The *mac1* Mutation Alters the Developmental Fate of the Hypodermal Cells and Their Cellular Progeny in the Maize Anther

William F. Sheridan,* Elena A. Golubeva,† Ludmila I. Abrhamova† and Inna N. Golubovskaya†

**Department of Biology, University of North Dakota, Grand Forks, North Dakota 58202-9019 and †N. I. Vavilov Research Institute of Plant Industry, St. Petersburg 190000, Russia*

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ABSTRACT

In angiosperm ovules and anthers, the hypodermal cell layer provides the progenitors of meiocytes. We have previously reported that the *multiple archesporial cells1* (*mac1*) mutation identifies a gene that plays an important role in the switch of the hypodermal cells from the vegetative pathway to the meiotic (sporogenous) pathway in maize ovules. Here we report that the *mac1* mutation alters the developmental fate of the hypodermal cells of the maize anther. In a normal anther a hypodermal cell divides periclinally with the inner cell giving rise to the sporogenous archesporial cells while the outer cell, together with adjacent cells, forms the primary parietal layer. The cells of the parietal layer then undergo two cycles of periclinal divisions to give rise to three wall layers. In *mac1* anthers the primary parietal layer usually fails to divide periclinally so that the three wall layers do not form, while the archesporial cells divide excessively and most fail to form microsporocytes. The centrally located mutant microsporocytes are abnormal in appearance and in callose distribution and they fail to proceed through meiosis. These failures in development and function appear to reflect the failure of *mac1* gene function in the hypodermal cells and their cellular progeny.

THE developmental pathway leading to sexual reproduction in flowering plants is regulated by genes that are expressed in the anthers and ovules. A large body of literature has accumulated concerning the morphogenesis and variety of forms of angiosperm anthers and ovules (see Maheshwari 1950; Bhandari 1984; Bouman 1984; and Greyson 1994 for reviews). The evolution of the anther and its possible homology with the ovule have received attention (Eames 1961; Favre-Duchartre 1984; D'Arcy and Keating 1996). The use of mutations to identify the genes regulating anther development has identified both nuclear (Cheng *et al.* 1979; Albertsen and Phillips 1981; Kaul 1988) and cytoplasmic factors (Warmke and Lee 1977) that affect microspore development. Attention has recently been focused on the role of genes in the morphogenesis of ovules (Grossniklaus and Schneitz 1998; Schneitz *et al.* 1998) where considerable progress has been made in identifying genes that regulate development of ovule primordia and integument formation.

A very early event in anther and ovule development is the switch in developmental fate from the mitotic sequence whereby somatic cells embark upon a meiotic destiny (Riley and Flavell 1977). Although there is evidence that meiocytes have an altered premeiotic cell cycle (Bennett *et al.* 1973), little is known about the genes that may regulate this switch in cell destiny (see Sheridan *et al.* 1996 for review). In their review of anther development Goldberg *et al.* (1993) noted that "no information exists about whether the differentiation of anther cells or tissues is induced (or inhibited) by signals originating from contiguous regions of a territory, or is controlled by sequestering morphogenetic factors during divisions of the L1, L2, or L3 layers, or both." A search for the genes that control these or other possible processes is warranted. A major function of these genes must be to control the separate destinies of those cells that will enlarge to form the archesporial cells, the precursors of the meiocytes, while at the same time maintaining the rest of the cells of the reproductive organs as somatic cells. In both the anthers and ovules of angiosperms, the archesporial cells composing the archesporium originate from the layer of cells, termed the hypodermal cells, located immediately beneath the epidermis of the anther and ovule primordia (Favre-Duchartre 1984).

Archesporial cells originate from hypodermal cells by different routes in maize ovules and anthers: In addition to the occurrence of multiple archesporial cells in each anther lobe and the presence of but a single archesporial cell in each ovule, two additional features are especially prominent in distinguishing the developmental patterns of these two organs of sexual reproduction in maize. In anthers the archesporial cells undergo a series of mitotic divisions to produce the microsporocytes

Corresponding author: William F. Sheridan, Department of Biology, University of North Dakota, Grand Forks, ND 58202-9019.
E-mail: wsherida@plains.nodak.edu

whereas, in each ovule, the single archesporial cell simply enlarges and differentiates directly into the megasporocyte. In all angiosperms examined to date, the archesporial cell of an ovule originates directly from the hypodermal layer (tenuinucellar and pseudocrassinucellar ovules) or is the inner product of the periclinal division of a hypodermal cell (crassinucellar), and there are different patterns of wall formation in anthers (Davis 1966). Maize displays the pattern that is shared by most monocots and conforms to the Monocotyledonous type of anther wall formation (Davis 1966; Favre-Duchartre 1984). In maize and other species of this type (Raghavan 1988), the hypodermal cells composing a single row in each lobe of the anther undergo a periclinal division to produce an internal sporogenous layer, the archesporial cells, and an external layer, the primary parietal cells (Kiesselbach 1949). In each anther lobe the primary parietal cell and adjacent cells (Raghavan 1988) form the primary parietal cell layer and the cells of this layer undergo a periclinal division resulting in the formation of the two secondary parietal layers. The outermost of these two layers differentiates into the endothecium, while the inner layer again divides periclinally to form the middle layer externally and the tapetum internally (Kiesselbach 1949; Davis 1966). The archesporial cells divide one or more times and their cellular progeny differentiate into the microsporocytes (Kiesselbach 1949). See Raghavan (1988) for a detailed analysis of this developmental sequence in rice.

