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# Pistil Development

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## INTRODUCTION

As implied by their name, angiosperms (from the Greek, "seeds within a vessel") were originally defined by the nature of their female reproductive structures in which the seeds are enclosed within an ovary. The angiosperms are both the dominant group of land plants and by far the most important plants for human use. Although numerous specific adaptations were involved in the rise of the angiosperms to their current dominance, it is not surprising that several of these adaptations relate specifically to the female parts of the flower. The unique morphology of angiosperm female reproductive structures has facilitated the evolution of highly diverse and sometimes complex mechanisms to ensure appropriate pollination. The ovary of angiosperms has also evolved into a range of elaborate forms that facilitate efficient dispersal of seeds by wind, water, or animals. In this paper, we review the structure and development of these critical components of the angiosperm flower and describe current efforts to determine the mechanisms governing their formation.

The female parts of an angiosperm flower are collectively referred to as the gynoecium, which consists of one or more ovule-bearing unit structures, the carpels. Evolutionary modification and fusion of carpels makes the boundaries between carpels indistinct in many modern plants. The term pistil is also commonly used in describing the female parts of a flower. It refers to a single carpel when individual carpels of the gynoecium are separate (simple pistils) or to a single structure formed by fusion of multiple carpels (compound pistil). Thus, the gynoecium can consist of a number of free pistils or a single pistil.

Although the overall morphologies of angiosperm gynoecia are highly variable, almost without exception the gynoecium occupies the central position or innermost set of whorls, or spirals, of a flower (Endress, 1992). In addition, most pistils exhibit a common set of structural features. At anthesis, the pistil can be considered to consist of three parts: the ovary, at the base of the pistil, which contains the ovules and which differentiates into the fruit following fertilization; the style, an extension above the ovary, through which the pollen tubes grow toward the ovules; and the stigma, at the top of the style, where pollen grains adhere and germinate (Esau, 1965). Figure 1 illustrates the basic components of the pistils of Arabidopsis and tomato, two representative plants that are currently used as model systems.

Ovules, the precursors to seeds, reside within the ovary and are themselves complex structures. Angiosperm ovules commonly consist of a central nucellus that contains the embryo sac (megagametophyte); one or two integuments, which enclose the nucellus; and a supporting stalk, referred to as the funiculus. These structures are indicated on sections of Arabidopsis and tomato ovules in Figure 2. Following fertilization, the ovule develops into a seed, with the embryo and endosperm forming from the embryo sac and the integuments differentiating into a seed coat.

## EVENTS IN PISTIL DEVELOPMENT

### Formation of the Ovary

Pistil development is initiated by the formation of the carpel primordia at the center of the floral meristem. A characteristic feature of early pistil development is the occurrence of carpel fusion. Although fusion is an obvious requirement for formation of a compound pistil from a group of carpels, it also occurs in plants with simple pistils, in which fusion of the carpel margins is necessary to form the closed pistil.

Two types of fusion are recognized as participating in the formation of pistils (Verbeke, 1992). Carpels are said to be congenitally fused when a compound pistil is directly produced as a single structure on the floral meristem. That the compound pistil consists of multiple carpels is inferred from the number and locations of vascular traces and rows of ovules or from other morphological and anatomical features. This type of fusion is also referred to as "phylogenetic fusion" because progressive fusion of the carpels is believed to have occurred during evolution from progenitors with less fused, or unfused, carpels. The contrasting process of postgenital fusion (also called ontogenic fusion) occurs when initially separate carpels meet and fuse to form a single structure. Postgenital fusion is commonly followed by redifferentiation of the contacting epidermal cells into normal parenchyma cells indistinguishable from other cells of the ground tissue of the ovary (Verbeke, 1992). This redifferentiation has been especially well studied in *Catharanthus roseus* (Madagascar periwinkle), in which the

