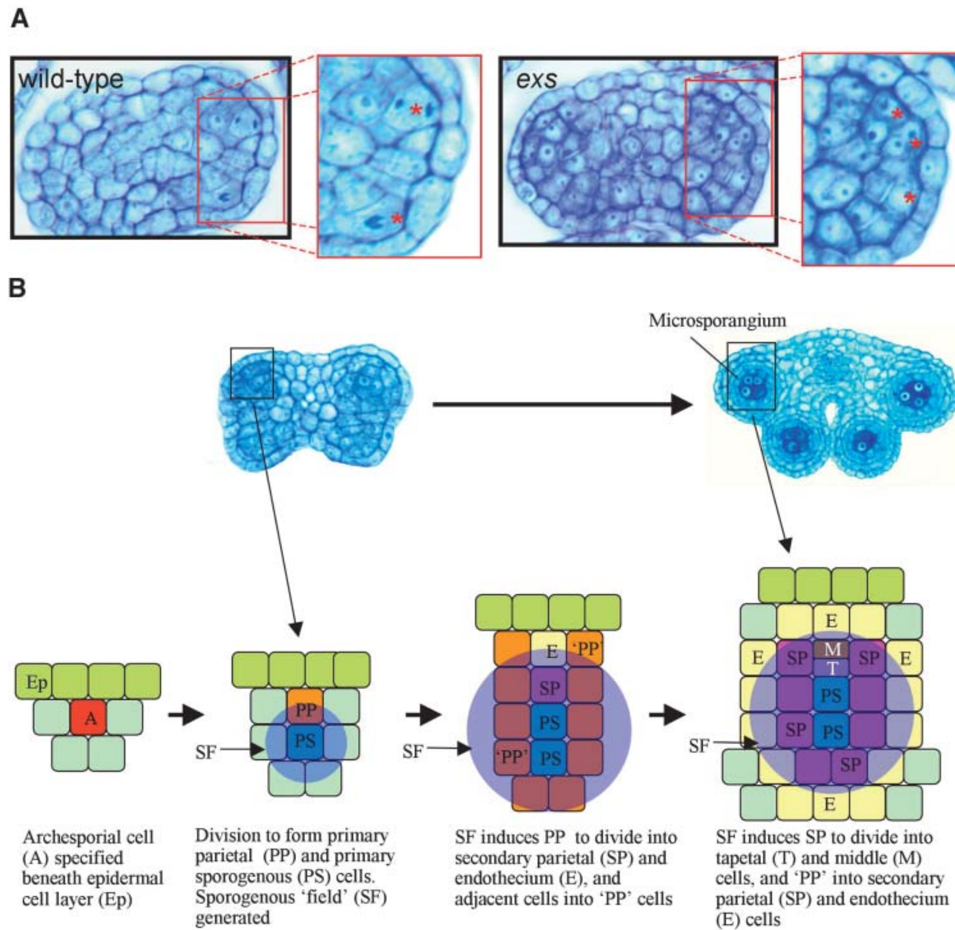

Figure 2. Microsporangium Development.

(A) Light micrographs of wild-type and *exs* anther primordia with archesporial cells marked by asterisks; extra archesporial cells arise in *exs* mutants (Canales et al., 2002).

(B) Model for the differentiation of the microsporangial cell layers in Arabidopsis.

EMS1 is itself required for tapetal specification, or whether the mass of extra sporogenous cells formed disrupts tapetal and middle layer differentiation, is unclear.

Periclinal divisions of the single archesporial cell thus give rise to a linear array of different cell types (Figure 2B). However, this process alone—even accompanied by anticlinal divisions—cannot generate the radially symmetrical microsporangium, for cells of the microsporangium adjacent to the connective have been shown to have a different origin from those on the outer face in a number of species (Nanda and Gupta, 1978; Goldberg et al., 1993). The model for microsporangial development most consistent with the data confers a key organizational role on the sporogenous cells (Figure 2). In this scheme, the PSC, after its formation from the archesporial cell, sets up a radial field of signals around itself. This field, which continues to be generated by the subsequent division products of the PSC, induces periclinal division and development in adjacent cells. Thus, the PPC, sister to the PSC, is induced to divide to form an endothecial cell and, adjacent to the source of signals, the meristematic SPC. The SPC then executes the final division of the program, forming tapetal and middle layer cells. However, these signals also are received and interpreted by other cells adjacent to the sporogenous cells, regardless of their origin, which then are recruited into the developmental pathway described above. This model may seem inconsistent with some observations—for example, the cell lineage described here for Arabidopsis is not accepted universally (Yang et al., 1999)—and certainly does not hold for all other species (Davis, 1966). However, whatever the division sequence followed by these cells, the sporogenous cells or their immediate antecedents are specified at an early stage in all plants and the wall layers subsequently develop radially around them. There are many examples in plants in which tissue organization is established in the absence of a coherent pattern of cell division (Torres-Ruiz and Jürgens, 1994). A second problem is a time disparity between the development of the inner- and outer-facing domains of the microsporangium (Nanda and Gupta, 1978). However, data from *exs/ems1* mutant anthers suggest that cells of the

