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The Role of the *ameiotic1* Gene in the Initiation of Meiosis and in Subsequent Meiotic Events in Maize

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ABSTRACT

Understanding the initiation of meiosis and the relationship of this event with other key cytogenetic processes are major goals in studying the genetic control of meiosis in higher plants. Our genetic and structural analysis of two mutant alleles of the *ameiotic1* gene (*am1* and *am1-pra1*) suggest that this locus plays an essential role in the initiation of meiosis in maize. The product of the *ameiotic1* gene affects an earlier stage in the meiotic sequence than any other known gene in maize and is important for the irreversible commitment of cells to meiosis and for crucial events marking the passage from premeiotic interphase into prophase I including chromosome synapsis. It appears that the period of *ameiotic1* gene function in meiosis at a minimum covers the interval from some point during premeiotic interphase until the early zygotene stage of meiosis. To study the interaction of genes in the progression of meiosis, several double meiotic mutants were constructed. In these double mutants (i) the *ameiotic1* mutant allele was brought together with the meiotic mutation (*afd1*) responsible for the fixation of centromeres in meiosis; and with the mutant alleles of the three meiotic genes that control homologous chromosome segregation (*dv1, ms43* and *ms28*), which impair microtubule organizing center organization, the orientation of the spindle fiber apparatus, and the depolymerization of spindle filaments after the first meiotic division, respectively; (ii) the *afd1* mutation was combined with two mutations (*dsy1* and *as1*) affecting homologous pairing; (iii) the *ms43* mutation was combined with the *as1*, the *ms28* and the *dv1* mutations; and (iv) the *ms28* mutation was combined with the *dv1* mutation and the *ms4* (*polymitotic1*) mutations. An analysis of gene interaction in the double mutants led us to conclude that the *ameiotic1* gene is epistatic over the *afd1*, the *dv1*, the *ms43* and the *ms28* genes but the significance of this relationship requires further analysis. The *afd* gene appears to function from premeiotic interphase throughout the first meiotic division, but it is likely that its function begins after the start of the *ameiotic1* gene expression. The *afd1* gene is epistatic over the two synaptic mutations *dsy1* and *as1* and also over the *dv1* mutation. The new *ameiotic*-485* and *leptotene arrest*-487* mutations isolated from an active ROBERTSON's *Mutator* stocks take part in the control of the initiation of meiosis.

THE problem of meiosis initiation may be viewed as requiring an understanding of two gene regulatory processes. The first switches the cell from the mitotic cell cycle to embark upon meiotic cell cycle. The second causes the meiocyte to enter into meiotic prophase I and to proceed with chromosome synapsis and the subsequent events that characterize meiosis. It is likely that the former process occurs in the G_1 phase of the cell cycle while the latter process must occur no later than during the G_2 phase. (See reviews by GOLUBOVSKAYA 1989; STERN 1990; JACOBS 1992; KLECKNER *et al.* 1991; and MURRAY 1992).

The control of switching from a mitotic sequence to a meiotic sequence has been extensively studied in the budding yeast, *Saccharomyces cerevisiae*. In this yeast the process of switching from a mitotic to a meiotic cycle is regulated by two genetic systems controlling the responses to mating type and nutritional conditions (MITCHELL 1988; MALONE 1990). The *MAT, RME1* and *IME1* genes regulate the mating type system. The stepwise order of gene action for switching cells into meiosis of *MAT* → RME1 → IME1 has been proposed by SIMCHEN and KASSIR (1989). Several genes control the yeast cell's response to nutritional conditions (MITCHELL 1988) but only the *IME1* gene (KASSIR, Granot and SIMCHEN 1988) and the *IME4* gene (SHAH and CLANCY 1992) regulate both of the genetic systems in *S. cerevisiae*.

The cloning of *cell division cycle* (*cdc*) genes of *S. cerevisiae* and the fission yeast, *Schizosaccaromyces pombe*, has led to the discovery that the *cdc28* and the *cdc2* gene are homologous genes in the two species; they both code for the protein kinase, p34. Furthermore, homologs to this gene and its encoded protein

This article is dedicated to the memory of MARCUS M. RHOADES.

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TABLE 1

Genetic and cytological analysis of the new allele of the *ameiotic1* gene and the other new meiotic mutations

Selfed F_1 individual parent plants	Pattern of inheritance[a] of progeny: Observed segregation progeny plants: Fertile normal	Sterile mutant	Total	χ^2 3:1 fit	Genotype of selfed parent
A. *pra1* a[b]	27	9	36	0.00	*pra1/+*
pra1 b	47	13	60	0.36	*pra1/+*
pra1 c	27	13	40	1.20	*pra1/+*
Total 3	101	35	136	0.04	
B. *am*485*	38	15	53	0.31	*am*/+*
C. *lar*487* a	17	4	21	0.40	*lar*/+*
b	10	6	16	1.33	*lar*/+*
c	8	1	9	0.92	*lar*/+*
Total 3	35	11	46	0.04	

	Allelic test: Observed segregation progeny plants: Fertile normal phenotype	Sterile *pra1* mutant phenotype	Total	χ^2 fit	Note
D. Crosses					
pra1/pra1 × *am1/+*	4	5	9	0.1	*pra1* is a new allele of Am1
pra1/+ × *am1/+*	70	15	85	2.45	
pra1/pra1 × *afd1/+*	20	0	20		*pra1* is nonallelic to *afd1*
pra1/+ × *afd1/+*	82	0	82		

Note: *pra1* is a new allele of *ameiotic1* gene and is designated *am1-pra1*; the dominance relationship is $am1^+ \rightarrow am1\text{-}pra1 \rightarrow am1$.
[a] All progeny were scored in the field for fertility (fertile) or male sterility (sterile) and also had their microsporocytes sampled and examined cytologically with the light microscope to determine normal or mutant meiotic phenotype.
[b] a, b and c designate individual fertile plants that were self pollinated and that proved to be heterozygotes when progeny tested.

are found in all eucaryotes including maize (COLASANTI, TYERS and SUNDARESAN 1991). The p34 protein complexes with another protein (cyclin) to form the Maturation or M-phase Promotion Factor (MPF), a cytoplasmic component long known to regulate meiotic progress in animal oocytes (DOREE, PEACELLEIER and PICARD 1983; DOREE, LABBE and PICARD 1989). It has recently become apparent that MPF complexes of p34 and cyclin regulate both the $G_1 \rightarrow S$ and the $G_2 \rightarrow M$ transition in both the mitotic and meiotic cells in all eukaryotes (see JACOBS 1992; PELECH, SANGHERA and DAYA-MAKIN 1990; and MURRAY 1992 for reviews).

It is conceivable that there are p34 or cyclin (or both) molecular species that are specific for the meiotic sequence and that play a fundamental role in its initiation and progress. The notion of a meiosis specific p34 protein controlling the $G_2 \rightarrow$ prophase I transition leads us to suggest that the chromatin condensation pattern characteristic of meiotic prophase I chromosomes (RHOADES 1950) may result from a changing pattern of histone phosphorylation during the leptotene-zygotene-pachytene stages. This suggestion is consistent with the fact that histones are a major substrate for p34 kinase activity (DOREE, LABBE and PICARD 1989; JACOBS 1992). However, other genetically regulated processes including the delayed replication of the zygotene-DNA (see STERN 1990 for review), the synthesis and assembly of the synaptonemal complex (see LOIDL 1990, 1991; KLECKNER, PADMORE and BISHOP 1991) and the recombination nodules (VON WETTSTEIN, Rasmussen and HOLM 1984; CARPENTER 1988) are undoubtedly important in the initiation of meiosis and its progress through prophase I.