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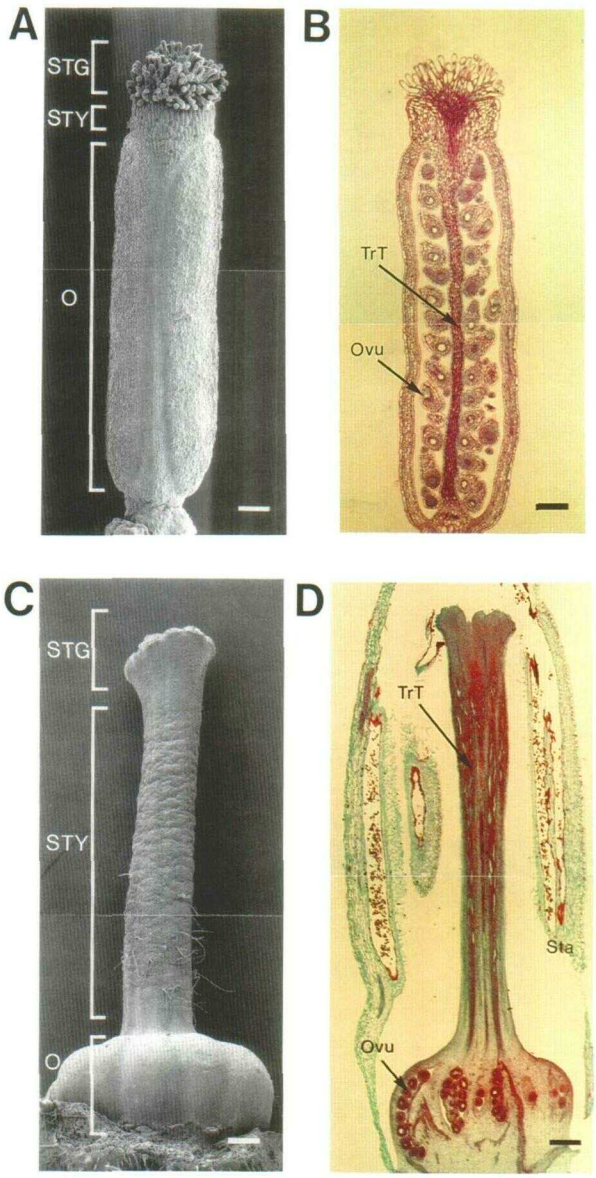


**Figure 1.** Pistils of Arabidopsis and Tomato at Anthesis.

**(A)** and **(C)** Scanning electron micrographs of Arabidopsis (Landsberg *erecta*) and tomato (VF36) pistils, respectively. STG, stigma; STY, style; O, ovary. Bars = 100 μm in **(A)** and 400 μm in **(C)**.

**(B)** and **(D)** Bright-field photomicrographs of longitudinal sections of Arabidopsis and tomato pistils, respectively. The Arabidopsis pistil was embedded in a glycol methacrylate resin, and the tomato pistil was embedded in paraffin. Ovu, ovule; TrT, transmitting tissue. Bars = 100 μm in **(B)** and 370 μm in **(D)**.

fusing surfaces are very large (Verbeke and Walker, 1985; see below).

The formation of an Arabidopsis pistil involves both congenital and postgenital fusion. The vascular arrangement in the Arabidopsis pistil and the presence of four rows of ovules indicate that it is made up of at least two fused carpels (Okada et al., 1989). However, it is initiated as a single hollow cylinder on the floral apex with no visible divisions between the congenitally fused carpels (Hill and Lord, 1989; Smyth et al., 1990). As the primordial cylinder extends, regions on opposite sides of the interior of the cylinder grow toward the center, where they meet and fuse postgenitally. This process forms a septum that divides the interior of the pistil into two locules (Hill and Lord, 1989; Smyth et al., 1990).

The pistil of the common cultivated tomato is formed from five carpels, which fuse postgenitally near their margins to form a complete pistil (Hayward, 1938; Chandra Sekhar and Sawhney, 1984). In addition, the central part of the floral meristem internal to the initial carpel primordia extends to form a column. This column undergoes postgenital fusion with each of the carpels to form the complete tomato ovary, which is divided into five locules.

The examples cited represent only a small portion of the fusion events that occur during formation of the ovaries of higher plants. In addition to more complex fusions between carpels, the gynoecium can also be fused to other floral organs such as stamens (as in orchids) or, in the case of plants with inferior ovaries, to a complex structure known as the floral tube (as in roses). Thus, in addition to being necessary for formation of pistils in nearly all angiosperms, fusion is also an important mechanism for generating diversity of floral form.