It is evident that the hypodermal cells of the maize ovule and anther differ in their behavior and in their immediate cellular fates. Whereas a single hypodermal cell of the ovule develops directly into the archesporial cell and it develops directly into the megasporocyte, a row of hypodermal cells in each lobe of an anther first divide mitotically to produce the progenitors of the anther wall cells and the archesporial cells, and then the latter divide mitotically prior to their differentiation into the microsporocytes. These differences in cell development patterns between the female and male reproductive cells are important for understanding the differing phenotypes of the *mac1* mutation in maize ovules and anthers.

Meiosis is normal in maize *mac1* ovules but fails in maize *mac1* anthers: Earlier we reported that in *mac1* mutant ovules several hypodermal cells (rather than the usual single cell) develop into archesporial cells, yet the resulting megasporocytes undergo a normal meiosis. In addition we noted that the sporophytic expression of this mutation resulted in ears on mutant plants showing partial sterility, apparently resulting from abnormalities in megaspore differentiation and embryo sac formation (Sheridan *et al.* 1996). Our initial studies on *mac1* microsporocytes suggested a defect in meiosis because these male meiocytes were unable to progress through prophase I of meiosis (Golubovskaya *et al.* 1992). But our subsequent observation that multiple megasporocytes proceed through a normal meiosis in mutant ovules (Sheridan *et al.* 1996) suggested that the failure of meiosis in mutant anthers might be a secondary effect resulting from some abnormality in anther development. Here we present the results of our analysis comparing the development of normal anthers with the cellular patterns observed during development of mutant anthers. We now report that the locules of mutant anthers contain a core of irregularly shaped microsporocytes with an abnormal distribution of callose, and that these anthers lack a clearly defined endothecium, middle layer, and tapetum. We attribute the inability of the microsporocytes to progress through meiosis to the lack of normal *mac1* gene expression in the archesporial cells and in their hypodermal cell progenitors. We interpret the differences in the effects of the *mac1* mutation on the function of the female and male meiocytes in terms of the differences in the patterns of behavior of the hypodermal cells and their cellular progeny in ovules and anthers.

MATERIALS AND METHODS

For genetic and developmental analyses, kernels were planted from self-pollinated ears that had been identified by previous progeny testing to have been produced by heterozygous plants. The resulting families of plants segregated, as expected, for fertile plants and male-sterile plants. Genetic stocks carrying the *mac1* mutation were used as kernel sources. In addition, genetic stocks carrying mutant alleles of the *ameiotic1* (*am1*) locus (Golubovskaya *et al.* 1997) and the *absence of first division1* (*afd1*) locus (Golubovskaya *et al.* 1992) were planted from progeny-tested ears to produce segregating families.

Cytological studies: For the cytological examination of microsporocytes, immature tassels were taken from fertile and male-sterile sibling plants and fixed in Farmer's (three parts ethanol:one part glacial acetic acid) fixative. After 48 hr the fixed samples were stored in 70% ethanol at 4° before analysis.

Anther developmental anatomy: For anatomical study, *mac1* mutant anthers and normal anthers were removed from immature tassels at different stages of development. Fixations were begun with immature plants containing anthers 0.5–0.8 mm in length and continued weekly for 6 wk. Fixations were performed on anthers from nine plants that were progeny of a self-pollinated ear of a known +/*mac1* heterozygote. Care was taken to remove only a portion of the tassels from each plant so sufficient tassel remained at flowering that it could be scored for male fertility (+/+ or +/*mac1*) or male sterility (*mac1*/*mac1*). Following the scoring for fertility and confirmation of the genotypes of the blindly fixed samples, fixed anthers from three normal and three mutant plants were embedded and sectioned. For each stage of development 8–12 anthers were sectioned and analyzed for both normal and mutant samples. The anthers were fixed for 24 hr at room temperature in FAA fixative (40% formaldehyde:glacial acetic acid:50% ethanol in a 5:5:90 volume ratio). The samples were embedded in plastic, sectioned, and stained as previously described (Huang and Sheridan 1994). Fixation of *am1* and *afd1* mutant and normal anthers was performed with anthers containing mature microsporocytes and processed in the same way as described above.

RESULTS

The formation of anther wall layers and the archesporium in normal anthers: Sectioning of normal anthers, from early in their development onward, revealed the expected pattern of wall layer formation. In the youngest anthers examined, the hypodermal cells located just beneath the epidermis had already divided periclinally to produce the archesporial cells to the interior flanked by the cells of the primary parietal layer to the exterior (Figure 1A). In this figure there is a pair of secondary parietal cells lying between the epidermis and the archesporial cells, having been recently formed by the periclinal division of a primary parietal cell. At a slightly later stage, following the completion of the periclinal division of the primary parietal cells, the archesporial cells can be seen to be partially encircled by the two readily distinguishable secondary parietal cell wall layers (Figure 1B). Although the outer of these two layers is destined only to undergo anticlinal divisions as this layer develops into the endothecium wall layer, the inner layer of secondary parietal cells will divide periclinally to produce the middle wall layer adjacent to the endothecium and the tapetal layer adjacent to the archesporial cells. One such division has already occurred and is evident in Figure 1B. As the anther continues to develop, the cells of the endothecium divide anticlinally and elongate in a plane parallel to the anther surface, while the inner secondary parietal layer continues to divide periclinally (Figure 1C).