The role of individual meiotic genes in initiating meiosis in higher plants is an intriguing problem (BAKER et al. 1976; GOLUBOVSKAYA 1979, 1989). Maize is a uniquely well-suited organism for both cytological and genetic analysis (RHOADES 1950). In this paper we explore the cellular functions encoded by the maize *ameiotic1* gene. Recovery of a new mutant allele (*am1-pra1*) of this gene and the light and electron microscopic characterization of its phenotype has elucidated the role of the *am1* gene locus in the control of meiosis initiation in maize. These studies indicate

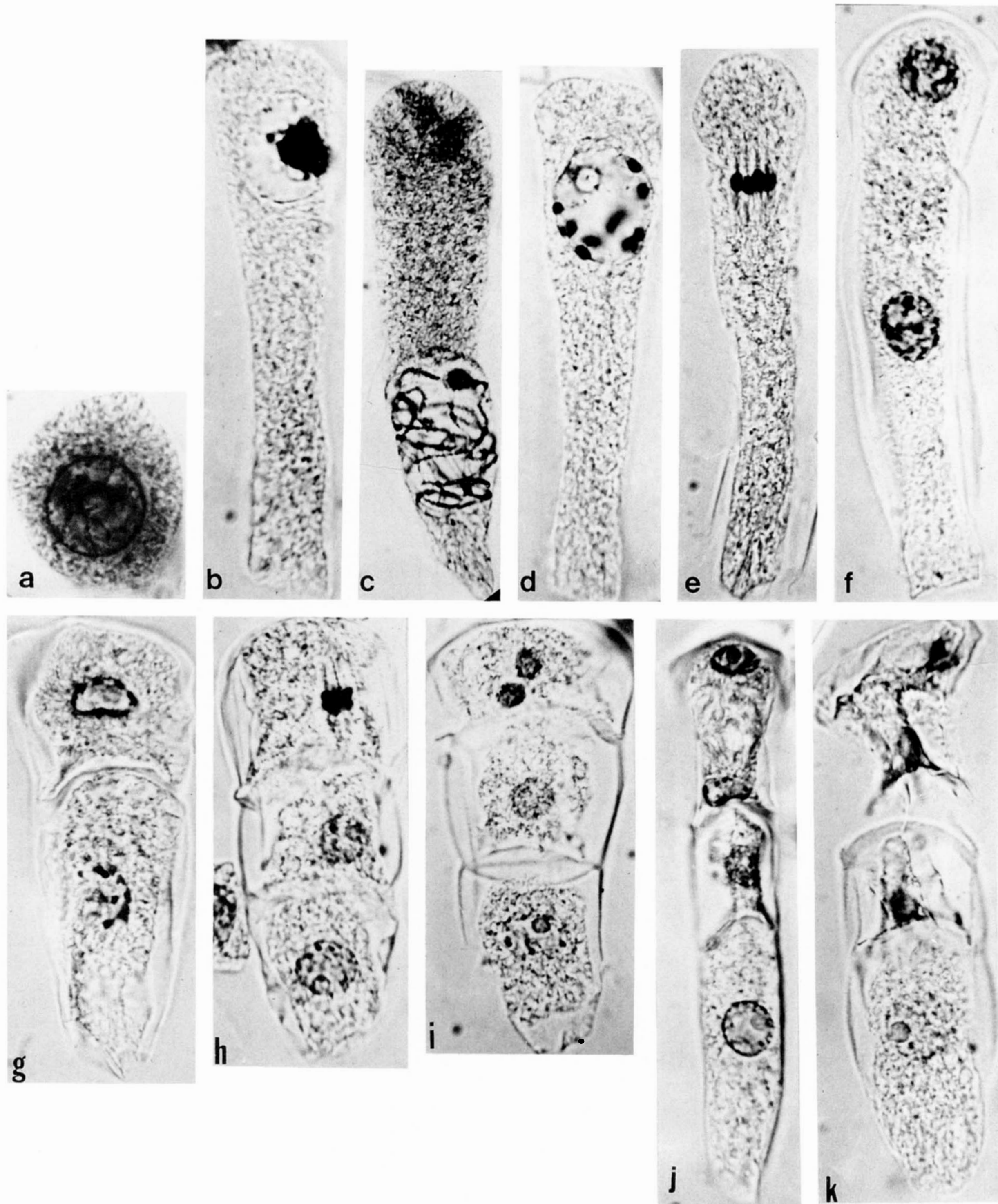

FIGURE 1.—Pattern of normal female meiosis in maize in squash smear slides as is seen under the light microscope. The picture of meiosis in megaspore mother cells is the same as in microspore mother cells as described by RHOADES (1950). Reprinted from GOLUBOVSKAYA, AVALKINA and SHERIDAN (1992) with permission of Wiley-Liss, a division of John Wiley & Sons. (a) Archeosporic cell proceeding into meiosis. (b–d) Prophase I: zygotene, pachytene, diakinesis. (e) Metaphase I. (f) Telophase I. (g–j) Second meiotic division. (k) Surviving megaspore (bottom), product of completed meiotic divisions.

that the function of the *am1* gene is important in the irreversible commitment of cells to meiosis and for crucial events of meiotic prophase I including chromosome synapsis. Genetic and cytological analysis of several combinations of double mutations has revealed the independence in gene action of some of the pairs of genes and in an epistatic interaction between others. In addition we have isolated two new meiotic mutations (*am*-485* and *lar*-487*) with ameiotic and leptotene arrest phenotypes of meiosis, respectively.

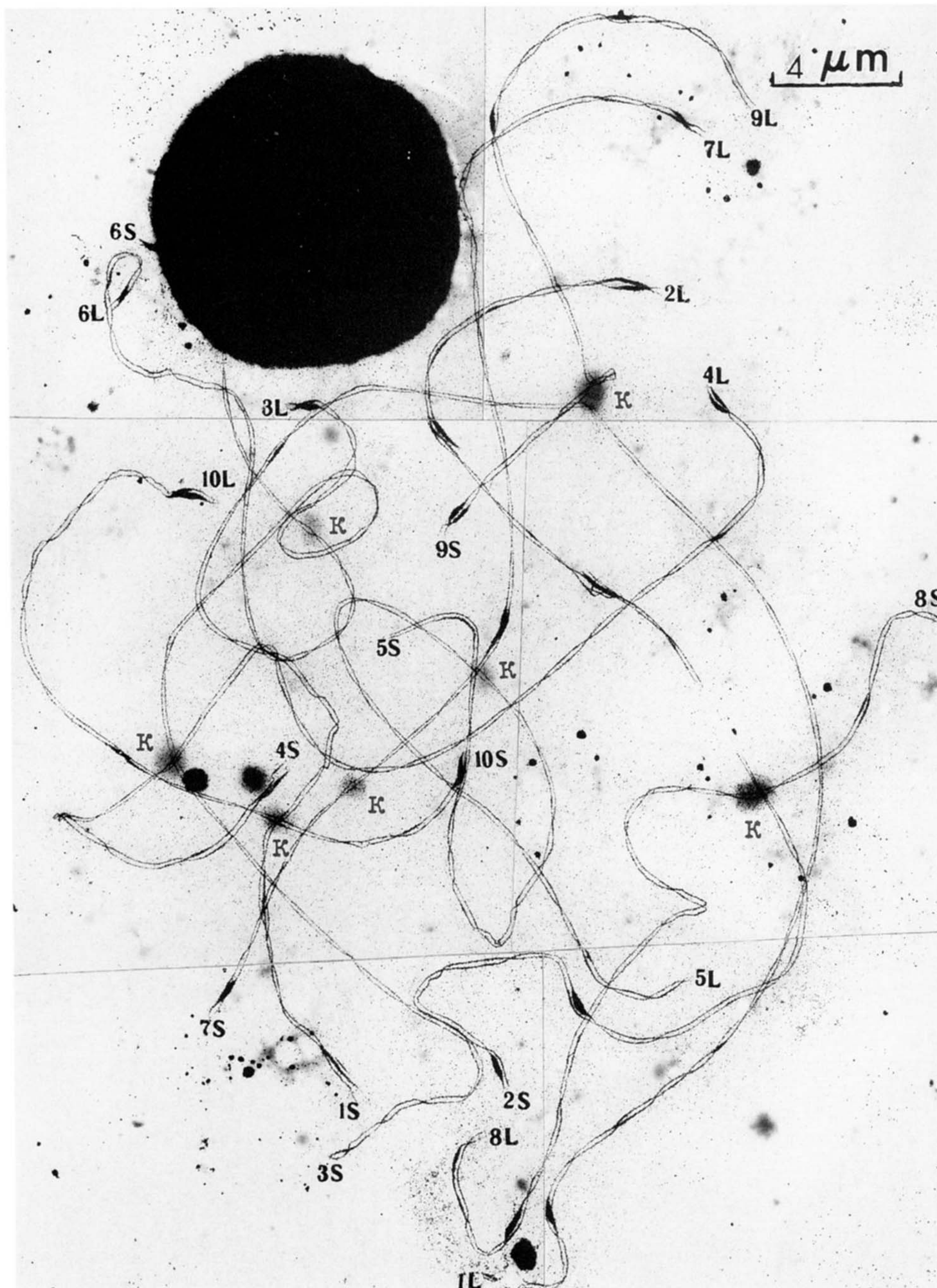

FIGURE 2.—Electron micrograph of an entire set of synaptonemal complexes in a spread normal pachytene nucleus. The normal homologous pairing in each of the ten pachytene bivalents is seen. Kinetochores (K) and both long (L) and short (S) arms of each numbered maize chromosomes are indicated. Bar shown.

We present evidence that they participate in the control of the initiation of meiosis. Because they were isolated from an active ROBERTSON's *Mutator* stock, it is likely that they are transposon tagged. This should facilitate our efforts to clone and molecularly characterize these mutations.