## Style Formation

Near the time of ovary closure, the tissues at the top of the ovary commonly begin to extend vertically to form one or more styles. This extension is achieved by a combination of cell division and cell elongation. Postgenital fusion may also occur at this point to assemble a single style from the apical regions of several carpels. The length and structure of the style(s) are highly variable. In some species, a single style is formed, whereas in others, each carpel has its own style, even when the carpels are otherwise fused into a single ovary. The purpose of stylar extension is to facilitate appropriate pollination, and the wide variety of stylar morphology reflects the variety of pollination strategies found among the angiosperms.

Arabidopsis and tomato pistils both have single styles that derive from the fusion of multiple carpels. However, the styles differ greatly in length. In Arabidopsis, the ovary is long and extended, and a short stylar region places the stigma in the proper location for pollination in this self-compatible species. By contrast, the style of cultivated tomato extends well above the rounded ovary, placing the stigma near the central region of the fused set of anthers in this species, which is also self-compatible. A simple illustration of the kind of modifications

necessitated by different pollination strategies is seen by comparing cultivated tomatoes to the closely related species *Lycopersicon peruvianum.* In this self-incompatible species, the style is much longer than that of cultivated tomato pistils, causing the stigma to be exerted from the fused cone of anthers. The exposed stigma is then able to receive pollen from adjacent plants. An even more extreme example of style elongation is found in maize, in which the styles (called "silks") from numerous female flowers on a single ear must extend as far as 20 cm to protrude from the sheathing leaves.

### Tissue Differentiation within the Pistil

Differentiation of tissues in the pistil is initiated during the morphological development of the ovary and style. Pistils share tissues with the vegetative plant body that are necessary for support, nutrition, and protection. These tissues include ground tissue, vascular tissue, and epidermis. Of more direct interest to those studying pistil development are tissues that are unique to the pistil, such as the stigmatic and transmitting tissues, as shown in Figure 1. These tissues are responsible for capture of pollen grains and facilitation of the passage of the pollen tube to the ovules, respectively.

The transmitting tissue most commonly differentiates from the inner epidermis or other layers near the inner surface of the carpels. Cells of the transmitting tissue are organized into vertical files. Each file consists of elongated cells that are connected end to end through plasmodesmata. These cells are highly secretory. The presence of their accumulated mucilaginous secretions, the stylar matrix, is another characteristic histological feature of the differentiated transmitting tissue. In some species, the style is hollow, and transmitting tissue on the inner surface of the stylar canal produces a layer of such secretions that line the canal. Pollen tubes then grow within these secretions rather than in the hollow core of the style. Other styles are solid, and the secreted material accumulates between the cell files. In these solid styles, the pollen tubes extend through the matrix between the cell files. In tomato, strands of transmitting tract are produced at each point of carpel fusion, resulting in a style that contains a variable number of transmitting tracts (Figure 1D). In Arabidopsis, the transmitting tract extends not only through the short stylar region but throughout the length of the septum (Figure 1B), facilitating pollen tube access to all of the ovules. Numerous variations in the location and extent of the transmitting tissue are found in angiosperms, but the cellular origin of the transmitting tissue and accumulation of a secreted matrix for pollen tube growth are conserved. Recent work by Sanders and Lord (1989; Lord and Sanders, 1992) indicates that the stylar matrix may be more than a simple pathway for pollen tubes and may actually provide the primary motive force for pollen tube extension.

The upper region of the style differentiates into a secretory structure, the stigma. This process includes cell proliferation, extension of the epidermal cells into papillae of varying lengths, and secretion of compounds involved in rejection of incompatible pollen and promotion of tube growth from compatible pollen. The secretions also have adhesive properties that facilitate the capture of pollen from the air or from animal pollinators. The stigmatic tissue is contiguous with the transmitting tissue, providing an uninterrupted pathway for pollen tube extension (Figure 1).

Although not usually considered a tissue, the placenta—the ovule-bearing region of the ovary—is worth mentioning because it is another differentiated structure that is specific to the pistil. The placenta develops on the interior of the ovary wall but is not as histologically well differentiated as the stigmatic and transmitting tissues. However, the fact that ovules arise only from specific regions of the ovary implies that some specialization of these regions has occurred. Additional structural and molecular studies on the placenta are warranted and should help to define its underlying nature.

### Ovule Formation

Ovules are initiated by periclinal divisions of cells in the L2 and/or L3 layers of the placental region(s) of the inner surface of the ovary (Bouman, 1984). The locations and arrangements of placental regions are highly variable among the angiosperms and are considered an important taxonomic feature. The ovule primordia extend into short fingerlike projections that initiate one or, more commonly, two integuments. The inner integument is initiated by periclinal divisions in an encircling band of cells partway up the primordium. The outer integument derives from epidermal and subepidermal layers located just below the inner integument, and it is often asymmetric from its inception, as a result of more frequent cell divisions on one side of the primordium (Bouman, 1984).

