Anther Development: Basic Principles and Practical Applications

Robert B. Goldberg,[1] Thomas P. Beals, and Paul M. Sanders

Department of Biology, University of California, Los Angeles, California 90024-1606

INTRODUCTION

Male reproductive processes in flowering plants take place in the stamen (Esau, 1977). This sporophytic organ system contains diploid cells that undergo meiosis and produce haploid male spores, or microspores. Microspores divide mitotically and differentiate into multicellular male gametophytes, or pollen grains, that contain the sperm cells. Figures 1 and 2 show that the stamen consists of two morphologically distinct parts—the anther and the filament. The filament is a tube of vascular tissue that anchors the stamen to the flower and serves as a conduit for water and nutrients. By contrast, the anther contains the reproductive and nonreproductive tissues that are responsible for producing and releasing pollen grains so that pollination and fertilization processes can occur within the flower.

Figure 1 shows that anther development can be divided into two general phases. During phase 1, the morphology of the anther is established, cell and tissue differentiation occur, and microspore mother cells undergo meiosis. At the end of phase 1, the anther contains most of its specialized cells and tissues, and tetrads of microspores are present within the pollen sacs (Figure 1). During phase 2, pollen grains differentiate, the anther enlarges and is pushed upward in the flower by filament extension, and tissue degeneration, dehiscence, and pollen grain release occur (Figure 1). The cellular processes that regulate anther cell differentiation, establish anther tissue patterns, and cause the anther to switch from a histospecification program (phase 1) to a cell degeneration and dehiscence program (phase 2) are not known.

The developmental events leading to anther formation and pollen release are exquisitely timed and choreographed (Koltunow et al., 1990; Scott et al., 1991). Cell differentiation and dehiscence events occur in a precise chronological order that correlates with floral bud size (Koltunow et al., 1990; Scott et al., 1991). This permits the mechanisms responsible for cell-type differentiation, tissue degeneration, and cell-specific gene activation within the anther to be explored with relative ease. During the past few years, there has been an explosive burst of interest in anther biology, both as a system to dissect plant developmental processes at the molecular and genetic levels (Koltunow et al., 1990; Gasser, 1991; McCormick,

1991; Scott et al., 1991; Chaudhury et al., 1992; Dawe and Freeling, 1992; Spena et al., 1992; van der Meer et al., 1992; Worrall et al., 1992; Aarts et al., 1993; Preuss et al., 1993) and for practical genetic engineering studies to improve crop plants (Mariani et al., 1990, 1992; Denis et al., 1993; Schmülling et al., 1993). In this review, we outline the major events that occur during anther development, using our studies of the tobacco anther as a focal point (Koltunow et al., 1990). We concentrate primarily on processes concerned with anther cell-type differentiation and dehiscence, because others in this volume have concentrated on the male gametophyte and have reviewed pollen grain biology and development (Mascarenhas, 1993, this issue; McCormick, 1993, this issue).

STAMEN INITIATION IS CONTROLLED BY HOMEOTIC GENE INTERACTIONS

As shown in Figure 2 and represented schematically in Figure 1, stamen primordia are initiated as a whorl of small bumps at specific locations on the floral meristem surface. The number of stamen primordia varies in different plant species, but their location on the floral meristem is fixed in what is referred to as the third whorl (Coen, 1991; Coen and Meyerowitz, 1991). In tobacco, for example, five stamen primordia are initiated in the third whorl (Figures 1 and 2) over a 1- to 2-day period (Koltunow et al., 1990). Stamen primordia appear after the sepal and petal primordia have initiated but prior to the initiation of carpel primordia (Figure 2). A short time after stamen primordia initiation occurs, the anther and filament compartments differentiate, two bilaterally symmetrical theca are established within the anther, major anther cell types and tissues form, and the anther takes on its characteristic paddlelike shape (Figures 1 and 2).

Stamen primordia initiation does not depend upon the presence of sepal or petal primordia, because surgical removal of these primordia does not prevent third-whorl primordia from becoming stamens (Hicks and Sussex, 1971). In addition, homeotic mutations that affect the identity of petal and sepal primordia do not affect stamen primordia initiation and differentiation (Coen, 1991; Coen and Meyerowitz, 1991). Thus,
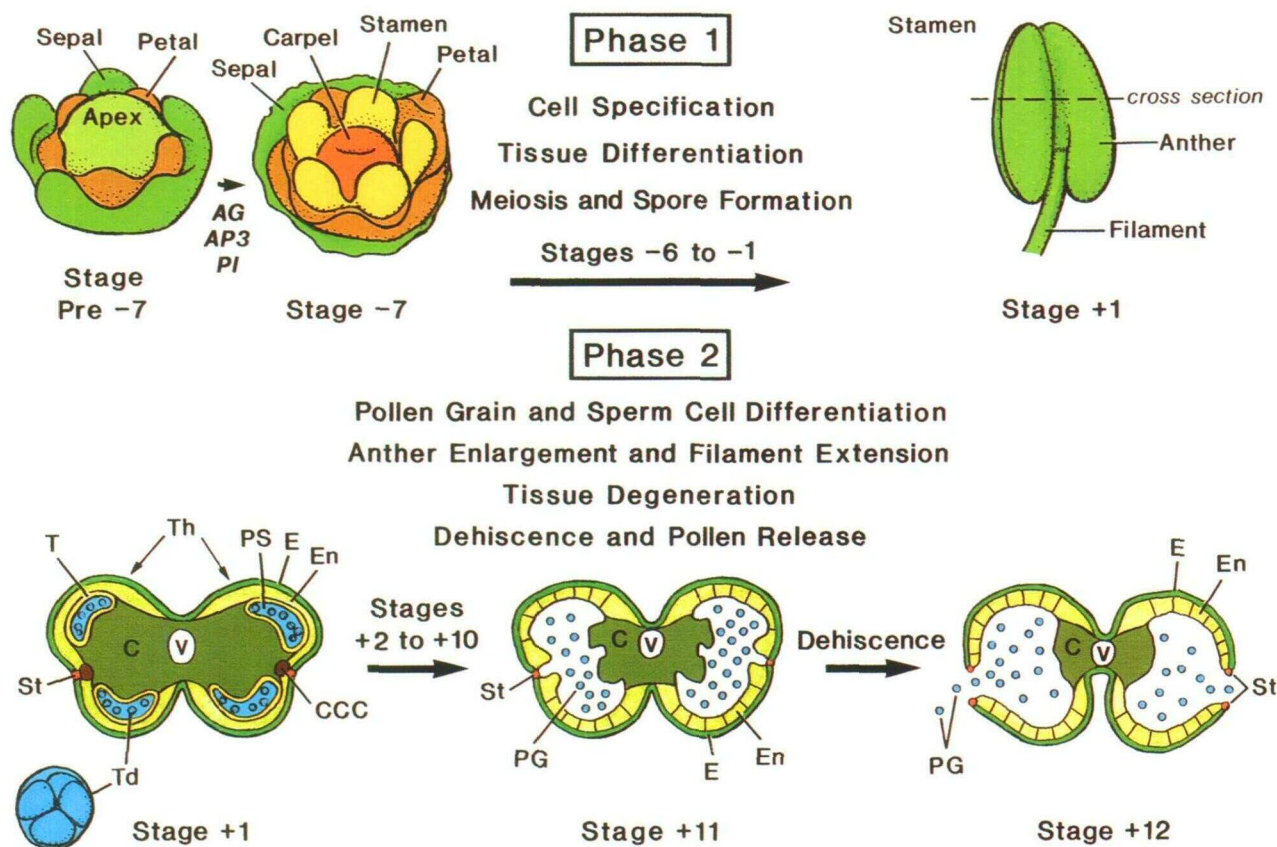
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[1] To whom correspondence should be addressed.