MATERIALS AND METHODS

The meiotic mutations, their sources, and the chromosome arm locations for the mutations examined in this study are: (i) three well-known meiotic mutations from the Maize Genetics Stock Center, RHOADES' *am1* (5S, PALMER 1971), *as1* (1S, BEADLE 1930), *dv1* (CLARK 1940); (ii) six meiotic mutants induced by treatment with N-nitroso-N-methylurea (GOLUBOVSKAYA 1989), *am1-pra1* allelic to *am1*, *afd1* (6L), *dsy 1*, *ms43 (8L)*, *ms28 (1S)*, *ms4*, which is allelic to BEADLE's *po1* (6S); and (iii) two new meiotic mutants isolated from an active ROBERTSON's *Mutator* stock, *am*-485* and *lar*-487*.

The normal alleles of the *am1*, *am1-pra1*, *am*-485* and *lar*-487* genes participate in control of meiosis initiation; in homozygotes of *am1* and *am*-485*, meiosis is omitted and replaced by a synchronized mitotic cell division cycle while in *am1-pra1* and *lar*-487*, meiosis is arrested at prophase I. In homozygotes of *afd1* there is the substitution of the first reductional meiotic division by an equational one with segregation of centromeres of sister chromatids at anaphase I.

The *as1* and *dsy1* loci participate in the control of homologous chromosome pairing (MAGUIRE and RIESS 1991; TIMOPHEEVA and GOLUBOVSKAYA 1991). The *dv1*, *ms43* and *ms28* mutations represent three independent meiotic genes that control segregation of homologous chromosomes; *dv1* is responsible for aggregation of microtubules at the Micro-

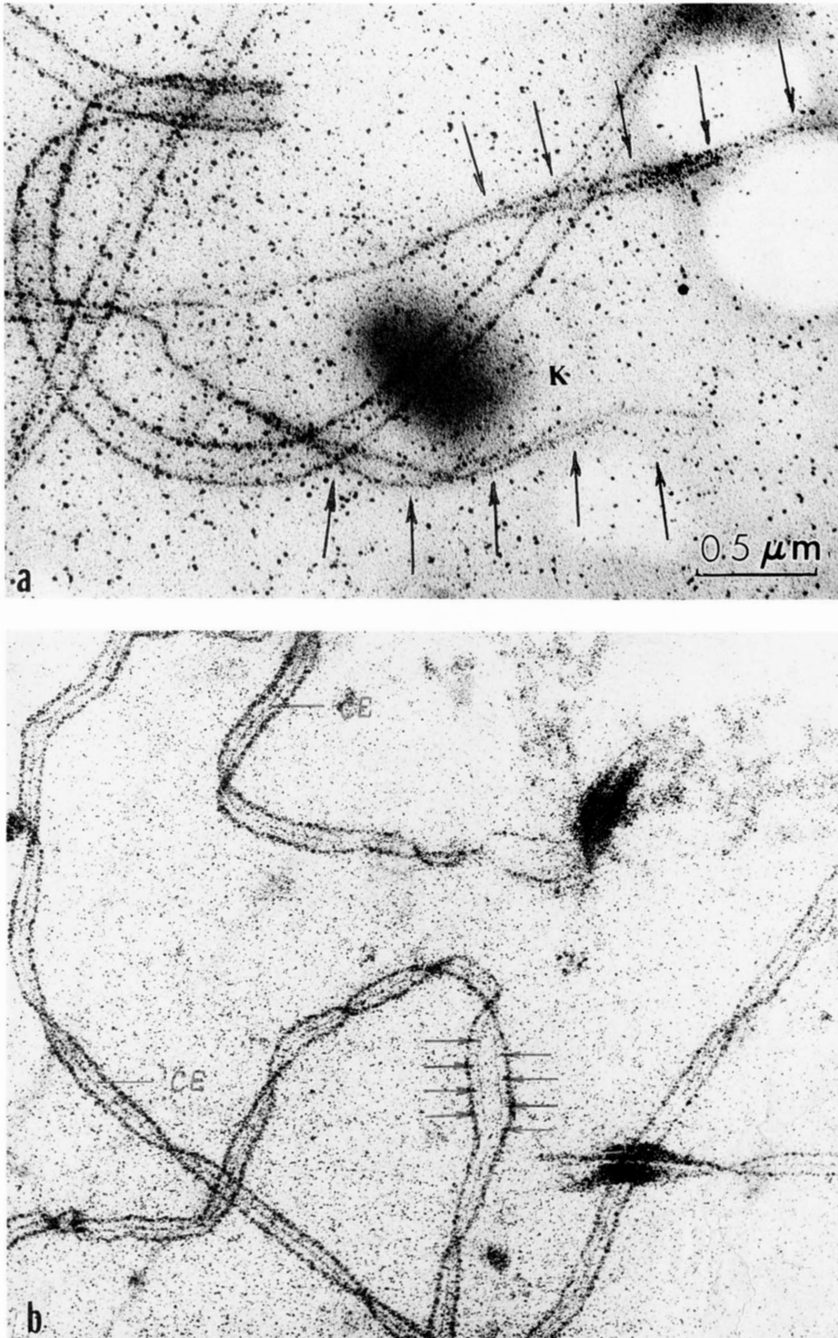

FIGURE 3.—Electron micrograph of a portion of a normal pachytene stage nucleus. (a) Each lateral element of a single synaptonemal complex is seen as a duplex structure (arrows). (b) A central element of a synaptonemal complex is separated into two strands (arrows). Bar shown.

tubule Organizing Center (MTOC) (STAIGER and CANDE 1990); *ms28* is responsible for depolymerization of the spindle apparatus following the first meiotic division, and *ms43* is responsible for the orientation of the spindle apparatus (GOLUBOVSKAYA 1989). The *ms4* mutation is a new allele at the *polymitotic1* locus and controls the initiation of postmeiotic mitosis. For more detailed information see (GOLUBOVSKAYA 1979, 1989).

Cytological procedures: For cytological analysis of microsporogenesis, with the light microscope immature tassels were placed in 3 parts 95% ethanol: 1 part glacial acetic acid. Meiotic analysis of microsporocytes was performed with acetocarmine squash slides of anthers. For cytological analysis of megasporogenesis with the light microscope, a squash technique following enzymatic digestion of isolated maize ovaries was used. A fixative mixture of 50% ethanol, glacial acetic acid, 40% formalin in the volume proportions of (90:5:5) and Feulgen staining were used (GOLUBOVSKAYA, AVALKINA and SHERIDAN 1992).

For electron microscopical analysis of the pattern of the homologous chromosome pairing, we used the technique of surface spreading of synaptonemal complexes (SC) described by GILLIES (1981) for maize.

Interaction of the meiotic mutations: Interactions of the different meiotic mutations were studied in the offspring of self-pollinated double heterozygotes. These were obtained by crosses among heterozygotes of different single mutations located on different chromosomes. Their heterozygous genotype was proven by self pollinating and progeny testing. A 9:3:3:1 ratio for any two independently assorting mutations as defined by cytological analysis of meiotic phenotype was expected in the self pollinated progeny of double