subepidermal L2 are ''primed'' for development and, together with the PPCs, may be able to respond to signals generated from the PSC earlier than other L2 cells and those of the connective tissue. The nature of the hypothetical radial signal is unknown, but mutants would have phenotypes resembling those of *exs/ems1* lines. The fact that transcripts of *TPD1*, a putative partner for the EXS/EMS1 receptor kinase, are concentrated in the spore mass (Yang et al., 2003b) provides a first indication of a radial disposition of signals centered on the developing microsporocytes.

MICROSPORANGIAL DEVELOPMENT

Development of the different cell types proceeds at varying speeds: although the micosporocytes and tapetal cells enlarge rapidly and display differing patterns of gene expression, the endothecial layer remains undifferentiated through meiosis and much of microsporogenesis. The middle layer, of which little is known, apparently is crushed by the expansion of the microsporocytes and the tapetum. After the initial phase of cell expansion, cytoplasmic contact is lost between the microsporocytes and the tapetum as plasmodesmata are ruptured, seemingly by the rapid synthesis of the microsporocyte callose wall (see below). Both the microsporocytes and the tapetum eventually develop into coenocytes. As the microsporocytes enter meiosis, plasmodesmata between them enlarge to form ''cytomictic channels'' up to 0.5 μm in diameter, through which cytoplasmic exchange must occur. These channels are believed to promote synchrony within the microsporocyte mass (Heslop-Harrison, 1966). Later in tapetal development, particularly in plants such as Arabidopsis with secretory tapeta (see below), walls between the tapetal protoplasts become gelatinous and interconnecting plasmodesmata enlarge to form irregular channels. Postmeiotic development thus involves crosstalk between two coenocytes, sealed within the microsporangium by a lipid/sporopollenin peritapetal membrane (Dickinson, 1970). Surprisingly, this interaction does not become essential until after meiosis, for *exs/ems1* microsporocytes progress to the tetrad stage in the absence of any visible tapetum, and it is believed to be the lack of tapetally derived β-1,3-glucanase, which releases the microspores from the tetrad callose walls (see below), that causes developmental arrest (Canales et al., 2002).

Meiosis in the Sporogenous Cells

The principal events of meiosis—chromosome pairing, recombination, and segregation—are common to all eukaryotes, and the genes involved have been the subject of a number of reviews (Vershon and Pierce, 2000; Rabitsch et al., 2001) and are the focus of an interkingdom database (www.germonline.unibas.ch). However, unlike in animals, meiosis in plants stands at the threshold not of gametogenesis but of the alternation of generations. The microspores formed as a product of male meiosis develop into pollen, an independent microgametophyte. Perhaps for this reason, developmental events surrounding plant meiosis, and some details of the process itself, differ from those in other kingdoms.

In plant microsporocytes, cytoplasmic reorganization accompanies nuclear events, including the dedifferentiation and division of plastids and mitochondria and dramatic decreases in rRNA and mRNA (Dickinson, 1987). Little DNA synthesis occurs during this period, except in the organelles, and prophase cDNA libraries reveal a steep decline in the numbers of nuclear genes expressed (Crossley et al., 1995). This de facto purging of sporophytic information from the microsporocyte cytoplasm has been interpreted either as facilitating gametophytic development after meiosis or freeing the germline of detrimental RNA species, including viruses and silencing elements (Dickinson, 1987). Organellar dedifferentiation and replication also may be caused by this decrease in information from the nucleus, but microsporogenesis is unusual in that it is highly sensitive to mitochondrial mutation. This is believed to result from a need for extreme efficiency in energy production by the tapetum, and mutations in mitochondrial DNA, and nuclear restorer genes that ''correct'' the defect, have been exploited in the development of cytoplasmic male sterility systems used in plant breeding (Kaul, 1988; Hanson and Bentolila, 2004).

As in all eukaryotes, homologous chromosomes pair during plant meiosis. Although there have been many reports of presynaptic alignment of homologs, little unequivocal data exist, save for work with polyploid cereals, in which homeologous pairing clearly takes place in floral tissue before meiosis (Martinez-Perez et al., 1999). The balance between homologous and homeologous pairing in cereals is regulated by the *Ph* locus (Vega and Feldman, 1998), and this premeiotic alignment of homeologs has been cited as evidence of an ancestral system of presynaptic alignment of homologs (Martinez-Perez et al., 1999). Evidence is now emerging of homologous pairing during interphase in somatic cells, as occurs in animals and microorganisms (Fransz et al., 2002), but any mechanistic relationship between these two types of pairing has yet to be established.