Figure 1. A Generalized Overview of Anther Development.

Schematic representations of anther developmental stages and cross-sections are based on scanning electron and light microscopy studies of tobacco anther development (Koltunow et al., 1990; Drews et al., 1992). The dashed line through the stage 1 anther drawn in the phase 1 portion of the figure represents the cross-section plane for anthers drawn schematically in phase 2. The vertical lines drawn through the endothecium at stages 11 and 12 represent fibrous cell wall bands. C, connective; CCC, circular cell cluster; E, epidermis; En, endothecium; PG, pollen grain; PS, pollen sac; St, stomium; T, tapetum; Td, tetrads; Th, theca; V, vascular bundle.

stamen primordia specification occurs independently of the presence of other floral organ primordia and vice versa. What processes control stamen morphogenesis and the differentiation of diverse stamen cell and tissue types are not known.

Genetic and molecular studies have shown that stamen primordia specification is controlled by the interaction of several homeotic genes (Figure 1), including *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*) in Arabidopsis and their counterparts *DEFICIENS* (*DEF*), *GLOBOSA* (*GLO*), and *PLENA* (*PLE*) in snapdragon (Schwarz-Sommer et al., 1990; Coen, 1991, 1992; Coen and Carpenter, 1993, this issue; Okamuro et al., 1993, this issue). Mutations in any one of these genes result in the loss of stamen primordia and the conversion of third-whorl primordia to a different floral organ type (Schwarz-Sommer et al., 1990; Coen, 1991, 1992; Coen and Meyerowitz, 1991). These homeotic genes also play a role in the specification of other floral primordia and are discussed in detail by Okamuro et al. (1993, this issue) and Coen and Carpenter (1993,

this issue). Related homeotic genes have also been identified in tobacco (Hansen et al., 1993), petunia (Angenent et al., 1992; van der Krol and Chua, 1993, this issue), tomato (Pnueli et al., 1991), and corn (Schmidt et al., 1993; Veit et al., 1993, this issue), indicating that the same genes control stamen primordia specification in distantly related plants.

Homeotic genes controlling stamen specification encode transcription factors related to those in yeast and mammals and contain a conserved MADS box region that probably represents the DNA binding domain (Coen and Meyerowitz, 1991). *DEF* (*AP3*) and *GLO* (*PI*) encode transcription factors that interact as heterodimers with their target DNA sequence motifs and, with the *PLE* (*AG*) gene product, activate genes required for stamen specification in the third whorl (Coen, 1992; Schwarz-Sommer et al., 1992; Tröbner et al., 1992). In situ hybridization studies have shown that these homeotic genes are expressed in the predicted pre-third-whorl territories of the floral meristem just prior to stamen primordia initiation and that they

continue to be expressed throughout stamen development in both the anther and filament (Schwarz-Sommer et al., 1990, 1992; Coen and Meyerowitz, 1991; Coen, 1992). Thus, the floral homeotic genes *DEF (AP3)*, *GLO (PI)*, and *PLE (AG)* set off a cascade of events that cause third-whorl primordia to follow a stamen differentiation pathway (Figure 1).

Although progress has been made in identifying genes required for stamen primordia specification, several crucial questions remain unanswered. First, which genes control the stamen primordia identity genes, and how do these upstream genes activate *DEF (AP3)*, *GLO (PI)*, and *PLE (AG)* in the proper third-whorl territories? That is, what processes regulate the position and number of stamen primordia on the floral meristem? Several homeotic genes have been identified in Arabidopsis and snapdragon that play a role in regulating floral meristem identity (Schwarz-Sommer et al., 1990; Coen, 1991, 1992; Coen and Meyerowitz, 1991; Coen and Carpenter, 1993, this issue), including *SQUAMOSA* and *FLORICAULA* in snapdragon (Coen et al., 1990; Schwarz-Sommer et al., 1990), and their counterparts *APETALA1* (Mandel et al., 1992) and *LEAFY* (Weigel et al., 1992) in Arabidopsis (Okamuro et al., 1993, this issue). It is not yet known, however, which genes and processes

function in the interval between the activation of floral meristem identity genes and those involved in stamen primordia specification. Second, and most important, it is not known what target, or downstream, genes are activated transcriptionally by the DEF (AP3), GLO (PI), and PLE (AG) proteins within the stamen primordia. That is, what are the genes whose products are required to carry out stamen morphogenesis and tissue differentiation programs after the stamen primordia have been specified? Among these downstream genes should be those that (1) regulate the differentiation of the stamen into anther and filament compartments, (2) control the shape of the anther and its size, (3) initiate cell and tissue specification programs required for the development of a mature anther, and (4) activate anther cell–specific gene sets. Clearly, the identification of the downstream genes and their products should provide insight into the detailed *mechanisms* responsible for anther morphogenesis and differentiation.

Figure 2. Scanning Electron Micrographs of Tobacco Stamen Primordia Development.

Floral buds and anthers harvested at the designated stages were photographed in the scanning electron microscope (Koltunow et al., 1990; Drews et al., 1992). Magnification factors and stage numbers are marked on each micrograph. Numbers have not been assigned to stages prior to −7, which are collectively referred to as pre −7 (Figure 1). A, anther; C, carpel; F, filament; P, petal; S, sepal; T, theca.

STAMEN DEVELOPMENT IS INFLUENCED BY NUCLEAR AND MITOCHONDRIAL GENOME INTERACTIONS

Several homeotic-like mutants have been identified in tobacco that affect stamen development (Rosenberg and Bonnett, 1983; Kofer et al., 1990, 1991, 1992). These mutants were identified originally as male-sterile progeny of crosses between different tobacco species (Sand, 1968; Hicks et al., 1977) and, more recently, in plants regenerated from tissue culture after fusion of protoplasts from different male-sterile cultivars (Kofer et al., 1990, 1991, 1992). Unlike male-sterile homeotic mutants in Arabidopsis and snapdragon that are caused by nuclear gene lesions, tobacco homeotic-like mutants are inherited cytoplasmically and are associated with alterations in specific mitochondrial DNA fragments (Kofer et al., 1991, 1992). This implies that mitochondrial genes play a role in the stamen differentiation pathway.