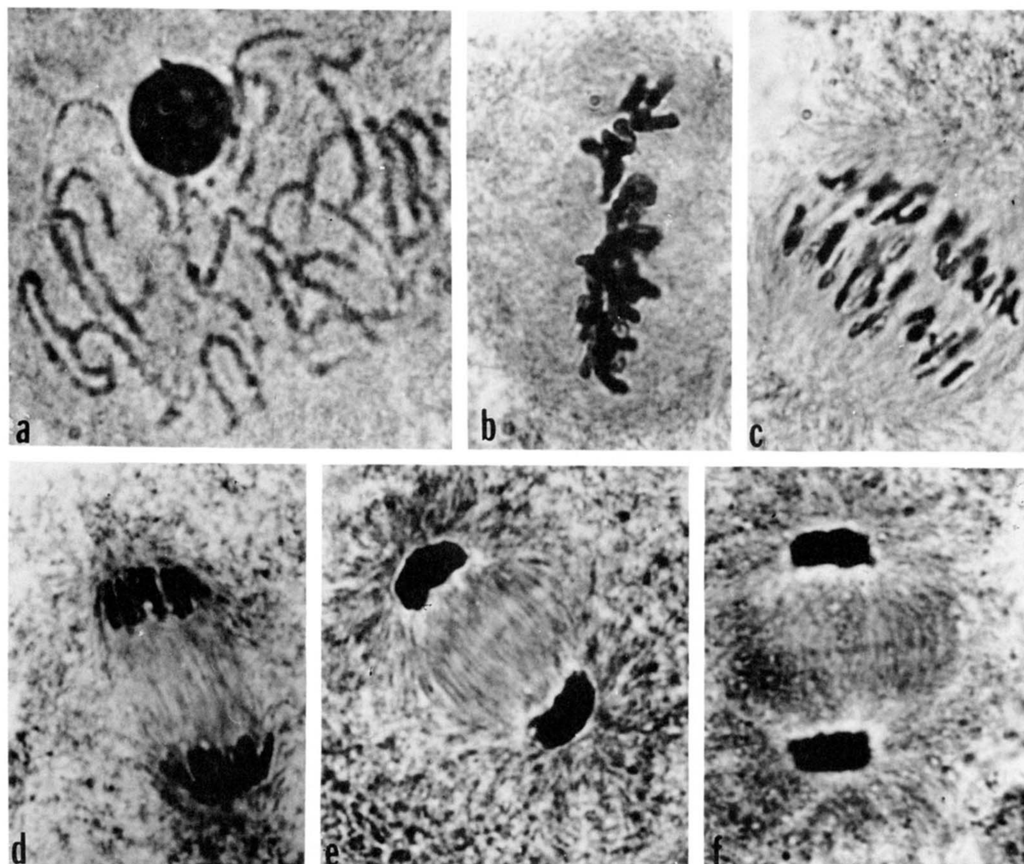

FIGURE 4.—The pattern of male meiosis in homozygotes of the mutant *am1* allele in maize. (a–e) The microspore mother cells do not enter into meiosis but undergo mitotic division with prophase (a), metaphase (b), anaphase (c and d) and telophase (e and f) stages.

heterozygotes assuming that the two mutations act independently of each other. In the case of epistasis, the expected ratio would be 9:4:3 because of the additional 1/16 of meiotic double homozygotes exhibiting the same mutant phenotype as that of the epistatic mutation. This would result from the assumption that the gene for the mutant phenotype with the "4" class is epistatic to the gene for the mutant phenotype with the "3" class. The differences between the expected and observed segregation ratios were tested by the chi-square test. All sterile plants in the epistatic class of segregants were microscopically analyzed to discern the appearance of the phenotype of the other meiotic gene in the background of the epistatical gene.

RESULTS

Isolation of the new *am1-pra1* allele of the *ameiotic1* gene: pattern of inheritance and allelic relationships: In 1988 a new meiotic mutant with an irreversible block of meiosis at prophase I termed *prophase I arrest (pra1)* was isolated in the homozygous state from the M7 generation after treatment with 0.25% N-nitroso-N-methylurea of dry seeds of the maize inbred stock A344. Segregation ratios among the progeny of three self pollinated individual plants (Table 1A) showed that the abnormal meiosis of *pra1* was responsible for the complete male and near complete female sterility observed and that it was inherited as a monogenic recessive.

Allelism tests (Table 1D) indicated the *pra1* meiotic mutation was allelic with *am1*; in the F_1 progeny (from both of the types of crosses shown in Table 1) segregations for both sterility and the mutant meiotic phenotype were observed in the expected ratios. Cytological examination revealed that the pattern of meiosis in a total of 74 fertile F_1 segregants was regular, but all 20 sterile segregants exhibited the *pra1* meiotic phenotype. Hence, the *pra1* mutation is a new allele of the well-known *am1* gene, and *am1-pra1* is the designation for this new allele. The occurrence for this important meiotic gene of allelic derivatives with distinguishable meiotic phenotypes gene should be helpful in understanding the function of this gene in meiosis. We have compared the meiotic pattern of the normal allele and of two mutant allelic derivates in pursuit of this goal.

Cytological effects of two mutant alleles of the *ameiotic1* gene on both male and female meiosis: *Normal meiosis:* The normal allele of the *am1* gene in either the homozygous or heterozygous state provides for a normal course of both male and female meiosis with a regular pattern of both pairing and segregation of homologous chromosomes, and the formation of four haploid products of meiosis. A distinguishable feature of female meiosis is that only one of the four

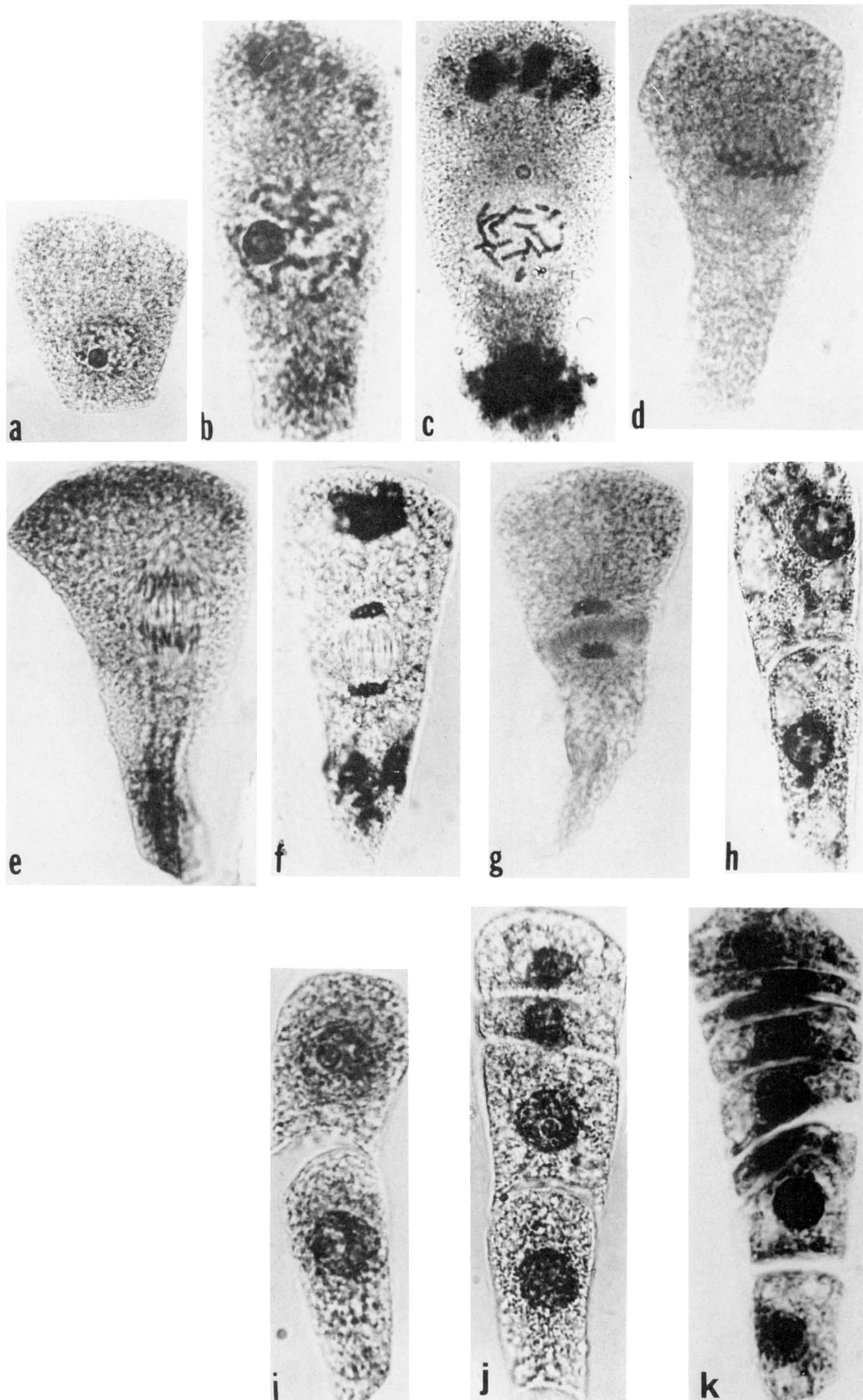

FIGURE 5.—Female meiosis in smear slides of isolated *am1* mutant megaspore mother cells. Reprinted from GOLUBOVSKAYA, AVALKINA and SHERIDAN (1992) with permission of Wiley-Liss, a division of John Wiley & Sons. (a–g) The megaspore mother cells (a) do not enter into meiosis but undergo mitotic division with prophase (b), metaphase (c, d), anaphase (e, f) and telophase (g) stages. (h, i) Two daughter cells as a result of completion of the first ameiotic cell cycle. (j) Four daughter cells as a result of completion of second round of ameiotic cell cycle. (k) Eight daughter cells as a result of completion of third round of ameiotic cell cycle.