Initiation of integuments delineates the nucellus at the tip of the developing ovule from the funicular stalk below. As shown in Figure 2, the integuments elongate and cover the nucellus, usually leaving a small pore, the micropyle, through which the pollen tube can enter. Within the nucellus, a single cell differentiates into a megasporocyte, which undergoes meiosis to produce four haploid megaspores. The megagametophyte (embryo sac) then develops from one or more of the megaspores (see Reiser and Fischer, 1993, this issue).

Further cellular differentiation can occur in ovules. In some species, the nucellus proliferates and can even form complex differentiated structures (Bouman, 1984). The integuments can also differentiate further. For example, in some species, including both tomato and Arabidopsis, one or more layers of the integument closest to the embryo sac differentiate into a densely cytoplasmic tissue called the endothelium or "integumentary tapetum" (Cooper, 1931; Kapil and Tiwari, 1978; Bowman et al., 1991a; Robinson-Beers et al., 1992). This structure may be involved in providing materials for embryo sac or embryo development (Kapil and Tiwari, 1978). Comprehensive descriptions of development of ovules in tomato and

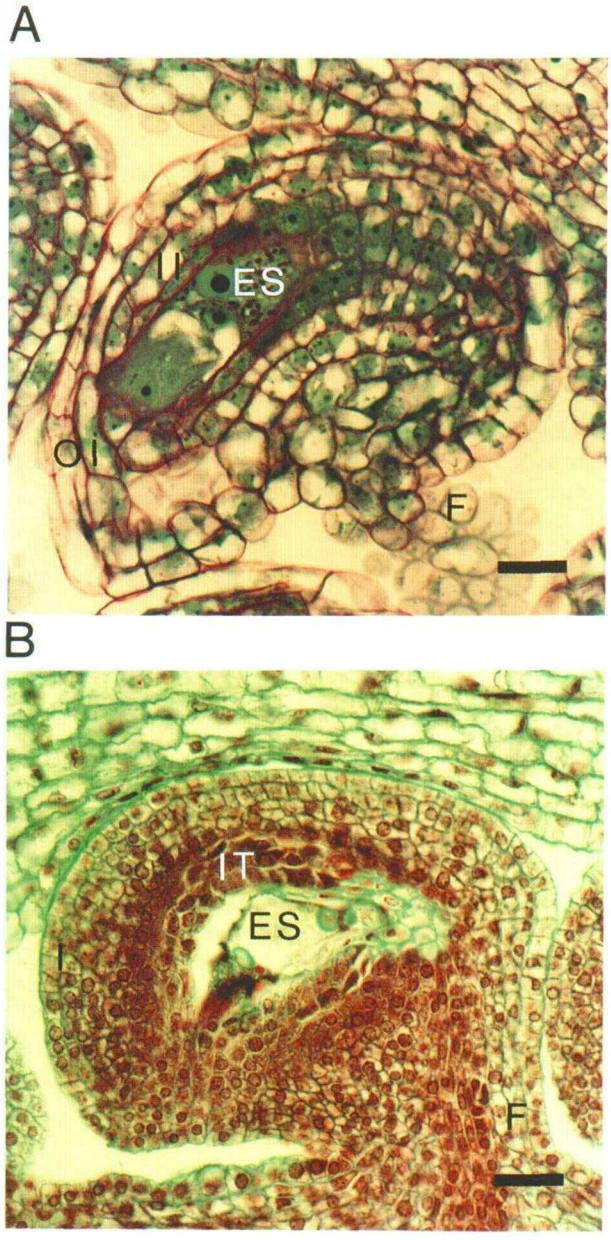


**Figure 2.** Arabidopsis and Tomato Ovules at Anthesis.

**(A)** Bight-field photomicrograph of a near-median longitudinal section of a plastic-embedded Arabidopsis ovule. The micropyle is not visible in this section, and the nucellus, which earlier surrounded the embryo sac, has nearly degenerated. Bar = 100 μm.