The phenotypes of tobacco homeotic-like mutants vary, but they correlate with the presence of a specific cytoplasm and mitochondrial genome (Kofer et al., 1991, 1992). For example, in some mutants, the third-whorl primordia emerge and then abort just prior to carpel primordia initiation (Rosenberg and Bonnett, 1983). In other mutants, the third-whorl primordia develop into petal-like structures or antherless stamens whose filaments have stigmatoid structures at their tips (Hicks et al., 1977; Rosenberg and Bonnett, 1983). The varied effects of the mitochondrial genome on stamen development suggest that the nuclear/mitochondrial gene interactions occur *after* stamen primordia specification (Hicks et al., 1977; Rosenberg and Bonnett, 1983). If so, this suggests that the altered cytoplasms interfere with the activation and/or function of the downstream genes required for stamen differentiation and morphogenesis. The question of how mitochondrial and nuclear gene interactions affect the stamen differentiation pathway remains unresolved.
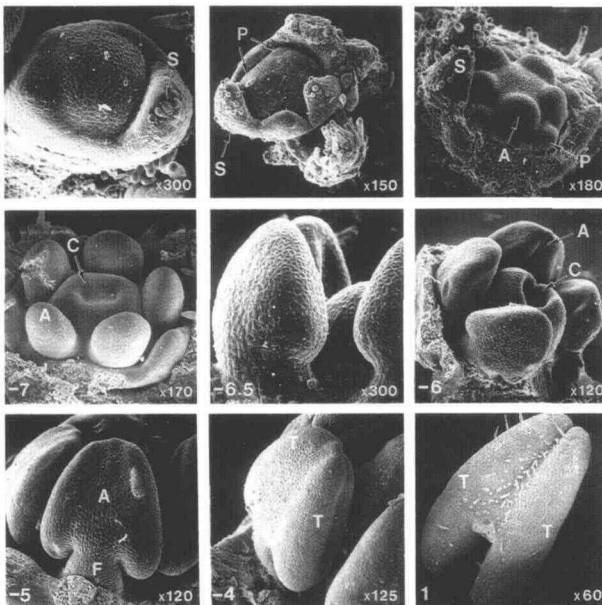
ANTHER CELL TYPES CAN BE TRACED TO SPECIFIC FLORAL MERISTEM LAYERS

At the end of phase 1 (Figure 1), a differentiated anther has several highly specialized cells and tissues that are responsible for carrying out nonreproductive functions (e.g., support and dehiscence) and reproductive functions (e.g., spore and pollen formation). Schematic representations of differentiated stage 1 tobacco anther cross-sections are shown in Figures 1 and 3, actual bright-field photographs are shown in Figure 4, and anther cell types and their functions are listed in Table 1. The nonreproductive tissues include the epidermis, endothecium, tapetum, circular cell cluster, connective, stomium, and vascular bundle (Table 1). Each of these tissues and cell types carries out specialized tasks (Esau, 1977). For example,

the stomium and circular cell cluster are involved in dehiscence (Bonner and Dickenson, 1989), the tapetum plays a role in pollen wall formation (Esau, 1977; Pacini et al., 1985), and the connective is responsible for anchoring the four pollen sacs together into a single anther structure (Weberling, 1989). In addition to these diploid sporophytic tissues, the anther also contains haploid microspores that fill the pollen sacs and differentiate into pollen grains (McCormick, 1991, 1993, this issue; Scott et al., 1991; Mascarenhas, 1993, this issue).

Cytochimera cell lineage studies performed with the Jimson weed (*Datura stramonium*), a solanaceous relative of a tobacco, showed that the floral meristem consists of three "germ" layers, designated L1, L2, and L3 (Satina et al., 1940), which give rise to different anther tissues following stamen primordia initiation (Satina and Blakeslee, 1941). Thus, once specified, the developmental fate of L1, L2, and L3 layer
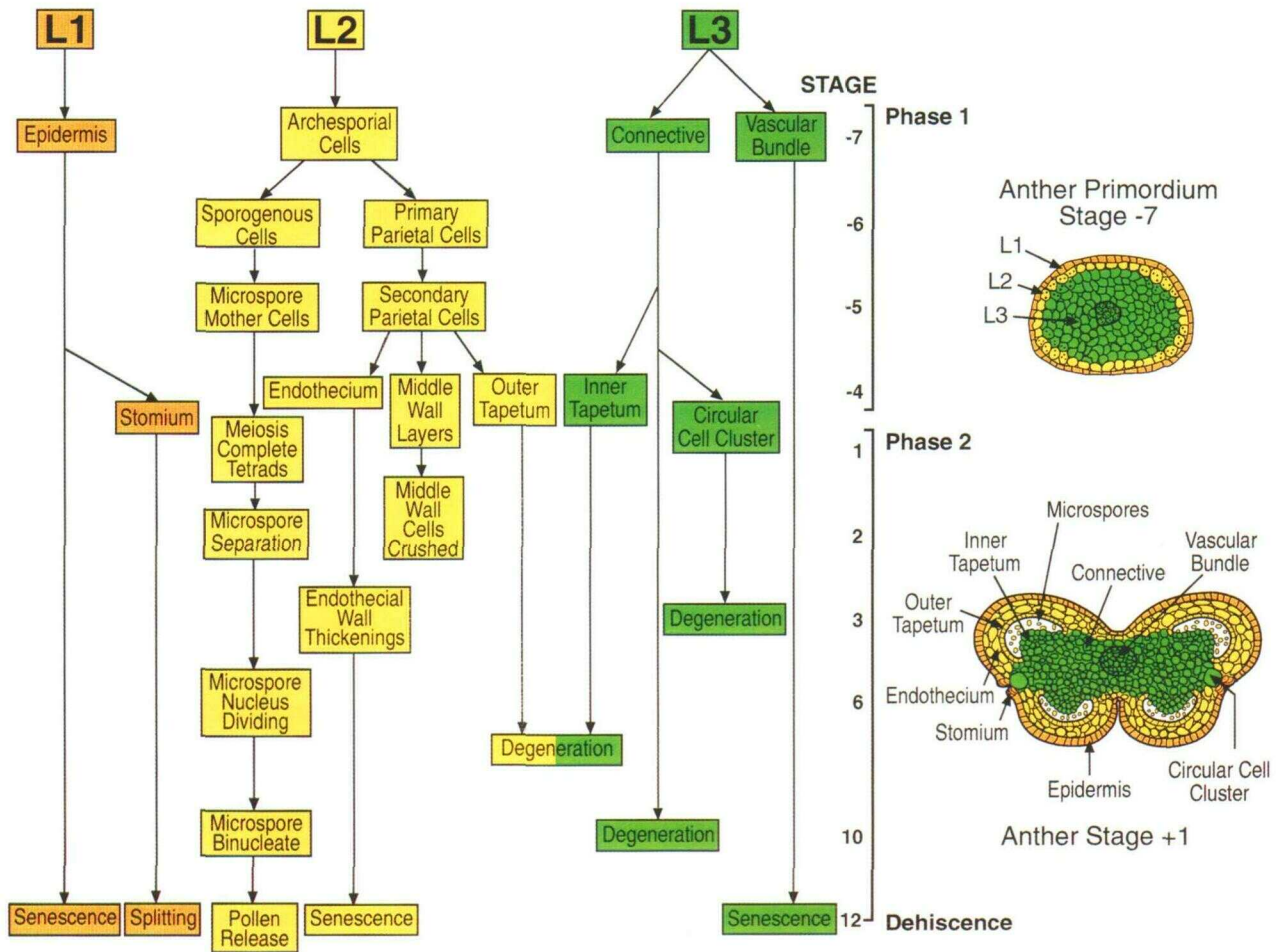

Figure 3. Cell Lineages and Major Events That Occur during Anther Differentiation and Dehiscence.