At the later stage shown in Figure 1D many of the cells of the inner secondary parietal layer have divided periclinally and one parietal cell can be seen at mitotic metaphase of a periclinal division. The archesporial cells have undergone mitotic divisions resulting in four large cellular progeny that are symmetrically arranged in the central region of the locule. The archesporial cells have not yet differentiated into meiocytes, the microsporocytes, and some are destined to undergo further mitotic division. The products of this division are evident in Figure 1E where the five centrally located microsporocytes are in the leptotene stage of meiotic prophase I. At this stage of anther development the three wall layers lying between the epidermis and the sporogenous tissue, the microsporocytes, are all formed so as to completely encircle the microsporocytes. The endothecium layer and the layer internal to it, the middle layer, consist of flattened cells elongated in the periclinal plane, while the innermost of the three layers, the tapetum, consists of cuboidal, palisade-shaped cells with a denser cytoplasm. The innermost surfaces of the microsporocytes are covered with dark-staining callose (Figure 1E). Subsequently, at a later stage, the tapetal layer becomes more dense in its cytoplasm and nuclear staining. The archesporial cells have advanced further into prophase I of meiosis, and they have become increasingly separated as callose has accumulated in the centermost region of the locule. Especially noteworthy is the greater density of the cells of the tapetum as compared to the cells comprising the endothecium and middle layers of the anther wall (Figure 1F).

The anther wall and the archesporium fail to develop normally in *mac1* anthers: Initially, in the very young anther the pattern of development in *mac1* anthers is like that in normal anthers. The hypodermal layer divides periclinally to produce a centrally located archesporium surrounded by the single-cell layer of primary parietal cells underlying the epidermis (Figure 2A). Soon, however, the pattern deviates from normal as a result of both a deficiency and an excess of cell divisions. The primary parietal layer increases in size as a result of anticlinal cell divisions but there is no evidence of periclinal cell divisions. Yet, the archesporial cells have undergone several cell divisions resulting in more numerous and smaller cells than those that normally comprise the archesporium (Figure 2B). At a later stage, the primary parietal layer continues to expand beneath the epidermis by undergoing anticlinal divisions so as to keep pace with the enlargement of the anther, but all of the cells interior to the single-celled primary parietal layer appear to be products of division of archesporial cells. This interpretation is supported by the apparent lack of periclinal divisions within the primary parietal layer as well as the wall alignments of the cells lying internal to that layer (Figure 2C). As anther development advances, the single-celled primary parietal layer encircles about five-sixths of the circumference of the mass of archesporial-derived cells occupying the central region of the locule, but the parietal cell layer does not appear to have undergone any periclinal divisions to contribute cellular progeny to the central region that it encircles. Moreover, although the cells occupying the central region vary greatly in size, their similarity in nuclear and cytoplasmic morphology, and particularly the planar orientation of their cell walls, indicate that all of these cells are the progeny of archesporial cell divisions (Figure 2D).

As the mutant anther further develops, the primary parietal layer proceeds to flatten and elongate in the plane parallel to the epidermis but shows no evidence of undergoing periclinal divisions. The outermost archesporial-derived cells proceed with both anticlinal and periclinal cell divisions resulting in some regions having a layered appearance. The innermost archesporial cells are enlarged and engaged in mitotic cell divisions (Figure 2E). At a more advanced stage, the most centrally located archesporial cells have differentiated into microsporocytes and the latter have entered into prophase I of meiosis. However, the microsporocytes are abnormal in their shape and in their wide range of sizes, as well as having an abnormal distribution of callose between and around them. The rest of the archesporial-derived cells vary substantially in size and shape. The primary parietal layer encircles the mass of arche-