**(B)** Bright-field photomicrograph of a median longitudinal section of a paraffin-embedded tomato ovule. The micropyle is not apparent because the single integument of the tomato ovule is tightly appressed around this opening. The more intensely staining inner layers of the integument comprise the integumentary tapetum. The nucellus has degenerated. Bar = 260 μm.

ES, embryo sac; F, funiculus; II, inner integument; OI, outer integument; I, integument; IT, integumentary tapetum.

Arabidopsis have been published elsewhere (Cooper, 1931; Robinson-Beers et al., 1992).

## EVOLUTIONARY ORIGINS OF COMPONENT PARTS

Some insight into pistil development is provided by examination of the evolutionary origin of its component parts. The classic view that carpels are highly modified leaves or leaflike structures (Gifford and Foster, 1989) remains consistent with recent evidence. One type of carpel, the "conduplicate carpel," has the appearance of a longitudinally folded leaflike structure with appressed margins. Conduplicate carpels are found in some early angiosperms (Drinnan et al., 1991) and in the extant genera *Degeneria* and *Drimys*, members of the family Winteraceae that also exhibit many other primitive characters (Bailey and Swamy, 1951). Conduplicate carpels are considered primitive and may be ancestral to the more specialized carpels of at least some modern angiosperm groups.

The appressed margins of conduplicate carpels are covered with glandular trichomes (hairs). Some of the hairs project out from the carpel margin and form a "stigmatic crest," to which pollen grains adhere and germinate (Bailey and Swamy, 1951). Where contact is made between the margins, the trichomes intermingle and their secretions merge, holding the carpels closed. Pollen tubes grow on the surfaces of these interlocking hairs (which constitute a loose transmitting tissue) toward the ovules, which are attached near the margins (Bailey and Swamy, 1951). If these carpels are representative of the ancestral form, then it would appear that the stigma and transmitting tissue have a common phylogenetic origin from glandular epidermal hairs. It is notable that other models for carpel evolution are also consistent with this origin for the transmitting tissue and stigma (Taylor, 1991). The stigmatic papillae still resemble such hairs, but the cells of the transmitting tissue have apparently become highly modified as pistil structure has evolved, maintaining only their secretory properties. Thus, the transmitting tissue and stigma appear to represent specialized tissues derived from the surface of the carpels.

In contrast to carpels, which are a defining characteristic of angiosperms, ovules are also found in gymnosperms such as conifers, cycads, and the Gnetales (Gifford and Foster, 1989). Gymnosperm ovules precede angiosperm ovules in the fossil record (Stewart, 1983). The nucellus and inner integument of the angiosperm ovule appear to be homologous to the nucellus and single integument, respectively, of most gymnosperm ovules. The origin of the outer integument is, however, less clear. Cladistic analyses indicate that two groups of gymnosperms, the extinct Bennettitales and the extant Gnetales, are the closest gymnospermous relatives of angiosperms (Crane, 1985; Doyle and Donoghue, 1986). Some members of the latter group have ovules with outer integument–like structures (Gifford and Foster, 1989), which may be homologous to the outer integument in angiosperm ovules. Alternatively,

the outer integument has been proposed to derive from the leafletlike cupule that often surrounded a group of several ovules in some fossil gymnosperms (Stebbins, 1974). Ongoing paleobotanical and phylogenetic investigations should help clarify the origins of both carpels and the components of angiosperm ovules.

## CURRENT STUDIES ON PISTIL DEVELOPMENT

### Molecular Signals in Postgenital Carpel Fusion

As noted above, *C. roseus* has proven to be a valuable model system for study of postgenital carpel fusion and of postgenital fusion in general. In this species, ~400 cells come into contact and undergo rapid redifferentiation when its two carpels meet (Verbeke and Walker, 1985).

The participation of intercellular communication in this process was first demonstrated by the observation that simple removal of one carpel or the placement of an impermeable barrier between the two carpels blocks the redifferentiation program (Walker, 1978; Verbeke and Walker, 1986). By contrast, the insertion of a permeable barrier between the two carpels allows normal redifferentiation (Verbeke and Walker, 1986). A porous barrier can even absorb the apparent communicating substance through contact with one carpel. When this barrier is then removed and brought into contact with the other carpel, normal redifferentiation ensues (Siegel and Verbeke, 1989). Subsequent experiments showed that each of the two carpels produces a unique signal that can only affect the other carpel (Verbeke, 1992). Thus, fusion of the two carpels of *C. roseus* requires two different factors. The accessibility and ease of manipulation of *C. roseus* carpels provide a unique opportunity for direct identification of morphogenic factors governing fusion and redifferentiation in the gynoecium. Characterization of these factors would be an important step toward a more general understanding of intercellular communication in higher plants.