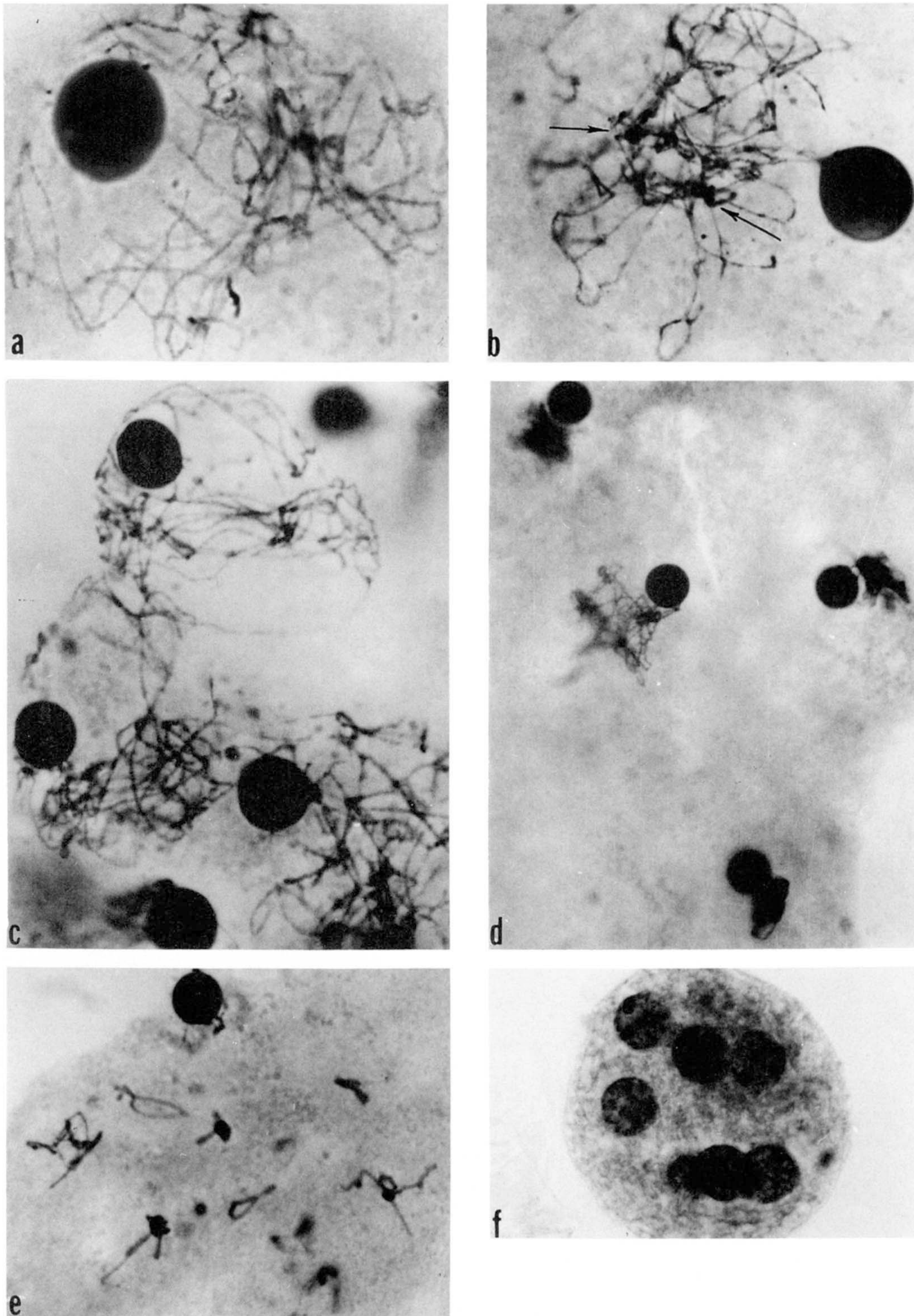

FIGURE 6.—Pattern of male meiosis in a homozygous *am1-praI* mutant as is seen under the light microscope. (a, b) Microspore mother cell entering into meiosis, but the progression of meiosis is arrested at early prophase I. (c–e) Chromatin and cell degradation occur following early prophase I, including the formation of a symplast containing numerous prophase I nuclei (c), pycnotic chromatin (d) and lysis chromosomes (e). (f) Multinucleate cells are often formed in this mutant.

haploid products of meiosis (megaspores) survives (Figure 1). This megaspore undergoes three successive postmeiotic nuclear divisions to produce the eight-nucleate embryo sac.

The following description of normal meiosis is based on the electron microscopic examination of 85 silver nitrate-stained spreads of early and late pachytene stage nuclei of microsporocytes obtained from plants either homozygous or heterozygous for the normal *am1* allele. The regular pattern of homologous

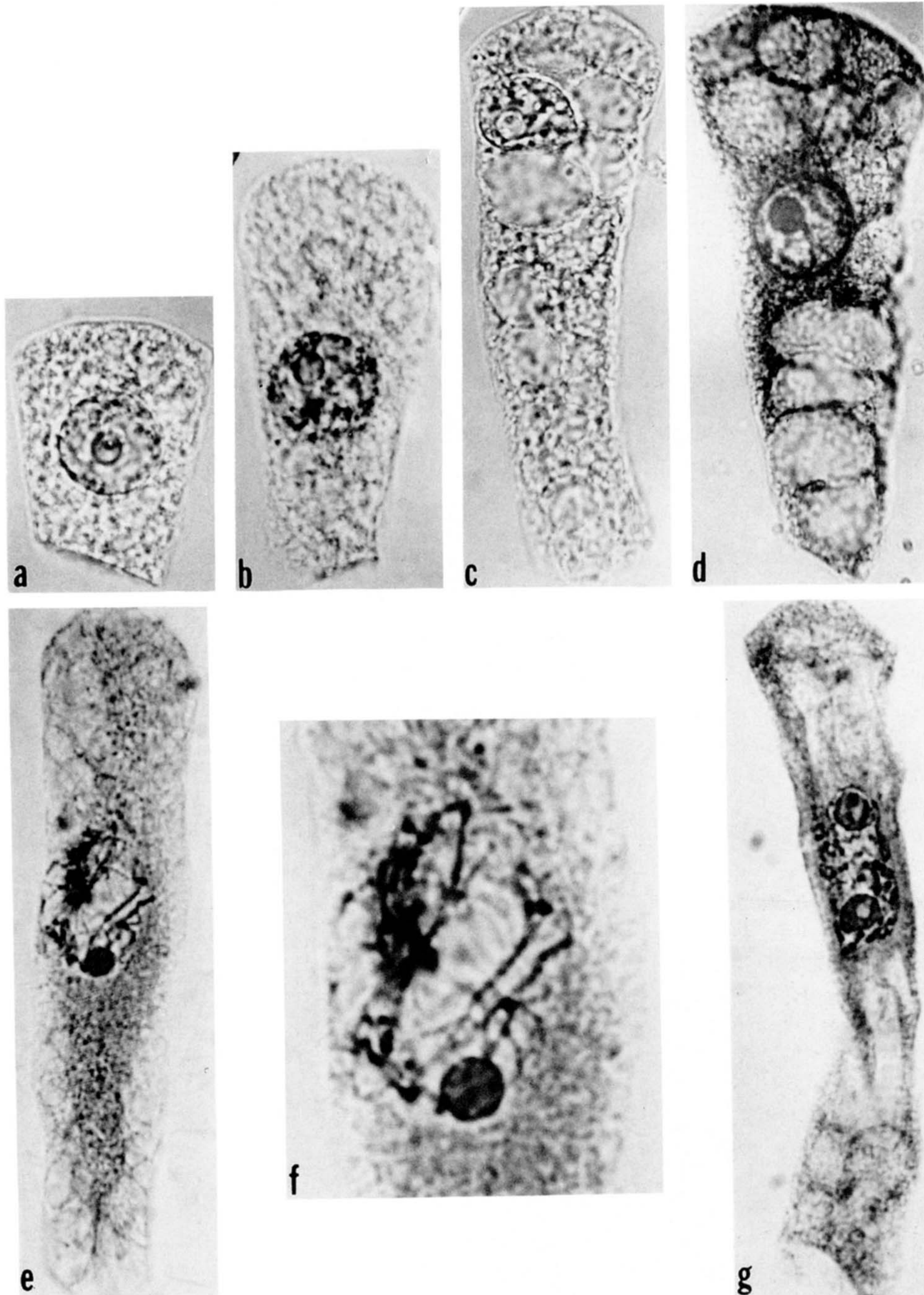

FIGURE 7.—Female meiosis in squash slides of isolated megaspore mother cells of the *am1-pra1* mutant under the light microscope. Reprinted from GOLUBOVSKAYA, AVALKINA and SHERIDAN (1992) with permission of Wiley-Liss, a division of John Wiley & Sons. (a) An archeosporic cell entering into meiosis. (b–d) Progress of the prophase I stage of meiosis. (e, f) The same megaspore mother cell at prophase I stage at a higher magnification. (g) Degrading vacuolating megaspore mother cells.

chromosome pairing was usually observed including the formation of a complete set of 10 synaptonemal complexes (SCs) (Figure 2). As a rule two lateral elements (LE) were aligned along the entire length of the bivalents and the kinetochores were clearly visible. Sometimes the typical tripartite structure of SCs (two LEs and one central element, CE) was observed (Figure 3b). We did not find homologous pairing abnormalities except in a few cases of interstitial or terminal separation of one or both LEs (Figure 3a), and one case of interstitial separation of LEs was accompanied by separation of the CE (Figure 3b). Hence, one dose of the normal allele of the *am1* gene is necessary and sufficient at that locus for normal meiosis in both male and female sporocytes.