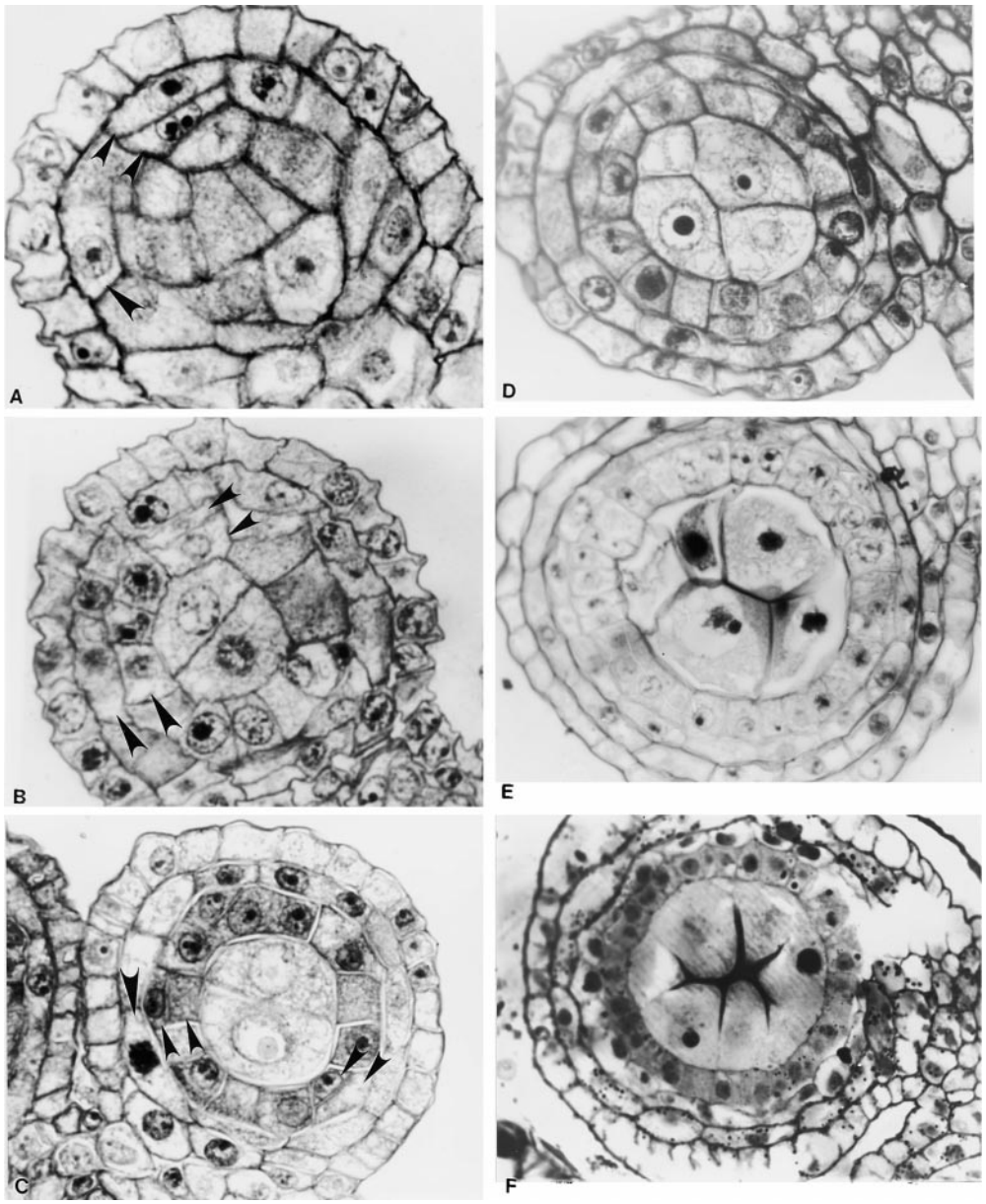

Figure 1.—Cross sections of normal anthers at different stages of development (×700). (A) Very young anther with a well-defined epidermis. Interior to the epidermis lies the primary parietal cell layer (large arrowhead) surrounding the archesporial cells. Note the two secondary parietal cell products (pair of small arrowheads) of the periclinal division of one of the subepidermal primary parietal cells. The archesporial cells occupy the central region and are relatively large in size compared to the parietal cells. (B) At this slightly later stage of anther development, beneath the epidermis are located the two secondary parietal layers (pair of large arrowheads) surrounding the archesporium. The two centrally located archesporial cells have larger nuclei than those of the wall cells. The periclinal division of the inner layer of secondary parietal cells has just begun as evidenced by the appearance of a cell of the middle layer (outer cell labeled with a small arrowhead) and a tapetum progenitor cell (inner cell labeled with a small arrowhead). (C) At this more advanced anther stage, two well-defined cell layers are readily distinguishable from each other. The outer layer is the outer secondary parietal cell layer and is located just internal to the epidermis. This layer develops into the endothecium, and it contains a mi-totic prophase stage cell (large arrowhead) that will divide anticlinally. The cells of the developing endothecium have elongated. The inner layer is the inner secondary parietal layer and it contains two sites (pairs of small arrowheads) where periclinal divisions have been completed to produce middle cell layer progenitors (outer cells) and tapetum progenitors (inner cells). Note the symmetrically arranged two archesporial cells occupying the central region. (D) At this later stage many cells of the inner secondary parietal layer have divided periclinally to form the progenitors of the middle cell layer (outer) and tapetal layer (inner). Four archesporial cells occupy the circular central region and their enlarged size make them easily distinguishable from the cells of the surrounding wall layers. Note that callose accumulation has not yet begun and that the archesporial cells are closely pressed together. (E) At this later stage, there are three wall layers located internal to the epidermis, and each displays its typical cell morphology. The endothecial and middle layers are flattened and elongated parallel to the circumference of the anther, while the tapetal layer consists of palisade-type cells that are slightly elongated along the anther's radial axis; some of the palisade-type cells are undergoing mitosis. Callose has begun to accumulate between the microsporocytes that are in the leptotene stage of meiotic prophase I. (F) In this more advanced stage the microsporocytes have progressed further into prophase I and have undergone partial separation as callose has increasingly accumulated. The components of each of the four tissues, the endothecium, middle layer, tapetum, and microsporocytes, all uniformly display the cellular morphology characteristic of each tissue, especially the greater nuclear and cytoplasmic density of the uninuclear cells comprising the tapetal layer.

sporial-derived cells but it shows no evidence of periclinal cell divisions. Although some of the cells located just internal to the primary parietal cell layer appear to be the products of periclinal cell divisions and some are elongated in the periclinal plane, their cytoplasmic appearance and their patterns of walls indicate their derivation from archesporial cells (Figure 2F).