### Examination of Pistil Gene Expression

The novel tissues in pistils are likely to be associated with the expression of genes unique to this organ system. Determination of the nature of the products of these genes and the mechanisms controlling their expression could greatly increase our understanding of the formation and function of pistil tissues. In early studies of genes expressed in the different organs of plants, Kamalay and Goldberg (1980) showed that the gynoecium contains up to 10,000 different mRNAs that are not present in other plant organs. The genes corresponding to these mRNAs would include regulatory genes responsible for controlling pistil development as well as "downstream" genes encoding proteins associated with differentiated cell types in the pistil.

In more recent work, researchers have used a variety of methods (reviewed in Gasser, 1991) to identify and isolate clones of genes that are expressed predominantly in pistils. Because these methods rely largely on detection of differences in specific mRNA levels between pistils and vegetative parts of plants they resulted in the isolation of genes preferentially expressed at relatively high levels. The majority of such genes are downstream genes that are not directly involved in control of development. However, as outlined below, these genes are proving to be useful tools for dissecting developmental processes and characterizing tissue differentiation in pistils.

Genes governing self-incompatibility and their homologs are one class of genes with pistil-predominant expression patterns that have been studied intensively. The properties of these genes are described in more detail elsewhere in this issue (see Nasrallah and Nasrallah, 1993, this issue; Newbigin et al., 1993, this issue) and will not be covered here.

Only a small number of additional genes expressed predominantly in pistils have been identified. The nature of the products of some of these genes has been determined immunologically or by comparison to previously sequenced genes or proteins. One such gene, *AGL1*, which appears to be expressed exclusively in the gynoecium of Arabidopsis, was isolated on the basis of its homology to known floral homeotic organ identity genes (Ma et al., 1991). Although little is known about this gene, the fact that it encodes a protein homologous to transcription factors and the specificity of its expression in the gynoecium make it a good candidate for a regulatory gene involved in pistil tissue differentiation.

Other genes isolated to date appear to encode downstream genes characteristic of differentiated pistil tissues. These include β-glucanase (Ori et al., 1990), pectate lyase (Budelier et al., 1990; McCormick, 1991), an extensin-like protein (Chen et al., 1992), a chitinase (Lotan et al., 1989, K. Harikrishna and C. S. Gasser, unpublished data), a proline-rich protein (Cheung et al., 1993), and a proteinase inhibitor (Atkinson et al., 1993). It should be noted that with the exception of the proteinase inhibitor gene, which is expressed in the stigmatic region, all of these genes are expressed primarily in the transmitting tissue. In addition, all of these proteins have been shown to be extracellular or to include putative signal peptides that could direct them to the outside of the expressing cells. Thus, the majority of the currently characterized proteins may be deposited in the extracellular secretions of the transmitting tissue or the stigma. The stigmatic secretions and stylar matrix are unique to the gynoecium, so it is not surprising that many pistil-predominant genes would be involved in the production of these materials.

The currently identified downstream pistil-predominant genes encode proteins that fall into two overlapping categories: those homologous to known pathogenesis-related proteins and those homologous to enzymes involved in cleavage of glycosidic bonds. Homologs of pistil-predominant chitinase, β-glucanase, proteinase inhibitor, proline-rich protein, and extensin genes have all been found to be induced during responses to pathogen attack or wounding and are hypothesized

to have defensive roles (Linthorst, 1991). The style provides an open, nutrient-rich pathway into the plant, and one possible function of stylar expression of these genes could be to protect the plant against infection by fungi or bacteria (Gasser, 1991). Three of the identified genes (pectate lyase, chitinase, and β-glucanase) encode homologs of proteins associated with cleavage of glycosidic linkages. Polysaccharide substrates for pectate lyase and β-glucanase are known to be present in the transmitting tissue. These enzymes have been proposed to facilitate pollen tube growth by digesting these components (Ori et al., 1990; McCormick, 1991). Substrates for chitinases have not been identified in higher plants. However, the recent observation that a chitinase can have a profound effect on somatic embryogenesis in carrot (De Jong et al., 1992) suggests that such substrates may exist or that plant chitinases may have activity against other plant compounds. Clearly, further work will be necessary to fully understand the roles of pistil-predominant genes.