Stages, timing of events, and anther schematic representations were taken from Koltunow et al. (1990) for tobacco anther development. Similar events take place during anther development in other plants (Esau, 1977). Cell lineages derived from the L1, L2, and L3 primordia layers were inferred from the histological observations of Satina and Blakeslee (1941), Esau (1977), and Koltunow et al. (1990).
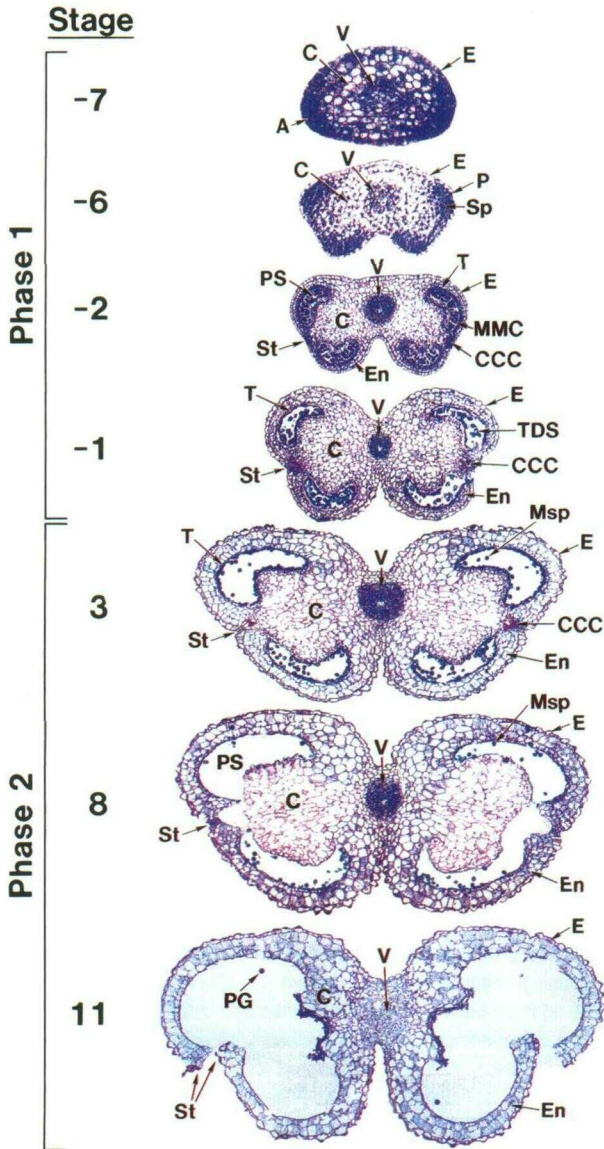
Stage

Figure 4. Bright-Field Photographs of Tobacco Anther Development.

Anthers at the designated stages were fixed, embedded in paraffin, and sliced into 10-μm transverse sections through the plane drawn schematically in Figure 1. The sections were stained with toluidine blue and photographed with bright-field illumination. Pictures are taken from Koltunow et al. (1990), except for stage 11. A, archesporial cells; C, connective; CCC, circular cell cluster; E, epidermis; En, endothecium; MMC, microspore mother cells; Msp, microspores; P, parietal cells; PS, pollen sac; Sp, sporogenous cells; St, stomium; T, tapetum; TDS, tetrads; V, vascular bundle.

derivatives is fixed. Figure 3 shows a schematic representation of a tobacco stamen primordium and the anther cell lineages that are inferred from histological analysis to be derived from the L1, L2, and L3 layers (Satina and Blakeslee,

1941; Esau, 1977; Koltunow et al., 1990). In most cases, individual tissues and cell types are derived from a single germ layer. For example, the L1 layer gives rise to the epidermis and the stomium; the L2 layer gives rise to the archesporial cells, microspore mother cells, endothecium, and middle-wall layers that lie between the epidermis and tapetum; and the L3 layer gives rise to the connective, vascular bundle, and circular cell cluster adjacent to the stomium (Figure 3). By contrast, both the L2 and L3 layers contribute to tapetum formation (Figure 3). Tapetal cells along the upper (inner) portion of the pollen sacs are specified from the L3-derived connective tissue, whereas those that line the lower (outer) portion of the pollen sacs are specified from the L2-derived archesporial lineage (Figure 3; Esau, 1977). The underlying mechanisms responsible for the differentiation of specific anther cell types *after* stamen primordia initiation occurs are not known.

CELL TERRITORIES MAY PLAY A ROLE IN THE ANTHER DIFFERENTIATION PROCESS

Figures 3 and 4 show that cells destined to become major anther tissues and cell types form at precise times and positions during anther development. For example, archesporial cells destined to differentiate into microsporangia (pollen sacs) and surrounding tapetum and endothecium tissues arise simultaneously in each corner of the anther primordium (Figures 3 and 4). By contrast, vascular tissues differentiate within the

Table 1. Functions of Anther Cell and Tissue Types

Cell or Tissue Type[a]	Major Function[b]
Connective	Join anther theca together; connect anther to filament; structure, support, and morphology
Circular Cell Cluster[c]	Dehiscence
Endothecium[d]	Structure and support; dehiscence
Epidermis	Structure and support; prevent water loss; gas exchange; dehiscence
Microspore	Pollen grain and sperm cell development
Middle Layer[d]	Structure and support; dehiscence
Stomium	Dehiscence
Tapetum	Pollen wall components; nutrients for pollen development; enzymes for microspore release from tetrads
Vascular Bundle	Connection between anther, filament, and flower; nutrient and water supply

[a] Cells present in a stage 1 tobacco anther (Figures 1 to 4).
[b] Taken from Esau (1977), Weberling (1989), and Koltunow et al. (1990).
[c] Also referred to as the crystal-containing idioblasts (Trull et al., 1991), intersporangial septum (Bonner and Dickenson, 1989), and hypodermal stomium (Horner and Wagner, 1992).
[d] Collectively referred to as the anther wall.

center of the anther primordium and establish a connection with the filament (Figures 3 and 4). Other tissues, such as the circular cell cluster and stomium, form at the boundaries between each microsporangial pair later in development (Figures 3 and 4). Thus, specific regions, or territories, are established early in anther development within which unique histodifferentiation events occur in a precise chronological order.

No information exists about whether the differentiation of anther cells and tissues is induced (or inhibited) by signals originating from contiguous regions of a territory, or is controlled autonomously by sequestering morphogenetic factors during divisions of the L1, L2, and L3 layers, or both. The territory-based differentiation of four microsporangia with identical tissue patterns and the differentiation of tapetal cells within each territory from two different cell lineages (Figure 3) suggest that cell–cell communication processes may play an important role in the anther histospecification process. For example, it is conceivable that a gradient of morphogens, synthesized by archesporial cells and interpreted by contiguous L2 and L3 cells, triggers the differentiation of tapetal and endothecial cells (Figures 3 and 4). Other morphogens appearing later at the boundaries of the microsporangia could be perceived by neighboring L1-derived epidermal cells and L3-derived connective cells and cause them to differentiate into the stomium and circular cell cluster, respectively (Figures 3 and 4). Interpretation of putative intercellular signals would lead ultimately to the transcriptional activation of genes responsible for establishing the differentiated fate of each cell type within the territory. Although speculative, this model leads to testable predictions.