After pairing, DNA processing in the male meiocytes is identical to that of other organisms, including the formation of double-strand breaks, resection, strand invasion, ligation, and resolution of the complexes formed via Holliday junctions. As in most eukaryotes, these events are accompanied by the formation of synaptonemal complexes and recombination nodules at zygotene/pachytene. Generally, the genes involved in meiotic processing in plants are orthologous with animal sequences, but differences in gene number and expression profile do occur (Bhatt et al., 2001; Caryl et al., 2003). Evidence from a range of meiotic mutants suggests that meiotic checkpoints also may differ between plants and other eukaryotes (Bhatt et al., 2001), with at least one checkpoint absent from male development in Arabidopsis (Bhatt et al., 1999). However, mutations in the homeodomain protein MALE MEIOCYTE DEATH, which cause meiocytes to arrest at diplotene/diakinesis (Yang et al., 2003c), suggest that some level of checkpoint control is present in anthers.

The Cell Wall in Male Meiosis

Cell wall formation (and dissolution) during and after meiotic divisions is another unique feature of male meiosis in plants. During prophase I of meiosis, the microsporocytes of most

angiosperm species secrete a wall of callose, a β-1,3-glucan, between the plasmalemma and the original cellulosic wall. At the end of meiosis, the walls separating the microspores also are composed of callose. Most monocot species undergo successive cytokinesis during male meiosis, in which a callose wall is formed between the dyad cells after meiosis I and the microspores of the nascent tetrad after meiosis II. As in mitotically dividing cells, these walls form centrifugally. By contrast, most eudicots, including Arabidopsis, undergo simultaneous microsporocyte cytokinesis, meaning that no walls are formed until the end of meiosis II. In simultaneous cytokinesis, intersporal walls first appear as ingrowths of callose from the parent wall surrounding the microsporocyte and expand centripetally until the microspores are separated (Brown and Lemmon, 1988, 2001; Owen and Makaroff, 1995).

Control of the division plane also occurs differently in sporophytic mitosis and in male meiosis. In meiotic divisions, the future site of cell separation is not marked by a preprophase band. Instead, after each meiotic division in species with successive cytokinesis, or after meiosis II in species with simultaneous cytokinesis, each microspore nucleus becomes surrounded by a radial array of microtubules that partition the surrounding cytoplasm into ''spore domains'' (Dickinson and Sheldon, 1984; Brown and Lemmon, 1988, 2001). Cytokinesis proceeds along the planes defined by the intersection of the arrays as vesicles contributing membranes and wall components coalesce at the spore domain interfaces. In *tetraspore/stud* (*tes/std*) mutants of Arabidopsis, there is partial or complete failure of male meiotic cytokinesis, so that all four microspore nuclei begin development in an undivided cytoplasm (Hülskamp et al., 1997; Spielman et al., 1997). The *TES* gene encodes a putative kinesin that appears to be required for the establishment of the radial microtubule arrays surrounding the microspore nuclei at the end of meiosis, because these are lacking in *tes* mutants (Yang et al., 2003a). The *tes/std* mutations are recessive, and *TES* is expressed throughout the anther before meiosis, indicating that male meiotic cytokinesis is under sporophytic control.

In wounded plant cells, callose is synthesized by a plasma membrane–localized glycosyl transferase UDP-glucose β-1,3-glucan synthase (callose synthase) (Delmer, 1987). Recently, Østergaard et al. (2002) described an Arabidopsis gene, *glucan synthase-like5* (*AtGsl5*), that encodes a plasma membrane protein homologous with yeast β-1,3-glucan synthase and that partially complements a yeast β-1,3-glucan synthase mutant. The gene is highly expressed in flowers and may represent the enzyme responsible for microsporocyte callose synthesis.

Several explanations of the biological functions of the callose wall have been advanced. Callose has been proposed to act as a barrier, or ''molecular filter,'' between the sporogenous cells and the rest of the sporophyte that is necessary for meiosis (Heslop-Harrison, 1964; Heslop-Harrison and Mackenzie, 1967). It also has been suggested that callose functions as a temporary wall that first isolates the products of meiosis to prevent cell cohesion and fusion and then releases free microspores upon its dissolution (Waterkeyn, 1962) or as a template for the formation of the species-specific exine-sculpting patterns seen on mature pollen grains (Waterkeyn and Bienfait, 1970). These hypotheses are not mutually exclusive. This last potential function is considered in more detail below.