In summary, the *mac1* mutant anther begins its devel-

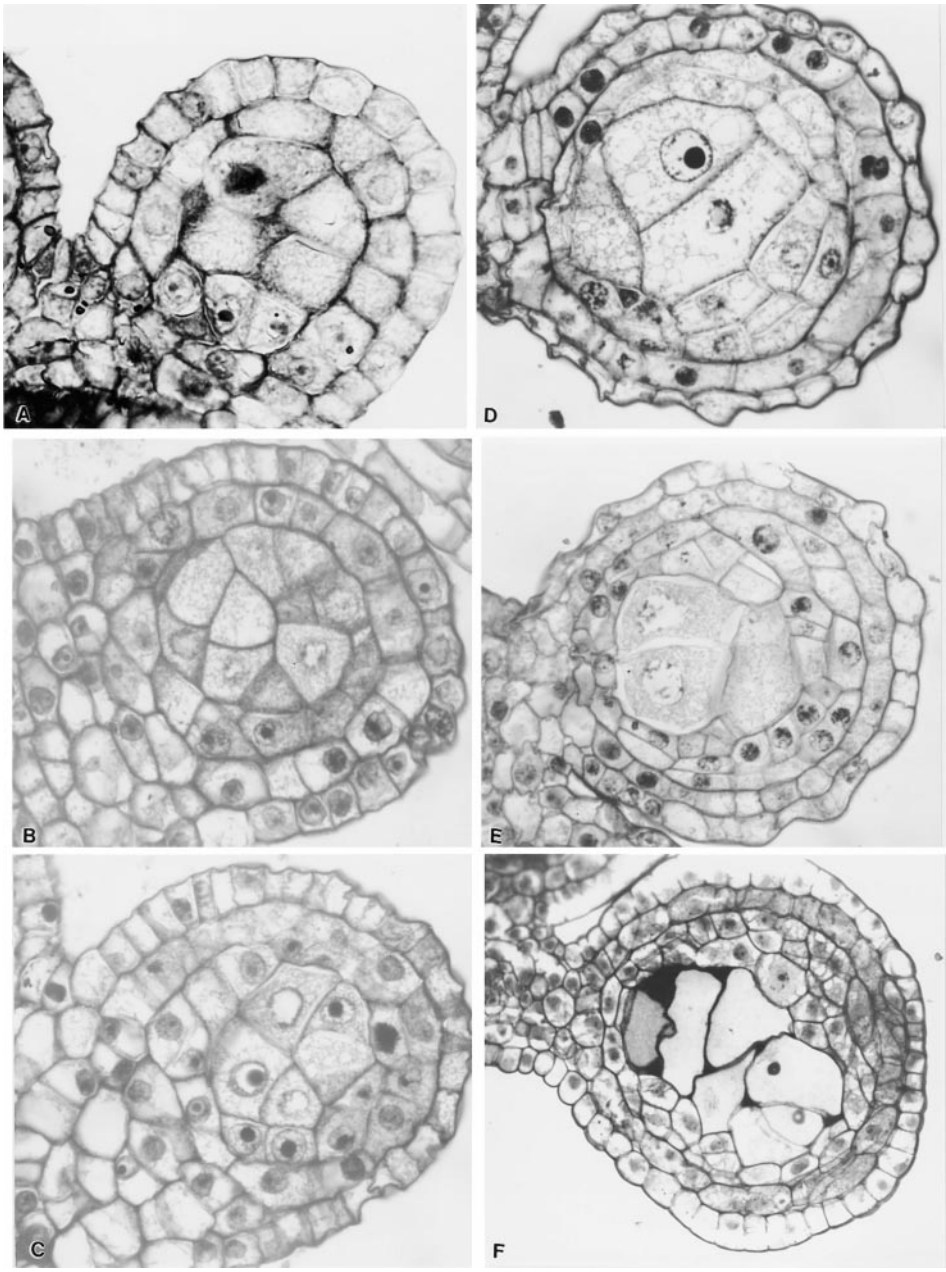

Figure 2.—Cross sections of *mac1* mutant anthers at different stages of development (×700). (A) Very young anther with a well-defined epidermis. Interior to the epidermis lies the primary parietal cell layer surrounding the archesporial cells. The cell patterns are like those of very young normal anthers. (B) At this later stage the primary parietal layer has increased in cell number by anticlinal divisions, but there is no evidence of periclinal divisions. The archesporial cells also have increased in number but vary in their size and shape, with the larger cells being more centrally located. (C) In this later-stage anther the primary parietal layer persists with no evidence of cell division except for an occasional indication of a periclinal division of its cells. The archesporial cells are more numerous than in a normal counterpart, but they vary considerably in their size and shape, with the largest archesporial cells being more centrally located. There are no readily distinguishable secondary parietal layers. (D) At this more advanced state, the primary parietal layer persists and some of its cells have elongated while others appear to be the products of recent anticlinal divisions. No products of a periclinal division of the primary parietal layer are evident. The cells located interior to the primary parietal cell layer are of archesporial origin, and some of these cells have undergone periclinal divisions resulting in cell arrangements superficially resembling inner wall layers. Note that these layered cells lack the cytological features of middle layer or tapetal layer cells. The two centrally located large cells have the typical appearance of archesporial cells. (E) At this later stage the primary parietal layer persists, and most of its cells have elongated. The archesporial cells have undergone further mitotic divisions to produce numerous cells varying in their size and planes of orientation, with only the three innermost cells having the large cytoplasmic and nuclear volumes typical of archesporial cells. Two of these three cells are in mitotic prophase as is a smaller adjacent cell. In some regions the outermost archesporial-derived cells are flattened and elongated so as to superficially appear to comprise a wall layer, but most, probably all, of these cells are of archesporial origin. As in Figure 2D, there is no evidence of the differentiation of a middle cell layer or tapetal layer. (F) In this more advanced stage those archesporial cells that had differentiated into microsporocytes have now entered prophase I of meiosis. Note the irregular shape and outline and the lack of radial orientation of the microsporocytes. The callose distribution is abnormal as compared to normal anthers at this stage. The remaining archesporial-derived cells are smaller than the microsporocytes and irregularly arranged internal to the primary parietal layer. The anther lacks well-defined wall layers, with no evidence of a typical endothecium, middle layer, or tapetum.