Several lines of evidence suggest that intercellular signals play an important role in determining the fate of cells within each floral meristem germ cell layer. These studies are reviewed by Sussex (1989) and by Huala and Sussex (1993, this issue). Genetic chimera experiments have indicated that the position of L1, L2, and L3 cells within the floral meristem is the primary factor in specifying their fate rather than prior cell ancestry (Sussex, 1989; Huala and Sussex, 1993, this issue). For example, rare divisions that enable L2 cells to penetrate or invade the L1 meristem layer cause these cells to follow an epidermal pathway. Conversely, penetration of L1 cells into the L2 layer causes these cells to have a fate identical to that of surrounding L2 cells. In addition, recent studies using gene markers specific for each germ layer have shown that the L3 layer influences the development of cells derived from the L1 and L2 layers (Szymkowiak and Sussex, 1992). These experiments suggest that cells within each meristem germ layer are conditionally specified (Davidson, 1991) and that the ultimate fate of these cells depends upon their position in the floral meristem. That is, position-dependent communication processes probably occur between adjacent meristematic cells, and these signaling events influence floral organ cell specification pathways. The extent to which cell-autonomous and position-dependent cell interaction processes play a role in the differentiation of specialized cell types derived from each anther primordium germ layer (Figure 3) remains a major unanswered question.

ANTHER DEHISCENCE INVOLVES THE PROGRAMMED DESTRUCTION OF SPECIFIC CELL TYPES

One of the major mysteries of anther development is how a differentiated anther switches from a histodifferentiation program (phase 1) to a cell degeneration and dehiscence program (phase 2) that leads ultimately to pollen release and stamen senescence at flower opening. The dehiscence program begins after the formation of tetrads, results in the sequential destruction of specific anther cell types, and is coordinated temporally with the pollen differentiation process (Figure 1). In tobacco, the entire dehiscence and cell degeneration process occurs over a 1-week period (Koltunow et al., 1990) and involves three major cell types that are formed during phase 1 from different anther primordium cell layers (Figure 3). These include the stomium, the endothecium, and the circular cell cluster (Table 1; Figures 1 and 4). The latter tissue, which is common in solanaceous anthers, is also referred to as the hypodermal stomium (Horner and Wagner, 1992), the intersporangial septum (Keijzer, 1987; Bonner and Dickenson, 1989) and, because it contains calcium oxalate crystals, the crystal-containing idioblasts (Trull et al., 1991; Horner and Wagner, 1992). The onset of the dehiscence program sets off an *ordered* series of events within the anther, including (1) the formation of fibrous band thickenings on the endothecial cell walls, (2) degeneration of the circular cell cluster and merging of the two pollen sacs in each theca into a single locule, (3) breakdown of the tapetum and connective, and (4) rupture of the anther at the stomium and pollen release (Figures 1 and 4; Bonner and Dickenson, 1989). Male-sterile tobacco anthers that lack pollen grains and tapetal cells undergo a normal dehiscence process (Koltunow et al., 1990; Mariani et al., 1990), indicating that dehiscence is not set into motion by signals derived from either the tapetum or differentiating pollen grains.

The dehiscence program probably requires the activation of many genes, including those that encode hydrolytic enzymes required for cell death, such as RNases, proteases, and cellulases (Keijzer, 1987; Bonner and Dickenson, 1989; del Campillo and Lewis, 1992). One marker for the dehiscence process in tobacco is the TA56 thiol endopeptidase gene (Koltunow et al., 1990). As shown in Figure 5, TA56 mRNA accumulates first in the circular cell cluster prior to its destruction, then in the stomium, and finally in the connective. Although the role of the TA56 thiol endopeptidase gene in the dehiscence process is not known, it marks the cell-specific degeneration events that occur prior to stomium rupture and pollen release from the anther (Figures 1 and 4; Koltunow et al., 1990). Recent experiments with chimeric genes containing TA56 5′ sequences showed that the sequential accumulation of TA56 mRNA in specific anther cell types is controlled primarily at the transcriptional level (T. P. Beals and R. B. Goldberg, unpublished results). Thus, the onset of the dehiscence program results in the transcriptional activation of genes that are inactive during the histodifferentiation phase of anther development (Figure 1). Which cellular events trigger the dehiscence program, activate dehiscence-specific genes, and coordinate pollen grain
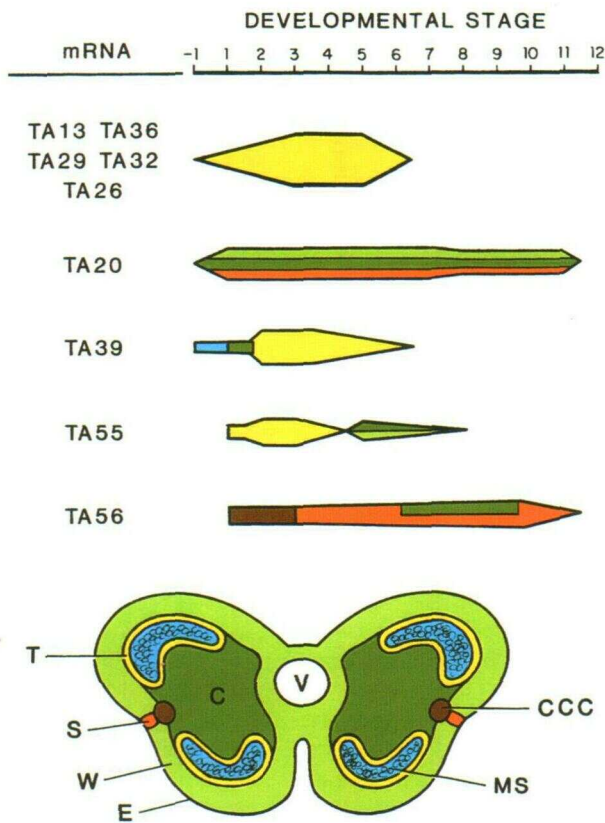
Figure 5. Temporal and Spatial Regulation of mRNAs during Tobacco Anther Development.

Data represent a summary of RNA gel blot and in situ hybridization studies performed in our laboratory with anther-specific cDNAs (Koltunow et al., 1990; T.P. Beals and R.B. Goldberg, unpublished results). Proteins encoded by these mRNAs and their cell specificities are summarized in Table 2. The colors present in each bar correspond with the cell and tissue types in which each mRNA is localized at that period of anther development. The tapering in each bar shows periods when the mRNA accumulates or decays, and the bar thickness approximates the relative mRNA prevalences. C, connective; CCC, circular cell cluster; E, epidermis; MS, microspore; S, stomium; T, tapetum; V, vascular bundle; W, wall. The wall includes the endothecium and middle layer (see Table 1). Figure adapted from Koltunow et al. (1990).

differentiation with the sequential destruction of specific anther tissues are not known.