Since these hypotheses were proposed, natural exceptions, mutants, and plants engineered to degrade the callose wall prematurely have provided more insight into the role of the callose wall. Several lines of evidence suggest that the callose wall is not required for male meiosis: both *Pandanus odoratissimus*, which naturally lacks a callose wall (Periasamy and Amalathas, 1991), and transgenic tobacco plants in which the callose wall is absent throughout meiosis as a result of the ectopic expression of an endo-β-1,3-glucanase (Worrall et al., 1992) successfully initiate and complete meiosis to produce a tetrad of microspores. The engineered absence of the callose wall in lettuce (Curtis et al., 1996), oilseed rape, tomato, and maize (R.J. Scott, unpublished data) also failed to disrupt meiosis.

At the end of meiosis, the external and intersporal walls of the tetrad are dissolved to release individual microsporocytes by a mixture of enzymes (''callase'') containing endoglucanases and exoglucanases secreted by the tapetum. In the anthers of petunia and lily, the expression and secretion of callase activity is under strict developmental control (Frankel et al., 1969; Stieglitz and Stern, 1973). Alterations in the timing of β-1,3-glucanase expression, or failure to express β-1,3-glucanase, leads to abnormal dissolution of the tetrad callose walls, which has been shown to be a primary cause of male sterility in cytoplasmic male-sterile lines of several species, including petunia (Izhar and Frankel, 1971). Callase also must contain cellulases to degrade the original cellulosic wall of the microsporocyte, because premature removal of the callose component of the wall does not release microspores into the locule until the normal time of callase production (Worrall et al., 1992).

Several candidates for genes encoding the endo-β-1,3-glucanase component of callase have been identified, although none has been confirmed directly (e.g., by knockout or knockdown analysis). The Arabidopsis *A6* gene encodes a polypeptide similar in sequence to β-1,3-glucanases, and reporter gene studies established that *A6* gene expression is tapetum specific and temporally correlated with callase activity (Hird et al., 1993). Transcripts of the tobacco *Tag1* gene, which encodes a polypeptide related to established tobacco β-1,3-glucanases containing the conserved pentapeptide motif of the active site of these enzymes, also show a callase-like pattern of expression (Bucciaglia and Smith, 1994). Although one or more of the genes described above may participate in callose wall dissolution, more effort is required to confirm this and then to understand how this key stage in pollen production is regulated. The availability of expression array data and gene knockout lines in Arabidopsis is likely to assist in this endeavor.

The formation and dissolution of the callose intersporal wall does appear to be important for the production of individual microspores. Species that naturally produce permanent tetrads (four microspores fused together), such as members of the Juncaceae, Ericaceae, and Oenotheraceae, develop little or no callose within the intersporal cross-walls of the tetrad (Blackmore and Crane, 1988). The microspores possess individual exine walls, but these are fused along the line of the cross-walls, which presumably prevents their separation. Blackmore and Crane (1988) proposed that the fusion of exines is

related to the extent and timing of callose wall deposition. The absence of callose in the tetrad cross-walls in some species that normally produce them also results in permanent tetrads (e.g., in tomato [R.J. Scott, unpublished data]). However, both *P. odoratissimus* (Periasamy and Amalathas, 1991) and engineered *Brassica napus* (R.J. Scott, unpublished data) produce individual microspores in the absence of cross-wall callose, suggesting that in some species callose is not essential to ensure that microspores remain separate during exine wall formation within the tetrad. By contrast, *quartet* mutants in Arabidopsis produce permanent tetrads despite apparently normal patterns of callose wall synthesis and dissolution, whereas pectic components are unusually persistent in the parent microsporocyte wall surrounding the tetrads, indicating that pectin dissolution also is necessary for microspore separation (Preuss et al., 1994; Rhee and Somerville, 1998).

Tapetal Development and Pollen Coat Formation

Although presumably acting as a nurse tissue to the developing meiocytes, the tapetum likely only provides nutrition and materials for the formation of the complex pollen wall. Developmental signaling, as occurs between nurse cells and the egg in *Drosophila* (St. Johnston and Nusslein-Volhard, 1992), is unlikely to take place because cytoplasmic connections between these groups of cells are lost at an early stage. However, microsporocytes and tapetal cells do share many developmental pathways (Dickinson and Bell, 1976), particularly during pollen wall formation. Exceptions are intense phases of protein synthesis early in development and meiosis itself, although significant nuclear changes do occur in the tapetum, with endomitosis and endoreduplication common in many species. In petunia, tapetal nuclei can attain DNA levels of 8C, requiring an intensity of nucleic acid synthesis that is probably unique to the tapetum and that, when combined with high levels of protein synthesis early in development, must create an exceptionally high demand for energy (Liu and Dickinson, 1989).