The ameiotic1 (am1) mutant allele: The effect of the *am1* allele on meiosis is well known (PALMER 1971; GOLUBOVSKAYA and KHRISTOLYUBOVA 1985). In the homozygous state this allele prevents the beginning of meiosis following the last premeiotic mitosis. Instead of entering meiosis, microsporocytes undergo a synchronized mitotic cell division. Sometimes some cells are involved in a second round of cell division and then meiocytes subsequently degenerate (Figure 4). In megasporocytes the same pattern of abnormalities was observed (Figure 5). Previous electron microscopic examination of thin sections of prophase nuclei

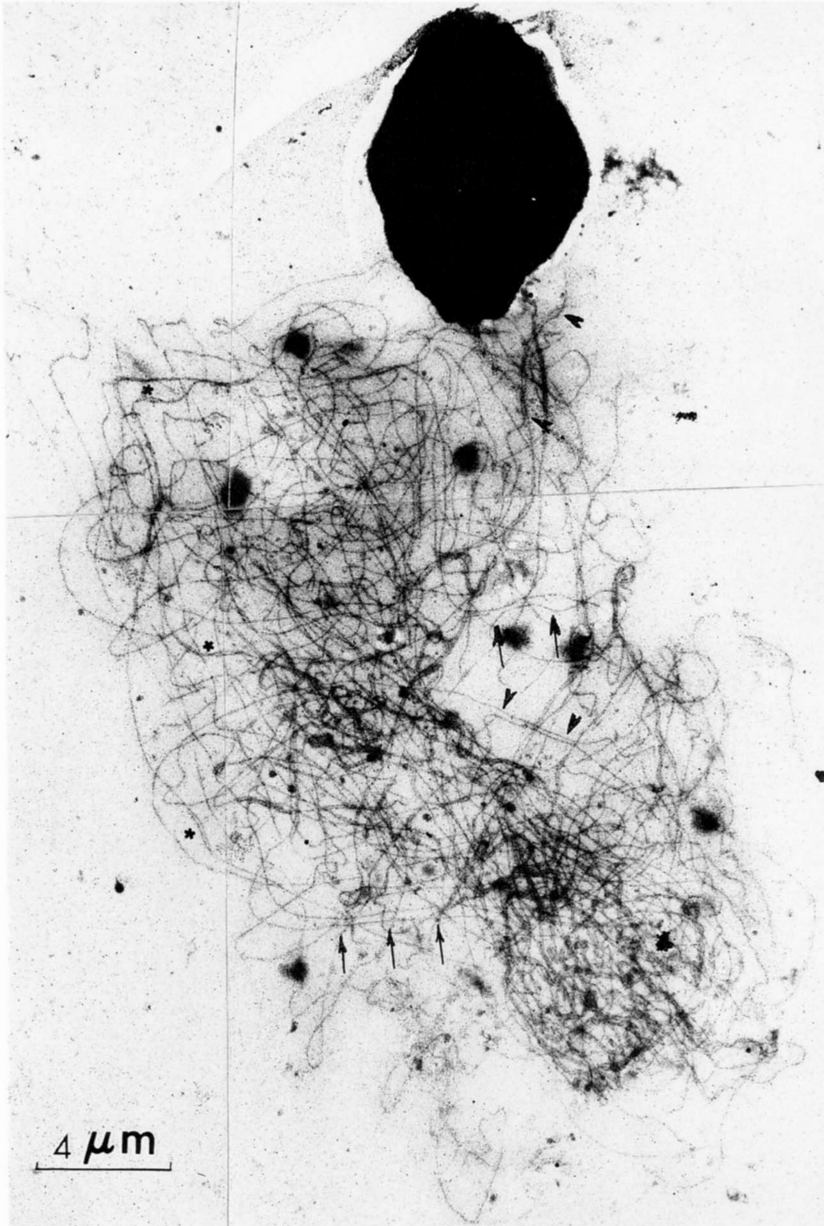

FIGURE 8.—Electron micrograph of an entire leptotene-early zygotene stage *am1-praI* mutant nucleus. Bar shown. Unpaired split axial elements are seen. Short pieces of closely aligned of homologous chromosomes are indicated by arrow.

of the *am1* mutant revealed no synaptic structures; hence, the prophase chromosomes in meiocytes of *am1* plants have the appearance of mitotic chromosomes (GOLUBOVSKAYA and KHRISTOLYUBOVA 1985).

The ameiotic1-prophase arrest (am1-praI) mutant allele: In both the homozygous condition and in combination with the *am1* allele, the *am1-praI* allele caused irreversible arrest in both male and female meiosis at the prophase I stage (Figures 6 and 7). The meiocytes in microsporogenesis degenerated and formed a multinuclear symplast in which lysis and pycnosis of chromatin occurred. Light microscopy did not allow us to define precisely the prophase I stage at which *am1-praI* meiocytes were arrested. We further characterized the state of meiotic arrest in mutant meiocytes by electron microscopic analysis of surface spreads of prophase I microsporocyte nuclei. The leptotene to early zygotene stages were the most advanced prophase I stages of meiosis observed. Among the 66 spread prophase nuclei analyzed, in 51 (77%) nuclei only axial elements characterizing the leptotene stage were observed (Figure 8). Meiosis in the other 15 (23%) nuclei proceeded until zygotene and both short pieces of SC structures and axial elements were observed in these nuclei (Figure 9A). Persistence of synaptic structures at the leptotene-early zygotene stages was not observed in this mutant and their breakdown appeared to start immediately after their formation. The degeneration of the synaptic structures included the splitting of unpaired axial elements and the sticking of some axial elements together with further transformation into amorphous bands. In contrast the SC pieces (paired lateral elements) were more resistant and were the last to disappear (Figure 9B).