GENE EXPRESSION IS TEMPORALLY AND SPATIALLY REGULATED DURING ANTHER DEVELOPMENT

RNA-excess DNA/RNA hybridization experiments with single-copy DNA indicated that there are ~25,000 diverse genes expressed in a stage 6 tobacco anther (Figure 4; Kamalay and

Goldberg, 1980). The vast majority of these genes encode rare mRNAs that constitute less than 0.001% of the mRNA mass when averaged over the entire anther mRNA population. Comparison of floral and vegetative organ system RNA populations showed that ~10,000 diverse anther mRNAs are undetectable in the nuclear RNA and mRNA populations of other organs and are anther specific (Kamalay and Goldberg, 1980, 1984). Although it is not known how many additional anther-specific genes are expressed during phase 1, when major histospecification events occur (Figure 4), these results indicate that the differential regulation of a large number of genes is required to specify and maintain the differentiated state of cells and tissues within the anther.

Experiments conducted in several laboratories have identified cDNA clones that represent prevalent mRNAs that are present exclusively, or at elevated levels, in the anther (Koltunow et al., 1990; Smith et al., 1990; Evrard et al., 1991; McCormick, 1991, 1993, this issue; Nacken et al., 1991; Scott et al., 1991; Shen and Hsu, 1992). Table 2 lists several tobacco anther-specific mRNAs studied in our laboratory. Anther-specific mRNAs have been shown to encode lipid transfer proteins, protease inhibitors, thiol endopeptidases, glycine-rich and proline-rich polypeptides with properties of cell wall proteins, pectate lyases, polygalacturonases, and chalcone synthase isoforms (Table 2; Koltunow et al., 1990; McCormick, 1991; Scott et al., 1991). Many anther-specific mRNAs, such as TA20, are found exclusively in sporophytic tissues (Table 2; Koltunow et al., 1990). Others are pollen grain specific or, like TA39 (Table 2), are present in both sporophytic and gametophytic cells (McCormick, 1991; Scott et al., 1991).

Experiments conducted with anther-specific cDNAs have demonstrated the striking degree to which different temporal- and cell-specific gene expression programs occur during anther development (Koltunow et al., 1990; McCormick, 1991, 1993, this issue; Scott et al., 1991). Figure 5 presents a

Table 2. Gene Markers for Tobacco Anther Cell Types

mRNA[a,b]	Protein Encoded	Cell Specificity
TA13	Glycine-rich	Tapetum
TA20	Unknown	Connective, stomium, endothecium, middle layer
TA26	Unknown	Tapetum
TA29	Glycine-rich	Tapetum
TA32	Lipid transfer	Tapetum
TA36	Lipid transfer	Tapetum
TA39	Unknown	Tapetum, microspore
TA55	Unknown	Tapetum, connective, endothecium, middle layer
TA56	Thiol endopeptidase	Connective, circular cell cluster, stomium

[a] Taken from Koltunow et al. (1990) and T.P. Beals and R.B. Goldberg (unpublished results).
[b] Gene markers for anther cells in other plants have been reviewed recently (Gasser, 1991; McCormick, 1991; Scott et al., 1991).

schematic representation of several different tobacco anther-specific gene expression programs that have been identified recently in our laboratory (Koltunow et al., 1990; T.P. Beals and R.B. Goldberg, unpublished results). One striking example is the coordinated accumulation and decay of the tapetal-specific TA13, TA26, TA29, TA32, and TA36 mRNAs during early phase 2 (Figure 5; Koltunow et al., 1990). This pattern, and others shown in Figure 5, probably represent only a few of the gene expression programs that occur during anther development (Evrard et al., 1991; McCormick, 1991; Scott et al., 1991). However, these patterns illustrate that (1) genes expressed in the anther are regulated by many different control programs that are partitioned with respect to both cell type and developmental time, (2) anther-specific gene expression programs correlate with the differentiation and degeneration of specific anther tissues and cell types, and (3) anther cell–specific mRNAs provide useful markers for dissecting the regulatory pathways that control the specification of different cell types during anther development. The mechanisms responsible for establishing cell- and temporal-specific gene expression patterns during anther development are not known.

TRANSCRIPTIONAL PROCESSES CONTROL ANTHER-SPECIFIC GENE EXPRESSION PROGRAMS

Solution hybridization experiments carried out with anther mRNA and nuclear RNA populations suggested that most anther-specific genes are under transcriptional control (Kamalay and Goldberg, 1984). Run-off transcription studies and transformation experiments with chimeric genes containing anther-specific gene promoters demonstrated directly that the temporal and spatial gene expression programs that operate in the anther are controlled primarily by transcriptional processes (Koltunow et al., 1990; McCormick, 1991, 1993, this issue; Scott et al., 1991). For example, the tapetal-specific TA29 gene (Table 2 and Figure 5) is not transcribed detectably in other plant organs, and chimeric genes with TA29 5' sequences are active only in the tapetum (Koltunow et al., 1990; Mariani et al., 1990, 1992). Chimeric genes driven by other anther-specific gene promoters, such as TA56 (Table 2 and Figure 5), produce transcription patterns that also reflect the cell- and temporal-specific expression programs characteristic of their parental, or endogenous, genes (McCormick, 1991; Scott et al., 1991; T.P. Beals and R.B. Goldberg, unpublished results). These results suggest that the mosaic of gene expression programs that function in the anther (Figure 5) reflects the distribution of transcription factors capable of activating unique gene sets in each specialized cell type. If so, this implies that anther cell differentiation events lead directly or indirectly to the expression of regulatory genes that are necessary to activate each cell-specific gene set. The identification of anther transcription factors and their corresponding genes should provide useful entry points into anther cell–specific differentiation pathways (Figure 3). How genes encoding anther transcription

factors are regulated with respect to cell type and time during anther development, activate batteries of cell-specific gene sets, and connect with regulatory circuits that ultimately trace back to homeotic genes controlling stamen primordia specification (Figure 1) remain major unanswered questions.

CELL ABLATION STUDIES WITH CYTOTOXIC GENES CAN BE USED TO STUDY ANTHER CELL DIFFERENTIATION PROCESSES

One powerful use of the anther cell–specific promoters identified in the gene expression studies (Table 2 and Figure 5) is to fuse them to cytotoxic structural genes, such as the diphtheria toxin A-chain gene (Palmiter et al., 1987), the RNase T1 gene (Mariani et al., 1990), or the barnase gene (Mariani et al., 1990), to selectively destroy specific cell types during anther development. This strategy, illustrated in Figure 6, is an extension of the microbeam laser cell ablation approach that (1) provided the first insights into the role of cell–cell interactions in Caenorhabditis elegans cell-type specification and (2) laid the foundation for molecular and genetic approaches that identified genes encoding components of different cell induction pathways (Sulston et al., 1983; Horvitz and Sternberg, 1991). Both of these strategies rely on the selective destruction of a specific cell type and an identification of the effect, if any, that the loss of this cell type has on the differentiation of contiguous cells (Palmiter et al., 1987). The microbeam laser approach is not readily applicable to studies of anther cell differentiation, because the anther consists of many cell layers and is contained within a closed flower bud (Figures 1 to 4). Thus, specific cell targets are refractory to identification and physical destruction by a laser beam. By contrast, ablation studies using "genetic lasers" in transgenic plants can, in theory, be applied to an unlimited number of cell types. All that is required is a set of cell-specific promoters that is active in a variety of anther cell types (Table 2).