Depending on the species, tapetal cells may be either secretory and remain at the periphery of the microsporangium throughout development or amoeboid/invasive and move into the locule and intermingle with the developing microspores (Pacini, 1990). In other plants, the tapetum fragments at an early stage, and components of the ruptured protoplast move into the locule (see below). In addition to its central role in pollen wall formation (see below), the tapetum also contributes a lipid-rich exine coating in many species. Thus, the secretory tapetum of *Lilium* deposits a blend of carotenins, flavonols, and lipids (termed pollenkitt) onto the exine surface (Reznickova and Dickinson, 1982), whereas the invasive tapetal protoplasts of the Asteraceae penetrate within the cavea of the complex chambered pollen wall (Howlett et al., 1975). Most is known of the complex coatings applied to the surface of crucifer pollen, termed tryphines (Dickinson and Lewis, 1973; Murgia et al., 1991). These are formed when tapetal fragments become applied to the pollen exine and contain a range of lipids, glycolipids, and proteins essential for the successful development of the pollen on the stigma surface (Ruiter et al., 1997). Arabidopsis mutants defective in coating components can fail to hydrate after pollination (Preuss et al., 1993), whereas in *Brassica* species, small, Cys-rich pollen coat proteins are involved in interactions with the stigmas and particularly with the female determinants of the self-incompatibility system (Doughty et al., 1998). Indeed, the male self-incompatibility determinants in these species (Schopfer et al., 1999) belong to the pollen coat protein family of proteins. The pollen coat and pollination are discussed in this issue by Edlund et al. (2004).

POLLEN WALL STRUCTURE, SYNTHESIS, AND PATTERNING

After male meiotic cytokinesis, the individual microspores of the tetrad initiate development of the pollen wall. The wall of a mature pollen grain is a multilayered structure consisting of a pectocellulosic intine surrounded by a sporopollenin-based exine, which itself contains two layers, the inner nexine and the outer sexine (terminology as given by Erdtman, 1969). The latter is the most complex of the layers, providing most of the species-specific variation in pollen wall patterning through the elaboration of its columellae and roof-like tectum (Figure 3).

The ephemeral callose wall is the first of several layers deposited at the microspore surface. This is followed by the primexine (a precursor of the sexine), the nexine, and finally the intine (Blackmore and Barnes, 1990). The primexine is composed largely of polysaccharide and apparently acts as a template that guides the accumulation of sporopollenin, the main structural component of the pollen wall. Worrall et al. (1992) showed that removal of the callose wall early in microsporogenesis disrupts pollen wall formation in tobacco, deleting the tectum to leave only exposed columellae; this finding suggests that the callose wall may provide a solid surface against which the tectum forms. Because correct columellae positioning occurs in *Brassica napus* in the absence of callose (R.J. Scott, unpublished data), the callose wall probably does not provide a stencil to guide the positioning of wall elements, as proposed by Waterkeyn and Bienfait (1970).

Until microspore release, sporopollenin is polymerized from precursors synthesized and secreted by the microspore; however, the bulk of sporopollenin precursors are secreted by the tapetum and incorporated into the wall after the dissolution of the tetrad (reviewed by Scott et al., 1991a). Sporopollenin confers on the exine an unparalleled combination of physical strength, chemical inertness, and resistance to biological attack; these features have greatly hampered progress in understanding both its chemical composition and details of its biosynthesis. Early literature frequently cites carotenoids as the main constituents of sporopollenin (Shaw, 1971; reviewed by Scott, 1994). However, the demonstration that a potent inhibitor of carotenoid biosynthesis, norflurazon, failed to prevent sporopollenin biosynthesis in *Cucurbita pepo* (Prahl et al., 1985) began a reevaluation of sporopollenin composition. Subsequently, a large body of experimental evidence (reviewed by Scott, 1994) established that sporopollenin consists mainly of long-chain fatty acids and a minor component of phenolic compounds, perhaps *p*-coumaric acid. More recent analyses have confirmed this view (Ahlers et al., 1999; Domínguez et al., 1999). The phenolic monomers are coupled by ester bonds characteristic of polyphenolics such as