opment normally by forming a primary parietal layer and archesporium. Later, however, the primary parietal layer fails to divide periclinally to produce the two secondary parietal layers that normally give rise to the endothecium, the middle layer, and the tapetum and, consequently, these three layers do not develop in mutant anthers. The cells of the archesporium divide excessively, resulting in many more archesporial cells than are normally present, and they vary greatly in size. Only the innermost of these archesporial cells differentiate

into meiocytes, and not only are they abnormal in shape and appearance, but they fail to proceed beyond early prophase I of meiosis.

The anther wall and archesporium develop normally in anthers of other meiotic mutants: To determine whether the failure in anther wall development might be a general feature of the failure of male meiosis in maize, we examined the anthers of the *absence of first division1* (*afd1*) and anthers of four alleles of the *ameiotic1* (*am1*) meiotic mutations. The mutant anthers containing mature microsporocytes were compared in their cytological features with those of their normal counterparts. The three well-defined anther wall layers that are present in normal anthers containing microsporocytes at prophase I of meiosis (Figure 3A) are also present in *afd1* anthers containing mutant microsporocytes at the abnormal prophase I stage of meiosis characteristic of this mutation (Figure 3B). The original *ameiotic1* mutant allele (*am1-1*) was first reported in 1956 (M. Rhoades, personal communication), and it results in both the male and female meiocytes undergoing mitosis and then degenerating (Palmer 1971). The two new mutant alleles, *am1-485* and *am1-489*, also result in mitosis replacing meiosis in male meiocytes but they display different degrees of blockage at interphase in female meiocytes (Golubovskaya *et al.* 1997). Sectioning of mutant anthers of *am1-1*, *am1-485*, and *am1-489* revealed that, in all three cases, the usual three well-defined anther wall layers are present in the mutant anthers (Figure 3, C, D, and E). A fourth *am1* allele, *ameiotic 1-prophase I arrest* (*am1-praI*), allows both the male and female meiocytes to enter prophase I of meiosis but they are then blocked at the leptotene/zygotene stages (Golubovskaya *et al.* 1993, 1997). Sectioning of *am1-praI* mutant anthers revealed that they too contained three well-defined anther wall layers (Figure 3F).

DISCUSSION

The results of this study reveal that the *mac1* mutation affects the development of the anther wall layers, so that the usual array of three wall layers between the epidermis and the archesporium is not formed. In addition, there is an increase beyond the normal number of mitotic cell divisions of the archesporial cells. Although the most centrally located and largest archesporial cells differentiate into microsporocytes, these meiocytes only enter prophase I of meiosis and then degenerate. Because the female meiocytes proceed through normal meiotic divisions in mutant ovules, it is evident that the *mac1* locus does not directly affect the course of meiosis, and the disablement of the meiocytes in their capacity to proceed through meiosis in mutant anthers most likely is the indirect effect of this gene's role earlier in anther development, rather than their suffering an intrinsic defect in the first meiotic division process. The observation that mutant anthers wherein the meiotic divisions are blocked by the *am1* and *afd1* mutations nevertheless have a normal structure with the usual three wall layers indicates that mutations that result in defects in the meiocytes do not necessarily result in a lack of tapetum or other wall layers.

Mutations that affect the meiotic divisions do not necessarily alter anther wall formation: The presence of the three normal-appearing anther wall layers in *afd1* mutant anthers indicates the independence of the genetic control of the events that are specific to the meiotic divisions from the control of wall layer formation. The studies with the *am1* mutant alleles were additionally revealing. The presence of the three wall layers in anthers wherein the initiation of meiosis is blocked and the meiocytes instead undergo mitosis (*am1-1*, *am1-485*, and *am1-489*) further indicates the independent control of the genesis of the wall layers from the onset of meiosis in the meiocytes that they normally surround. Finally, the analysis of the *am1-praI* provided additional insight. In anthers affected by the *am1-praI* mutation the archesporial cells differentiate into meiocytes and these cells enter into prophase I and progress into the leptotene or zygotene stages, much as is observed with the *mac1* meiocytes that differentiate in the central region of the archesporium; the *am1-praI* meiocytes then degenerate, as do the *mac1* meiocytes. Yet, despite this strong similarity in their meiocyte behavior, the *am1-praI* anthers contain the usual three wall layers that are absent from *mac1* anthers. Taken together, these results strongly suggest that the effect of the *mac1* mutation on anther wall development is specific for this locus among the meiotic mutations and that a defect in anther wall formation is not a usual accompaniment of a disturbed meiosis in anthers.