Another distinctive characteristic of most currently identified pistil-predominant genes is that their expression is confined to specific subsets of cells within the pistil. Each of these genes therefore represents a specific biochemical marker for the tissue in which it is found. In some cases, the expression patterns of the genes define novel compartments within a previously identified pistil tissue. For example, the tomato gene designated *9612*, which encodes a protein with homology to pectate lyases (Budelier et al., 1990; McCormick, 1991), is expressed only in the upper two-thirds of the outer layers of the transmitting tissue of the style (Budelier et al., 1990). This region is not histologically different from the remainder of the transmitting tissue, but it is shown to be a biochemically distinct compartment by virtue of *9612* gene expression. Formulation of a complete model of control of gene expression in pistils will require that all such compartments be defined.

Promoter regions from genes expressed in specific tissues of pistils may also prove useful in characterizing the control of pistil differentiation. For example, Budelier et al. (1990) showed that the promoter region of the tomato *9612* gene directs expression of an attached β-glucuronidase coding region in the upper region of the transmitting tissue of the style in transgenic tomato plants. Thus, this construction could be used to begin characterizing the promoter sequences necessary for production of this expression pattern. Surprisingly, the same construct showed a completely different pattern of expression in transgenic tobacco, indicating significant differences between control of gene expression in these two members of the same family.

A novel use for promoter regions that direct tissue-specific expression of chimeric genes has recently been described. Using the promoter from a *Brassica* self-incompatibility gene, Thorsness et al. (1991) targeted expression of the diphtheria toxin to the stigmatic and transmitting tissue regions of transgenic tobacco. Any cells expressing this chimeric gene would be killed by the action of the toxin. In addition to lacking stigmas, the pistils of the transgenic plants showed varying defects in other aspects of pistil morphology. All expressing plants showed some shortening of the style. In the most extreme cases, the style was absent and fusion of the carpels was disrupted. These observations provide evidence of a relationship between the stigmatic and transmitting tissues and the process of organ fusion. The isolation of additional promoter regions with different tissue specificities within the pistil will facilitate further dissection of pistil development by this potentially powerful method.

## Genetic Studies on Pistil Development

Using genetic approaches, several laboratories have recently made significant progress in identifying the determinants of floral organ identity. These studies on Antirrhinum and Arabidopsis have shown that the developmental fates of floral organ primordia are determined by a small set of conserved genes encoding putative transcription factors (see Coen and Carpenter, 1993, this issue; Okamuro et al., 1993, this issue; van der Krol and Chua, 1993, this issue). Models of floral organ identity resulting from these studies indicate that once the floral program has been initiated, a single gene, referred to as *AGAMOUS* (*AG*) in Arabidopsis and *PLENA* in Antirrhinum, is a primary determinant of carpel identity (Coen and Meyerowitz, 1991; see Coen and Carpenter, 1993, this issue; Okamuro et al., 1993, this issue). This single factor cannot, however, direct the differentiation of tissues and structures within the gynoecium. Indeed, stigmatic tissues and ovules can be seen to form in *ag* mutants of Arabidopsis if this mutation is present in combination with mutations in other classes of floral organ identity genes (Bowman et al., 1991b). Thus, additional, as-yet-undefined genes must govern the later stages of pistil development.

Several laboratories have initiated research to isolate and characterize mutants that may illuminate factors governing pistil development. Okada et al. (1989) have isolated mutants that affect the gross morphology of pistils in Arabidopsis. In several of these mutants, septal fusion is aberrant, and the pistils have a single locule. One such mutant, *fl-89*, exhibits the additional feature of having a terminal bifurcation of the pistil, resulting in the formation of two stigmas. Further analysis of these and other pistil morphology mutants will aid in understanding the determinants of pistil form and the relationship between tissue differentiation and morphological development.