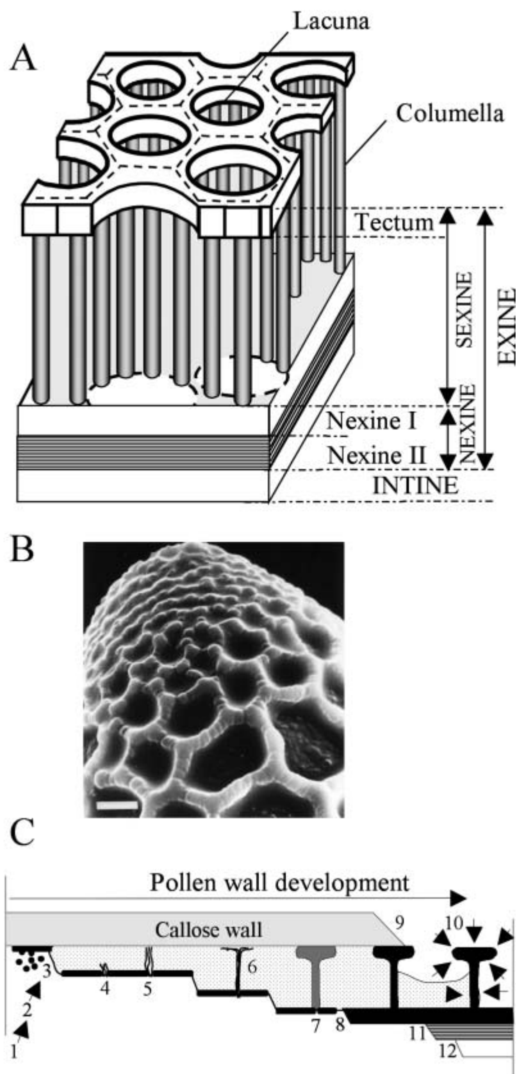

Figure 3. Pollen Wall.

(A) Scheme of the main architectural features of a generalized mature pollen wall. The terminology is according to Erdtman (1969).

(B) Scanning electron micrograph of a *Lilium* pollen wall showing a reticulate pattern formed by the fused heads of the columellae. The nexine I is visible through the lacunae. Bar = 5 μm.

(C) Model of pollen wall development based on *Lilium* (after Scott, 1994). Events begin before meiosis and proceed left to right until the mature wall is formed. 1, Transcription of "pattern" genes in the premeiotic microsporocyte nucleus. 2, Insertion of labile pattern information into the plasma membrane via endoplasmic reticulum–derived vesicles, followed by tessellation to produce a negative stencil of plates. 3, First phase of primexine synthesis over the entire microspore surface except areas destined to become apertures. 4, Conversion of primexine to a sporopollenin-receptive state through the activity of a factor(s) secreted from sites between the plates. 5, Second phase of primexine synthesis, more rapid than the first, resulting in limited primexine conversion and specification of columellae. 6, Nascent columellae are apparent as lamellated strands that lack substantial sporopollenin. 7, Consolidation of columellae by the appearance of partially polymerized (proto)sporopollenin on the receptive surfaces. 8, Final phase of primexine synthesis, during which the pattern stencil dissipates or is circumvented to produce the solid nexine I. 9, The callose wall is dissolved. 10, Wall elements are further consolidated by tapetally derived sporopollenin. 11, Nexine II is synthesized without the participation of the primexine. 12, Intine synthesis is initiated.

lignin and suberin, which places sporopollenin together with cutin, suberin, and lignin in a family of functionally (protective) and compositionally related biopolymers (Scott, 1994).

In addition to layering, pollen wall patterning also involves the development of a consistent number of precisely positioned germinal apertures and arrays of columellae (Figure 3). The pollen wall above the apertures consists of intine only, and consistent with the proposed role for primexine, this matrix is not deposited in areas destined to become apertures. Heslop-Harrison (1963) proposed that primexine synthesis is prevented by the apposition of a colpal shield (a plate of endoplasmic reticulum) to the plasma membrane at the position of the future aperture. Subsequently, Dover (1972) and Sheldon and Dickinson (1986) implicated the meiotic spindle in aperture positioning, perhaps by forcing cytoplasmic components against the plasma membrane. Despite the huge diversity in pollen wall patterning, a basic design feature shared by many species is the reticulate arrangement (frequently as hexagons) of the columellae to form a series of interlocking lacunae (Figure 3).