Comparison of the effects of the *mac1* mutation in ovules and anthers: The effect of the *mac1* mutation in ovules is to increase from one to about nine the number of archesporial cells formed from the hypodermal cells in each mutant ovule. We have suggested that this increase in sporogenous cells (resulting in multiple female meiocytes, all of which undergo a normal meiosis) is likely to be the result of the *mac1* locus playing an important role in the commitment of hypodermal cells of the ovule to the meiotic pathway (Sheridan *et al.* 1996). Whereas in a maize ovule a hypodermal cell directly develops into an archesporial cell and eventually differentiates into a megasporocyte, in an anther the hypodermal cells first divide mitotically in a periclinal plane, and it is the inner of the resulting two daughter cells that functions as an archesporial cell. This archesporial cell and its immediate cellular progeny undergo one or more mitotic divisions before they differentiate into the meiocytes. In considering these differences in the immediate fates of the hypodermal cells of the ovules and anthers, it seems likely that insight might be obtained in the role of the *mac1* locus in ovule and anther development and in the commitment of the hypodermal cells or their progeny to the meiotic pathway. The lack of a normal *mac1* allele in mutant plants does

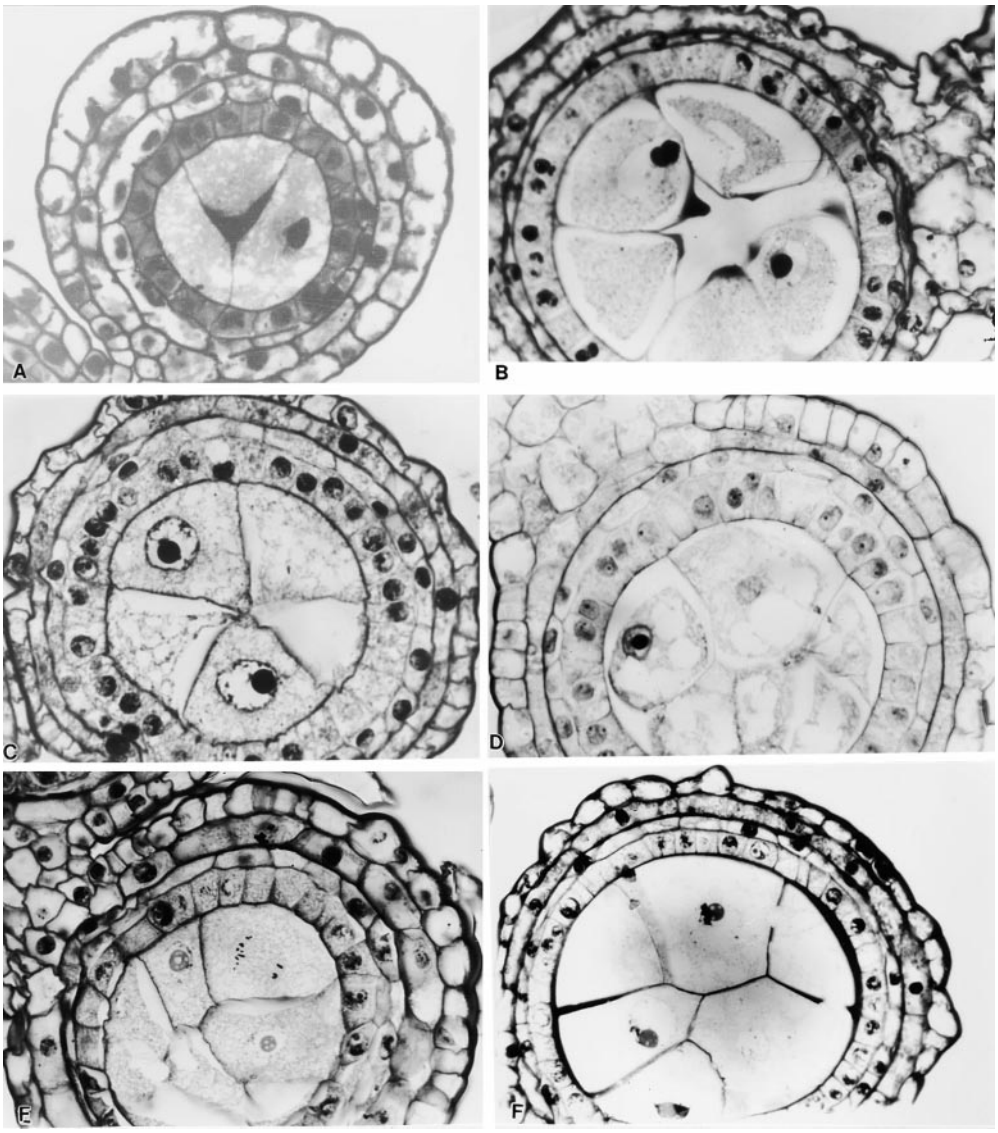

Figure 3.—Cross sections of mutant and normal anthers at meiosis (×700). (A) Section of normal anther with microsporocytes at the prophase I stage of meiosis. Note the three well-defined wall layers surrounding the microsporocytes and the pattern of callose distribution typical of a normal prophase I anther. (B) Section of *absence of first division1* mutant anther with microsporocytes at an abnormal prophase I stage of meiosis typical for this mutation. Note the three well-defined wall layers surrounding the microsporocytes. The latter are normally shaped and arranged, but callose is reduced in amount, although normally located in the central region. (C) Section of *ameiotic1-1* mutant anther with microsporocytes in a mitotic prophase typical for this mutation. Note the well-defined three wall layers. The shape and orientation of the microsporocytes appear normal, but the callose deposition typical of normal meiocytes is absent. Note that the shape of the tapetal layer is normal, but these cells lack the cytoplasmic density characteristic of these cells in normal anthers. (D) Section of *ameiotic1-485* mutant anther with microsporocytes in interphase or early mitotic prophase typical for this mutation. Note the well-defined three wall layers. Although the microsporocytes have a normal shape they lack any associated callose. The tapetal layer resembles a normal tapetum but lacks its typical density. (E) Section of *ameiotic1-489* mutant anther with microsporocytes in varying stages of mitotic divisions typical for this mutation. Note the well-defined three wall layers. The microsporocytes have a normal shape but the anther lacks the callose and density of tapetum characteristic of normal anthers. (F) Section of *ameiotic1-praI* anther with microsporocytes in meiotic prophase I at the leptotene-zygotene stage typical of this mutation. Note the well-defined three wall layers and the presence of callose, with the latter being abnormally distributed around the normally oriented microsporocytes. The tapetal layer is normal in morphology but lacks the density typical of normal anthers.

not appear to affect the immediate behavior of the hypodermal cells in mutant anthers, inasmuch as these cells divide periclinally to produce primary parietal cells and archesporial cells. But the fate of both of these tissues appears to be modified: the cells of the primary parietal layer fail to divide periclinally to produce secondary parietal cells, the progenitors of the three wall layers that fail to form in mutant anthers, and the archesporial cells enlarge but then subdivide excessively. Moreover, those archesporial cells that do differentiate into meiocytes and enter into meiosis fail to proceed past early prophase I and then degenerate.