We fused the tobacco tapetal–specific TA29 gene promoter (Table 2 and Figure 5) to several cytotoxic genes to selectively destroy the tapetum during anther development (Figure 6; Koltunow et al., 1990; Mariani et al., 1990). These experiments showed that the tapetum is critical for pollen development but that it is not necessary for the function and/or degeneration of other anther cell types during phase 2 of anther development (Koltunow et al., 1990; Mariani et al., 1990). That is, the tapetum functions in a cell-autonomous manner following its formation during phase 1 of anther development (Figures 3 and 4), and tapetal cells are not required for the induction and/or completion of the dehiscence program (Figure 1). Of particular interest, of course, is how the loss of cells formed during the early stages of anther development would affect the differentiation of adjacent cell types (Figures 3 and 4). For example, how would the loss of primary parietal cells affect the differentiation of contiguous sporogenous cells within developing microsporangial territories? These ablation studies,
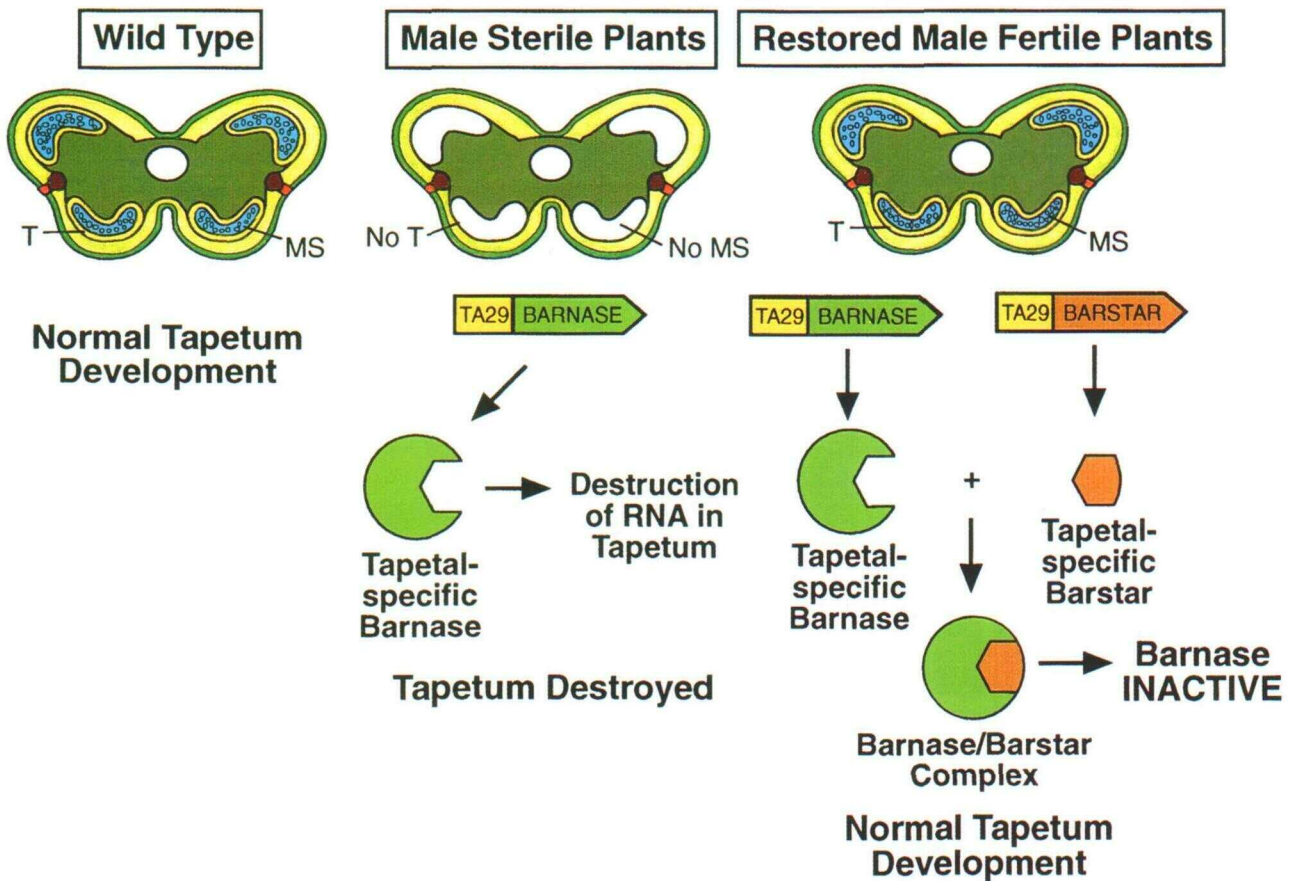
Figure 6. Genetic Engineering for Male Fertility Control.

A schematic representation of the male sterility and male fertility restoration experiments described by Mariani et al. (1990, 1992). MS, microspores; T, tapetum.

however, will require promoters that are active in specific cell types of the developing anther primordium, such as the archesporial and parietal cells (Figures 3 and 4). The availability of anther primordium–specific promoters should permit a thorough investigation of the lineage relationships, cell functions, and cell–cell interaction processes that occur during anther development (Figure 3).

MALE-STERILE MUTANTS CAN BE USED TO GENETICALLY DISSECT ANTHER DEVELOPMENT

Cell ablation studies can provide important conceptual information about the processes that control the differentiation of anther cell types. They cannot, however, identify genes that are responsible for directing cell specification events. To accomplish this will require, in part, a genetic approach analogous to that used in Drosophila and *C. elegans* to identify genes involved in both position-dependent and cell-autonomous

differentiation pathways (Horvitz and Sternberg, 1991; Krämer et al., 1991).

Male-sterile mutants have been identified in many plants, including Arabidopsis (Kaul, 1988). Although most male sterility genes and their wild-type alleles have not been cloned and studied (Aarts et al., 1993), phenotypic and histological analyses suggest that some male sterility mutations affect the differentiation and/or function of many anther cell types, including those in the stomium, tapetum, endothecium, and the archesporial and sporogenous layers of the anther primordium (Kaul, 1988; Chaudhury, 1993, this issue). Arabidopsis male-sterile mutants are easy to identify because they flower for a longer time period, grow taller, and remain in a green or nonsenesced state longer than their male-fertile, wild-type counterparts (Feldmann, 1991; Chaudhury et al., 1992; Forsthoefel et al., 1992; Aarts et al., 1993; Preuss et al, 1993). Because large numbers of mutagenized plants can be screened readily (Feldmann, 1991; Chaudhury et al., 1992; Forsthoefel et al., 1992), genetic studies in Arabidopsis have the potential to dissect gene pathways that control both the

histodifferentiation program (phase 1) and the dehiscence and cell degeneration program (phase 2) of anther development (Figure 1).

Recently, we screened a large number of Arabidopsis lines that were treated with either T-DNA (Feldmann, 1991; Forsthoefel et al., 1992) or ethyl methanesulfonate (EMS) (Koornneef et al., 1982) for male-sterile mutants. We were particularly interested in anther mutants that failed to dehisce and those that had defects in the anther differentiation pathway. Figure 7 shows two male-sterile mutants that were identified in our EMS screen. One mutant, designated as *undeveloped anther*, has stamens with normal filaments but anthers that lack pollen and have an undeveloped, club-shaped appearance (Figure 7A). The selective effect of this mutation on anther development suggests that distinct genes control anther and filament differentiation following stamen primordia specification. By contrast, the other mutant, designated as *late dehiscence*, has stamens that undergo a normal differentiation pathway and contain anthers that are morphologically similar to their wild-type counterparts (Figure 7B). *Late dehiscence* anthers, however, fail to dehisce on time (Figure 7B) and release their pollen grains after the stigma is no longer receptive to pollination. These male-sterile mutants and others with analogous phenotypes should permit the isolation of genes controlling anther developmental processes in the near future.