Several lines of evidence suggest that the genes responsible for species-specific pollen wall patterning are transcribed within the diploid nucleus of the premeiotic microsporocyte and that the pattern information is inherited by the microspores (Heslop-Harrison, 1963, 1968; Rogers and Harris, 1969). There is no evidence for the participation of any subcellular organelle in specifying the columellae position. However, centrifugation experiments with developing lily microsporocytes suggest that the agent(s), potentially protein-coated vesicles, responsible for imposing pattern on the primexine appears in the cytoplasm at the beginning of meiosis and is inserted into the plasma membrane as meiosis progresses (Sheldon and Dickinson, 1983; Dickinson and Sheldon, 1986). These vesicles may insert protein, or another substance, into the plasma membrane that somehow influences the position of the columellae. Heslop-Harrison (1972) made the following observation: "Sometimes when we contemplate biological pattern it is difficult to imagine how great a part of it could arise from physical causes, and correspondingly easy to slip into accepting that genetical control extends down to all detail. Yet this is obviously not so." Sheldon and Dickinson (1983) suggested that vesicles fuse randomly with the plasma membrane and the deposited protein undergoes self-assembly into pattern-specifying units. This would occur in a manner analogous to the behavior of oil droplets on water, or the cracks in the drying of a uniform surface such as mud, or more dramatically in the basalt layers that produced geological formations such as the Giants Causeway (Ireland), which naturally generate hexagonal patterns (reviewed by Scott, 1994). In the droplet model, the vesicles insert material into the membrane that eventually forms hexagonal plates, with the columellae forming along the interfaces. In the cracking model, the vesicular material forms a uniform layer, which upon

shrinkage forms the hexagonal arrays; the columellae are specified along the fracture lines.

There are very few mutations reported to affect pollen wall patterning. However, in the recently described *defective in exine formation1* (*dex1*) mutant of Arabidopsis, primexine deposition is delayed and reduced significantly and pollen wall patterning is disrupted (Paxson-Sowders et al., 2001). The *DEX1* gene encodes a predicted membrane-associated calcium binding protein that may act as a nucleation site for sporopollenin.

ANTHER DEHISCENCE

Mature pollen is released from the anther by dehiscence, a program of cell destruction culminating in rupture of the stomium, a furrow separating each pair of anther locules (Figure 1). Sanders et al. (1999) codified the major events that occur during the dehiscence program in Arabidopsis. These begin with the degeneration of the middle layer and tapetum, expansion of the endothecial layer, and deposition of fibrous bands (wall thickenings) in endothecial and connective cells. Degeneration of the septum generates a bilocular anther, which is followed by stomium cell breakage. In tobacco, the dehiscence process is very similar, differing only in the greater degree of connective breakdown. An elegant series of cell ablation experiments in tobacco showed that a functional stomium region is essential for dehiscence (Beals and Goldberg, 1997). Although events associated with dehiscence normally are coordinated with pollen development, male-sterile tobacco anthers that lack pollen and tapetum undergo a normal dehiscence process (Goldberg et al., 1993), indicating that dehiscence does not require signals derived from these cell types.

Comparative anatomical studies, microscopic observations, micromanipulation experiments, and measurements of water movement in stamens of a range of species suggested a model in which the endothecium contributes to two steps in the dehiscence process (Keijzer, 1987; Bonner and Dickinson, 1989). Just before dehiscence, concomitant with lysis of the cells of the stomium, the endothecial and epidermal cells become turgid (Figure 4A). This generates an inwardly directed force in the anther wall that causes the rupture of the weakened stomium. Subsequent desiccation of the endothecium causes differential shrinkage of thickened and unthickened regions of the cell wall, creating an outward bending force that leads to the retraction of the anther wall and full opening of the stomium to permit pollen release (Keijzer, 1987). The *ms35* mutant of Arabidopsis (Dawson et al., 1999) provides supporting evidence for the role of the endothecium in this second phase of dehiscence. Mature anthers of *ms35* mutants contain viable pollen grains, breakdown of the septum and stomium occurs normally, and the pattern of water movement is apparently also normal. However, the endothecial cells fail to develop lignified secondary wall thickenings and do not undergo the shrinkage normally associated with retraction of the anther wall. The *MS35* gene (renamed *MYB26*) encodes a putative R2R3-type MYB transcription factor (Steiner-Lange et al., 2003). Because other MYBs are known to regulate the phenylpropanoid pathway (e.g., AtMYBb4 [Jin et al., 2000]), the role of MYB26 may be to activate this pathway in endothecial cells to provide lignin residues for wall thickening.

Perhaps the most significant recent advance in understanding the control of dehiscence has been the discovery of the involvement of jasmonic acid (JA), a lipid-derived signaling compound distributed widely in the plant kingdom. All known mutants in JA biosynthetic or signaling pathways exhibit a similar phenotype, with (1) reduced elongation of the filaments, (2) delayed anther dehiscence, and (3) reduced pollen viability resulting in male sterility. Several mutants in JA biosynthetic enzymes have been isolated that cause male sterility and can be rescued by the application of JA (McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000; Park et al., 2002; von Malek et al., 2002). The role of JA in male fertility is supported by the JA signal transduction mutant *coronatine-insensitive1*, which is male sterile and insensitive to JA treatment (Feys et al., 1994; Xie et al., 1998).