The meiocytes of the *mac1* mutant anther fail to proceed through meiosis: It is conceivable that the reason the microsporocytes do not go through meiosis is because of the absence of a tapetum surrounding them. The anther is the distal fertile part of the stamen and the stamen is viewed by Eames as "the most highly specialized of floral organs, resembling an ancestral laminar sporophyll far less than the carpel, and showing much greater variety of form. Adaptation to various types of pollen distribution has brought about not only great range in external form but in the histological structure of the anther" (Eames 1961, p. 137). Ovules do not contain a tissue counterpart to the tapetal layer of the anther (Bouman 1984). However, it appears to us that the failure in meiosis is the result of a defect in the development of the archesporial cells and it is the

defective development of these sporogenous progenitors that results in the failure in function of the meiocytes. Two lines of reasoning support this hypothesis. First, the abnormal number and patterns of cell divisions of the archesporial cells result in an excessive number of cellular progeny and a wide range of cell size and shapes, suggesting that the regulation of the developmental pathway leading to meiocytes has gone awry in the *mac1* archesporial cells. Second, the regulation of the developmental fate of the archesporial cells and their meiocyte progeny most likely resides within the archesporial cells themselves. In view of the unique developmental fate of the archesporial cells and their progeny it is simpler (and therefore more attractive) to suggest that their highly specialized mode of development is regulated within and among these meiocyte precursors, rather than from without by the tapetum or some other tissue. The success of meiosis in the ovule that lacks the specialized wall layers of the anther supports the view that the meiocytes control their own fate.

Why does the *mac1* mutation have opposite effects on the primary parietal layer cells and the archesporial cells? An explanation for the lack of periclinal cell division in the primary parietal layer and the increase in cell division of the archesporium in mutant anthers may possibly be found in considering the role of the *mac1* gene in the archesporium of the normal anther. Once the archesporium has formed, only the cell layer closest to the archesporium divides periclinally in the normal maize anther (Kiesselbach 1949). That is to say, only the primary parietal layer and the inner secondary parietal layer undergo periclinal divisions, while the epidermal layer and the outer secondary parietal layer undergo only anticlinal divisions, thereby enabling these latter two tissues to maintain their continuity as the anther enlarges (Cheng *et al.* 1983). With regard to the possible role of the archesporial cells in regulating angiosperm anther wall development, Goldberg *et al.* (1993) noted that "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." In a mutant maize plant, the hypodermal cells and the archesporial cells lack a functional *mac1* allele, and the failure of *mac1* gene expression by the archesporial cells may result in a lack of self-inhibition of their excessive cell division and a lack of stimulation of the periclinal division of the cells of the adjacent primary parietal layer.

If the *mac1* locus codes for a molecule that acts through a signal transduction mechanism, then future investigation of maize anthers may possibly reveal that both the archesporial cells and the cells of the primary parietal layer may share a common ligand receptor component but differ in their response component. Such a difference in the response components might explain the differences in the phenotypic responses of the two cell types to mutation of the *mac1* locus. Cloning and molecular analysis of the *mac1* gene should shed light on the mechanism of action of this interesting locus.

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