ANTHER GENE EXPRESSION STUDIES HAVE LED TO THE GENETIC ENGINEERING OF CROP PLANTS FOR MALE FERTILITY CONTROL

The anther plays a prominent role in crop production because it is responsible for carrying out male reproductive processes necessary for generating seeds that will produce the next plant

Figure 7. Arabidopsis Male-Sterile Mutants That Are Defective in Anther Development.

(A) *undeveloped anther.*
(B) *late dehiscence.*
A, anther; F, filament; OV, ovary; P, petal; S, sepal; SL, style; ST, stigma.

generation (Figure 1). One of the major factors contributing to increases in crop productivity over the past few years has been the breeding of hybrid varieties in crops such as corn and oilseed rape (Brewbaker, 1964; Feistritzer and Kelly, 1987). Crosses between inbred plant lines give rise to hybrid progeny that, at times, exhibit heterosis or hybrid vigor (Brewbaker, 1964). Although the molecular basis of hybrid vigor is not known, hybrids with a heterotic phenotype are more resistant to disease, less susceptible to environmental stress, and generate higher yields than their inbred parents (Brewbaker, 1964; Feistritzer and Kelly, 1987). Increased crop yields are particularly important to keep the world's food supply sufficient to meet the needs of an ever-expanding population (Klein, 1987).

The reproductive biology of higher plants makes it difficult to perform directed crosses between inbred lines on a scale large enough for hybrid seed production. Because stamens and carpels (Figures 1 and 2) are present within the same flower (e.g., tobacco, oilseed rape, Arabidopsis) or are contained within separate flowers on the same plant (e.g., corn), a high level of self-pollination can occur within individuals of each parental line. To overcome this problem and ensure that crosses occur *between* inbred lines, several breeding strategies have been used, including (1) manual and/or mechanical removal of stamens from the flowers of one parental line, (2) use of self-incompatibility alleles that prevent self-pollination, and (3) employment of dominant cytoplasmic or nuclear male sterility genes that disrupt pollen development (Feistritzer and Kelly, 1987). Each strategy has limitations that prevent its use as a general approach for hybrid seed production in all crop plants. For example, many crop plants do not have self-incompatibility alleles, manual removal of stamens is labor intensive and impractical for crops with small bisexual flowers (e.g., oilseed rape), and male sterility genes require a restorer system.

An important practical spin-off of anther cell differentiation and gene expression studies has been the development of a genetically engineered system for male fertility control that is applicable to a wide range of crop plants, including oilseed rape, tomato, lettuce, and corn (Mariani et al., 1990, 1992). This system, which is illustrated schematically in Figure 6, takes advantage of the tapetal-specific transcriptional activity of the tobacco TA29 gene (Table 2 and Figure 5; Koltunow et al., 1990) and an RNase/RNase-inhibitor defense system utilized by the bacteria *Bacillus amyloliquefaciens* (Hartley, 1989). The genetically engineered male fertility control system was developed as a collaborative effort between our laboratory and Plant Genetic Systems in Gent, Belgium (Mariani et al., 1990, 1992). In brief, barnase is an extracellular RNase that is produced by *B. amyloliquefaciens* to protect itself from microbial predators (Hartley, 1989). Barstar, on the other hand, is a barnase-specific RNase inhibitor that is used intracellularly by *B. amyloliquefaciens* to protect itself from the cytotoxic effects of barnase (Hartley, 1989). As illustrated in Figure 6, a cytotoxic chimeric gene consisting of the TA29 gene promoter and barnase gene coding sequences leads to the selective ablation of anther tapetal cells and the production of pollenless, male-
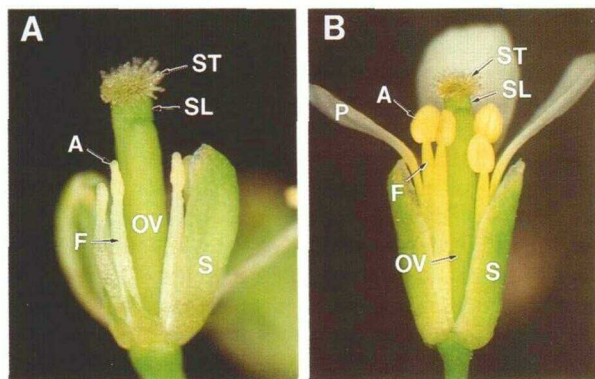
sterile plants (Mariani et al., 1990). By contrast, an anticyto-toxic chimeric gene containing the same TA29 gene promoter and the barstar gene coding region does not affect tapetal cell development and is a dominant suppressor of the TA29/barnase gene (Figure 6).

When male-sterile plants containing the TA29/barnase gene are crossed with male-fertile plants containing the TA29/barstar gene, progeny are produced that contain both genes, and these progeny are male fertile (Mariani et al., 1992). The presence of barstar within the tapetal cells of F_1 progeny restores male fertility by forming a complex with barnase that prevents barnase cytotoxic activity from destroying the tapetal cells within the anther (Figure 6). Because the TA29 gene promoter is regulated correctly in a wide range of dicot and monocot crop plants (Mariani et al., 1992), the barnase/barstar system illustrated in Figure 6 has the potential for being universally applicable for hybrid seed production in a wide variety of crops (Mariani et al., 1990, 1992). This system can also be used for cell ablation studies that investigate anther cell specification processes because it overcomes the need for cell-specific promoters to ablate a specific cell type. That is, a combination of chimeric barnase and barstar genes containing promoters with overlapping cell specificities can be used to selectively ablate single cell types (Table 2; T.P. Beals and R.B. Goldberg, unpublished results).

Recently, other strategies have been developed for the production of male-sterile plants. These include (1) the use of a chimeric tapetal-specific glucanase gene to prematurely disrupt microspore development (Worrall et al., 1992), (2) antisense inhibition of flavonoid biosynthesis within tapetal cells to disrupt pollen development (van der Meer et al., 1992), (3) tapetal-specific expression of a chimeric Agrobacterium rhizogenes rolB gene that increases auxin activity in the anther and causes developmental abnormalities (Spena et al., 1992), and (4) overexpression of a chimeric cauliflower mosaic virus 35S atp9 mitochondrial gene that disrupts anther development (Hernould et al., 1993). Clearly, knowledge gained by studying anther development at the molecular and genetic levels will reveal other approaches that can be used to genetically engineer novel varieties of hybrid crop plants.

CONCLUSION

The anther represents an outstanding system to study the differentiation of plant cell types. Excellent progress has been made in describing many of the gene expression events that occur during anther development as well as genes controlling stamen primordia specification. However, much remains to be learned about the mechanisms that are responsible for anther cell specification and those required for the activation of cell-specific gene sets within specialized anther cells and tissues. Molecular and genetic tools are now available that should reveal new insight into the processes controlling anther development in the near future.

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