The *DEFECTIVE IN ANTHER DEHISCENCE1* (*DAD1*) gene encodes a phospholipase A1 that catalyzes the initial step of JA biosynthesis (Ishiguro et al., 2001). Desiccation of *dad1* anthers appears delayed so that at the time of flower opening, when dehydration and shrinkage of the endothecium and connective cells break the stomium in wild-type anthers, the endothecium and connective cells of *dad1* anthers are fully expanded and the locules are filled with liquid. Expression studies suggest that DAD1 activity is restricted to the anther filament immediately before flower opening; therefore, the filament may represent the primary source of JA within the flower. Ishiguro et al. (2001) proposed a model in which JA synthesized in the filaments regulates water transport in the stamens and petals to bring about the coordinated opening of flowers, filament elongation, and anther dehiscence (Figure 4B). In this model, JA is required for the expression of genes involved in water transport in anthers. Interestingly, AtSUC1, a plasma membrane H^+-sucrose symporter, which is theoretically capable of transporting sucrose to increase water uptake, accumulates in some of the connective cells surrounding the vascular tissue during the final stages of anther development (Stadler et al., 1999). Filament and petal

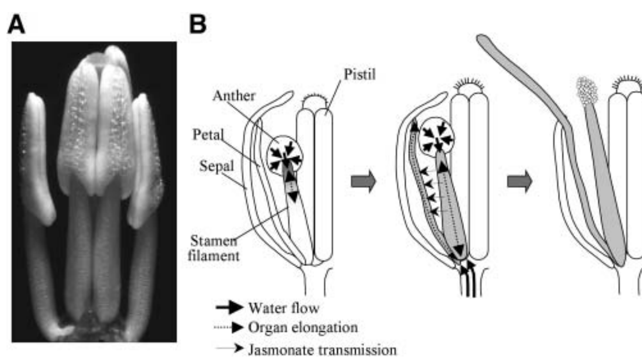

Figure 4. Anther Dehiscence.

(A) *Brassica oleracea* stamens before dehiscence showing fluid-filled epidermal cells on the abaxial surface of the anther.

(B) Model for the synchronous regulation of pollen maturation, anther dehiscence, and flower opening by jasmonic acid. The shaded areas represent regions that actively take up water and elongate in response to jasmonic acid (adapted from Ishiguro et al., 2001).

elongation also are inhibited in *dad1* flowers, suggesting that JA regulates water transport into these organs. This finding accords well with the observation that the dehiscence of onion anthers correlates with the extension rate of the filament (Keijzer, 1987) and with the suggestions that anther dehiscence is preceded by dehydration of the locules and that water is exported through the filaments to the petals (Bonner and Dickinson, 1990). An alternative model suggests that JA regulates programmed cell death in the anther as part of the dehiscence process (Zhao and Ma, 2000).

Rieu et al. (2003) reported evidence for the involvement of ethylene signaling in dehiscence. Tobacco plants insensitive to ethylene developed structurally normal anthers, but dehiscence was late and no longer synchronized with flower opening, in correlation with delays in the degeneration of stomium cells and dehydration of the anther. Treatment of nearly mature anthers with ethylene accelerated dehiscence in wild-type plants. Ethylene and JA may perform the same role in tobacco and Arabidopsis, respectively, or act redundantly in both species. The fact that Arabidopsis mutants such as *dde1*, which cannot synthesize JA within the stamens, eventually undergo dehiscence might represent evidence in support of redundant pathways.

CONCLUSION

Ten years ago, little was known about the genetic control of stamen development beyond the specification of stamen primordia by floral homeotic genes. Now, footholds have been established in several areas, notably patterning of the microsporangium, regulation of meiosis, and anther dehiscence. Other processes, such as the genesis of the pollen wall, have proved less tractable, perhaps because of a lack of informative mutants. Important questions remain to be addressed, particularly those concerning the establishment of cell fates within the microsporangium, the molecular regulation of the very different nuclear events in the meiocytes and tapetal cells, the link between sporophytically controlled callose degradation and male gametophyte development, the "molecular mechanics" of pollen wall formation, and bridging the gap between JA levels and water movement during dehiscence. Understanding stamen development is key to the successful exploitation of plant breeding systems. In a world of diminishing resources and an increasing need for efficient food production, solving these questions should be accorded a high priority.

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NOTE ADDED IN PROOF

A recent paper shows that severe reduction of RNA and protein levels of the tobacco β-1,3-glucanase *Tag1* does not affect tetrad dissolution:

Bucciaglia, P.A., Zimmermann, E., and Smith, A.G. (2003). Functional analysis of a β-1,3-glucanase gene *Tag1* with anther-specific RNA and protein accumulation using antisense RNA inhibition. J. Plant Phys. **160,** 1367–1373.