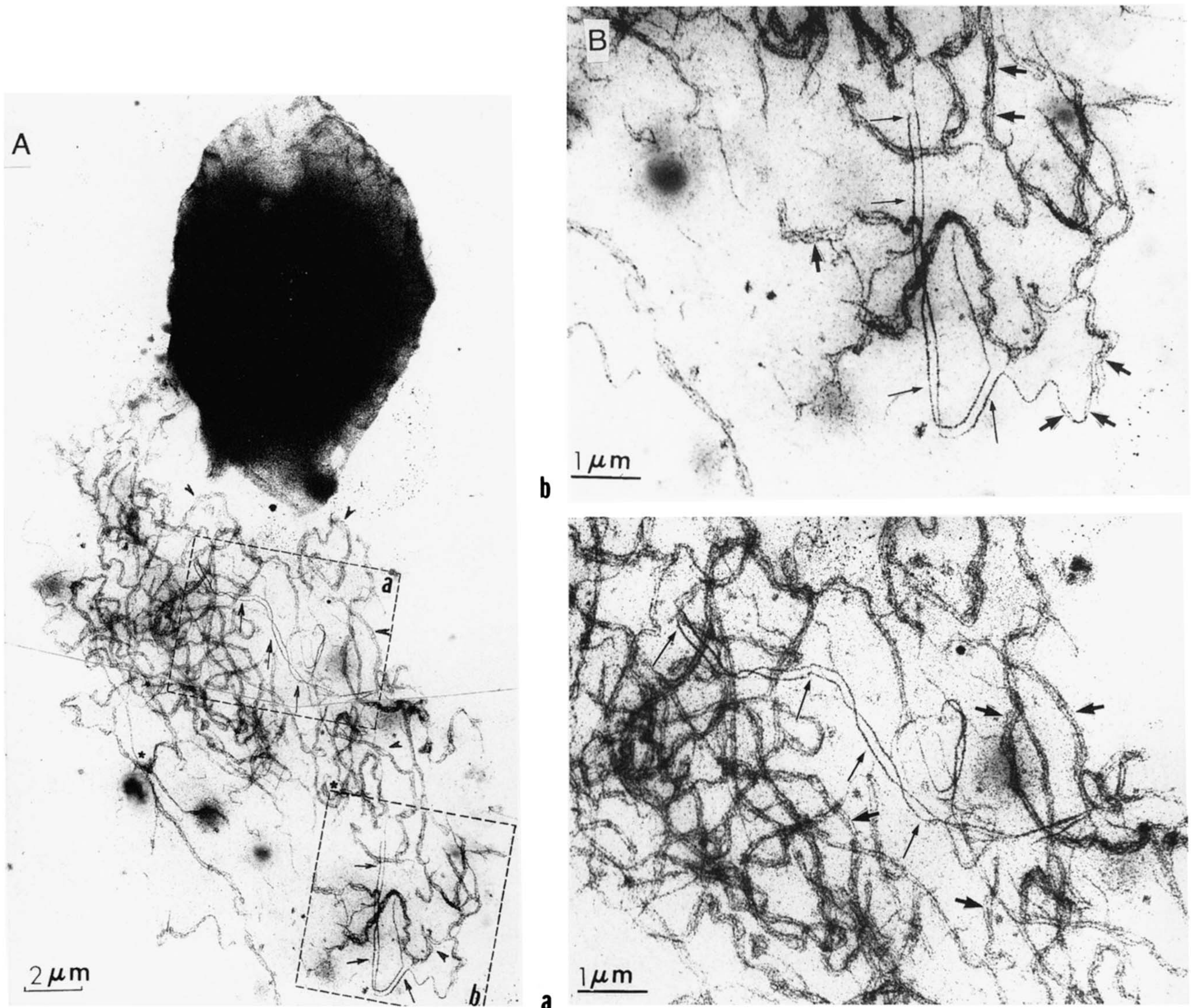

FIGURE 9.—(A) Electron micrograph of an entire zygotene *am1-praI* mutant nucleus. Bar shown. An early zygotene stage is seen, only short pieces of synaptonemal complexes are formed but the SC structure appeared more stable and persisted longer than the unpaired axial elements. (B) Electron micrograph of portions of the same zygotene nucleus shown in (A) but at higher magnification. Bar shown. All unpaired structures are being degraded as evidenced by the splitting and amorphous conditions of axial elements.

The new meiotic mutants: ameiotic-485 (am*-485) and leptotene arrest*-487 (lar*-487):* To provide an opportunity for molecular analysis of meiotic genes, we have isolated several meiotic mutations from an active Robertson's *Mutator* stock. These include two interesting recessive mutations, *ameiotic*-485* and *leptotene arrest*-487*. Genetic analysis of the two new meiotic mutants showed that both of them are recessive monogenic mutations (Table 1B and C). The meiotic phenotype of homozygous *am*-485* mutant plants grown in the greenhouse and in the field is similar in detail with that of Rhoades' *am1* mutant. In pollen mother cells an ameiotic cell cycle occurred instead of normal meiosis (Figure 10), and the mutant plants displayed complete male and nearly complete female sterility.

The phenotype of *lar*-487* is similar to that of the *am1-praI* mutant but is distinguishable from it. In mutant plants grown in the greenhouse, the mutant phenotype was very severe; most of the pollen mother cells stopped in meiosis at the leptotene stage (Figure 11), all nuclei were in a common symplast and they degenerated. Only a few nuclei progressed on into later stages of meiosis but they also degraded. In plants grown in the field, the same meiotic phenotype was observed, but in some anthers the expression of the mutant phenotype was not as strong and some cells progressed in meiosis as far as the pachytene stage. In all cases, however, there was no completion of meiosis because of their irreversible arrest at prophase I. Further genetical analysis including allelism tests and analysis of double mutants should reveal whether these new mutations are in the same or a different pathway controlling the initiation of meiosis as that controlled by the *am1* gene.

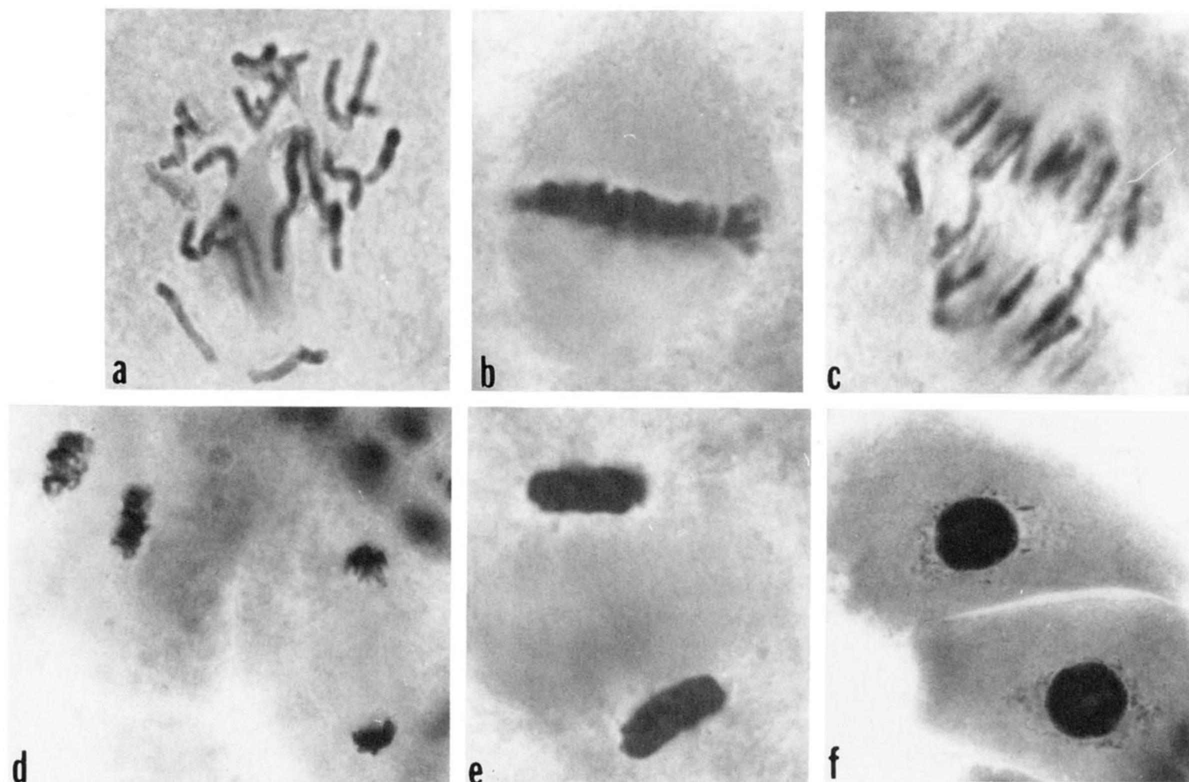

FIGURE 10.—Pattern of meiosis in a homozygous *ameiotic (am*485)* mutant microsporocytes.

Double meiotic mutant analysis: At the present time a challenge to the understanding of meiosis is the integration of the meiotic genes into a coherent pattern governing the same or different pathways of the meiotic program. The construction of 12 combinations of double heterozygotes of the meiotic mutations and our detailed analysis of their progenies from selfing of all of them has revealed much about the interaction of the meiotic genes. The population of self pollinated progeny from each of them were analyzed in the field for male sterility as well as cytologically for meiotic phenotypes (Table 2).

Independent effects of the meiotic mutations: Independence of gene action was observed for five pairs of meiotic mutations, and in all cases the four expected meiotic phenotypes were distinguished among the progenies. The *as1* mutation, which affects homologous chromosome pairing (MAGUIRE and RIESS 1991), and the *ms43* mutation, which affects the control of chromosome segregation, each manifested their own effects in the double meiotic homozygotes; the desynapsis of chromosomes was observed at the first meiotic division and abnormal chromosome segregation characteristic of the *ms43* mutant was observed in anaphase I and II stage cells from the same mutant plants (Table 2).

Three meiotic mutations affect the control of chromosome segregation: *ms43* disturbs the orientation of the spindle apparatus, *dv1* has an effect on MTOC aggregation, and *ms28* is responsible for depolymerization of the spindle apparatus. We analyzed the double homozygous segregants for all three possible combinations of these three mutations and we observed the independent expression of both mutant phenotypes in each combination (Table 2). We also analyzed the combination of the two mutations *ms28* and *ms4* (*ms4* is allelic to *polymitotic1* and responsible for initiation of the postmeiotic cell division cycle) and observed the independent expression of both mutant phenotypes (Table 2) in double homozygous segregants.

Epistatic interaction of meiotic genes: The identification of the correct sequence of genes that act in a single genetic pathway, wherein each gene sequentially regulates the next gene in the pathway, can be obtained by the analysis of double mutants. For a pathway of genes that controls the initiation and stepwise progress of meiosis, this would mean that each mutation is epistatic to the one aligned next in the ordered series of meiotic genes.