A fascinating Arabidopsis mutant that may help illuminate the processes of postgenital fusion and stigma differentiation has recently been described by Lolle et al. (1992). In this mutant, *fiddlehead* (*fdh*), leaves and all of the floral organs engage in postgenital fusion with adjacent structures. Fusion of adjacent floral organs distorts the inflorescences into the characteristic curled shape after which the mutant is named. As noted above, postgenital fusion is usually observed in Arabidopsis only in the developing septum of the pistil and in tissue that will form the stigma. The fusion of vegetative and floral organs in *fdh* mutants appears to occur by the same mechanism that is responsible for normal fusion of the septum of

the pistil. Further characterization of the *fdh* mutant has shown that in addition to its fusion competence the epidermis of the mutant supports both pollen hydration and pollen tube germination (Lolle and Cheung, 1993). In wild-type plants, this capacity is confined to the stigma.

Thus, the entire epidermis of *fdh* plants exhibits two properties that are normally confined to epidermal regions of the gynoecium. More specifically, *fdh* epidermis has properties of the stigma and of the region of the septum that will give rise to the transmitting tissue, which is likely to be related to the stigma (see above). On the basis of these observations, it has been hypothesized that the wild-type *FDH* gene encodes a factor that normally restricts development of the unusual epidermal properties to specific regions of the gynoecium (Lolle and Cheung, 1993). The disruption of this gene leads to expression of the program for organ fusion and pollen activation in all of the epidermis. Thus, the *FDH* gene appears to directly control some aspects of tissue differentiation in the pistil of Arabidopsis. This mutant further supports the relationship between the stigma/transmitting tract and organ fusion implied by the cell ablation experiments described above. Additional analysis of *fdh* mutants will help to illuminate the processes of postgenital fusion and pollen tube germination and may lead to important insights into control of tissue differentiation in the gynoecium.

In our laboratory, we have taken a direct approach to the identification of mutations that affect the differentiation of pistil structures necessary for female fertility: the stigma, transmitting tissue, and ovules. Mutants altered in formation or differentiation of these structures are isolated by initially screening a mutagenized population of Arabidopsis for infertile mutants (i.e., those plants with fruits that fail to expand). This is followed by reciprocal crosses to differentiate between male- and female-sterile mutants. The first two female-sterile mutants we have characterized, *short integuments* (*sin1*) and *bell* (*bel1*), both affect ovule development (Robinson-Beers et al., 1992). Homozygous *sin1* mutants have short integuments that fail to cover the nucellus at anthesis. It can be seen that this phenotype results from a failure of the integumentary cells to elongate normally because the number of cells in the integuments of *sin1* mutants is similar to the number in wild-type integuments (Robinson-Beers et al., 1992). This indicates that the processes of cell division and cell elongation, both of which are necessary for normal morphological development of ovules, are regulated independently in this structure. Within the nucellus of *sin1* mutants, a megasporocyte differentiates, but meiosis does not occur. Thus, *sin1* mutants are affected in both integument development and megasporogenesis, indicating that these two processes are interconnected or interdependent.

*bel1* mutants appear to initiate only an outer integument (Robinson-Beers et al., 1992). Further development of the single integument is aberrant, resulting in the formation of a thick collar of tissue that grows up around the nucellus. The collar of tissue can also produce small outgrowths that superficially resemble organ primordia. In *bel1* mutants, a megasporocyte develops and appears to undergo meiosis, but subsequent development of the gametophyte is aberrant. Thus, *bel1* mutants further indicate connections between integument and embryo sac development.

In collaboration with D. Preuss (Stanford University), we have now isolated more than a dozen additional female-sterile mutants with a variety of defects in pistil development, the majority of which affect ovule development (C. S. Gasser, K. Robinson-Beers, and D. Preuss, unpublished data). Other laboratories have recently reported the identification of T-DNA insertion mutations affecting pistil (Sessions et al., 1993) and ovule (Haughn et al., 1993) morphology. Because facile methods are available for isolating genes mutated by T-DNA insertion (Yanofsky et al., 1990), it is likely that sequence information for some genes governing pistil development will soon be available. The combination of a broad range of mutants in pistil formation and the isolation of clones of a subset of genes governing this process provide the necessary tools for genetic and molecular dissection of pistil development.

## PERSPECTIVE

The prospects for new insight into understanding pistil development seem promising. Ongoing paleobotanical investigations are providing new information on the nature of primitive carpels and the evolution of the gynoecium. Biochemical, histological, and molecular analyses have allowed further refinement of the description of differentiated compartments within pistils. Mutants in genes responsible for controlling pistil differentiation have now been identified that will allow direct examination of the factors governing this process. Application of these new tools should provide a dramatic increase in our understanding of this critical organ system in the near future.

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