The *am1* mutation is epistatic over the *afd1*, which is responsible for transformation of the reductional first meiotic division into an equational one (for "centromere fixation" in the sense of STERN and HOTTA 1967), and also over the *dv1*, and the *ms43* and the *ms28* mutations. The *afd1* mutation is epistatic over the two desynaptic mutations *dsy1* and *as1* and also over the *dv1* mutation (Table 2). These results indicate that the gene product specified by the normal allele of the *ameiotic1* gene is needed for the occur-

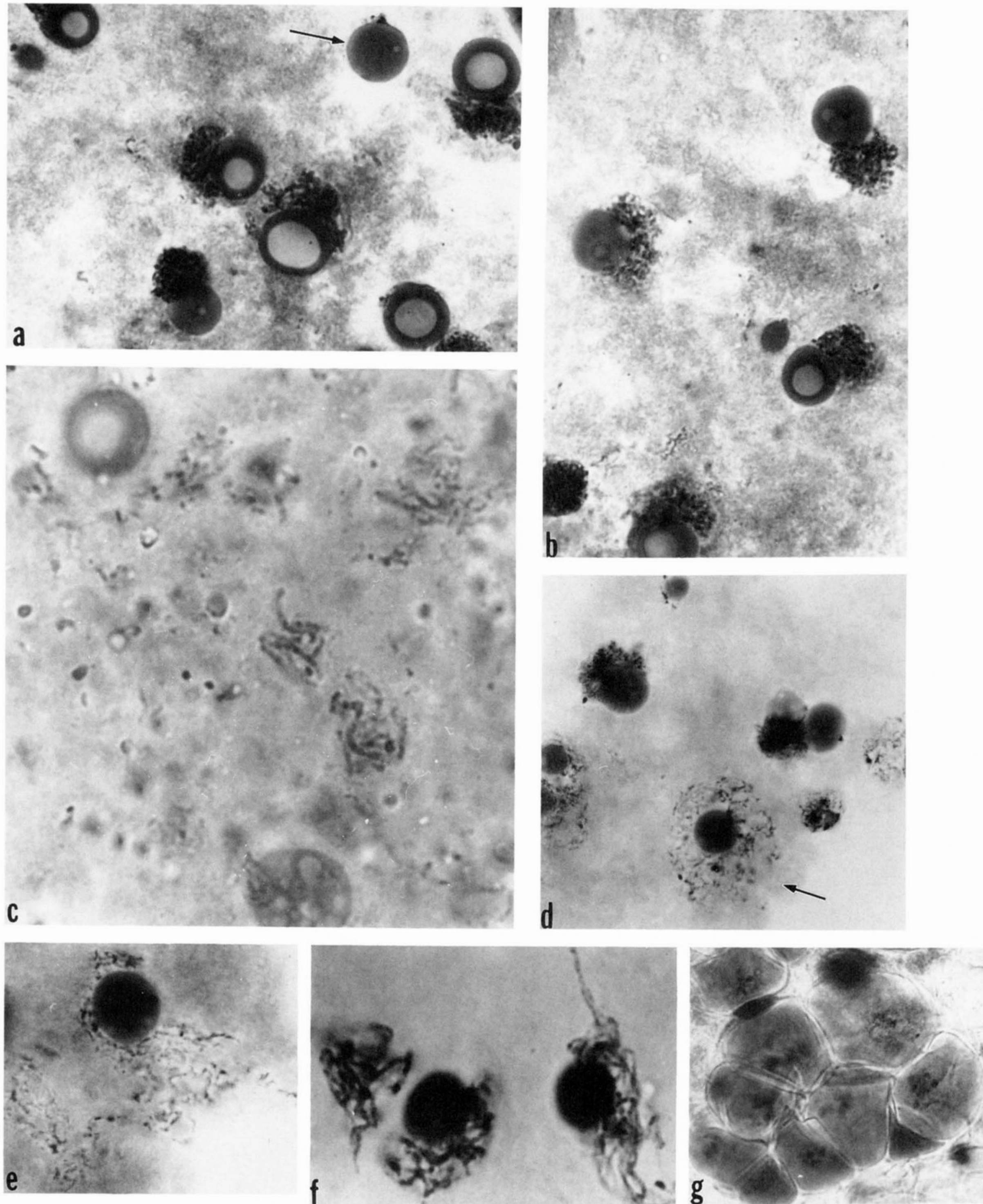

FIGURE 11.—Pattern of meiosis in a homozygous *lar*-487* mutant microsporocytes. (a, b) The leptotene stage in microspore mother cells, the meiocytes lack cell walls and membranes and as a result the individual nuclei random are arrayed in a common coenocytic tissue. In most meiocytes the nuclei did not advance beyond the leptotene stage. (c) Degradation of chromosomes in later meiotic stages, and disorganized chromatin material are seen. (d, e) Process of the despiralization of the chromosomes and as result the loosening of the chromosomes from their association with the nucleolus is shown. (f, g) Degrading meiocytes at more advanced stages: an abnormal pachytene stage (f) and the process of envelope formation (g).

rence of events controlled by the *afd1*, *ms43*, *ms28* and the *dv1* genes. The *afd1* gene product is required for occurrence of the events under control of both the desynaptic genes *dsy1* and *as1*. In other words, the *am1* gene acts earlier in meiosis in maize than these other genes, and it is involved in shifting the genetic program from the mitotic division cycle to the meiotic division cycle. The other main key cytogenetic events

TABLE 2

Character interactions between meiotic genes based upon cytology of progeny from selfing double heterozygotes

Genotype of double heterozygote (a)	(b)	Segregation in field: Fertile 9/16	Sterile 7/16	χ^2 fit	Segregation of meiosis type (cytology): Fertile, normal	Sterile, mutant (a)	(b)	(ab)	Sum	χ^2 fit	Epistasis *vs.* independent relation between *mei* genes
1. *am1/+*	*afd1/+*	140	116	0.25	140:	64 *am1*:	52 *afd1*:	0	256	0.44	*am1* over *afd1*
2. *am1/+*	*ms43/+*	127	87	0.83	59:	27 *am1*:	19 *ms43*:	0	105	0.05	*am1* over *ms43*
3. *am1/+*	*dv1/+*	89	60	0.73	89:	33 *am1*:	27 *dv1*:	0	149	0.84	*am1* over *dv1*
4. *am1/+*	*ms28/+*	196	172	1.34	116:	69 *am1*:	26 *ms28*:	0	211	9.72	*am1* over *ms28*
5. *afd1/+*	*dsy1/+*	179	141	0.01	179:	77 *afd1*:	64 *dsy1*:	0	320	0.38	*afd1* over *dsy1*
6. *afd1/+*	*as1/+*	105	75	0.32	50:	24 *afd1*:	13 *as1*:	0	87	0.93	*afd1* over *as1*
7. *afd1/+*	*dv1/+*	72	46	1.09	65:	27 *afd1*:	14 *dv1*:	0	106	2.22	*afd1* over *dv1*
8. *as1/+*	*ms43/+*	32	21	0.37	32:	7 *as*:	8 *ms43*:	6	53		Independent
9. *ms43/+*	*ms28/+*	98	70	0.30	45:	12 *ms43*:	6 *ms28*:	5	68		Independent
10. *ms43/+*	*dv1/+*	59	39	0.62	36:	14 *ms43*:	10 *dv1*:	1	61		Independent
11. *ms28/+*	*dv1/+*	56	33	1.61	20:	7 *ms28*:	4 *dv1*:	1	32		Independent
12. *ms28/+*	*ms4(po)/+*	118	77	1.44	49:	10 *ms28*:	16 *ms4*:	8	83		Independent

of meiosis–the pairing and the segregation of homologous chromosomes–require the prior activity of this gene.

DISCUSSION

The results of this study merit discussion in three respects. These are the role of the *am1* locus, the sequence of gene action in meiosis, and the significance of the *am*-485* and *lar*-487* mutations.

The first described mutant allele at the *ameiotic1* locus (*am1*) was discovered by MARCUS RHOADES and cytologically analyzed by PALMER (1971). This recessive mutation prevents the entry of maize microsporocytes into meiosis but does not block passage of meiocytes from G_2 into the M phase of the cell cycle. The mutant cells proceed with a mitotic prophase, metaphase, anaphase and telophase, and then degenerate. As long as *am1* was the only mutant allele available for analysis, it remained unclear whether the normal allele functions in switching from a mitotic cell cycle to a meiotic cycle and therefore it is an essential gene for the initiation of meiosis, or whether it simply acts at this juncture to terminate the mitotic sequence. It has therefore remained conceivable that the *am1* locus does not have a direct role in the initiation of the characteristic cytological events of meiotic prophase I: the leptotene → zygotene → pachytene sequence of chromosomal behavior. The work of STAIGER and CANDE (1992) revealed a preprophase band of microtubules in *am1* cells that these authors interpreted as demonstrating that this gene acts at or before the G_2 phase of the cell cycle.

The most significant result presented here is the isolation and cytological characterization of the new mutant allele at the *am1* locus. Because the *am1-praI* recessive allele only blocks meiosis after the meiocytes are well advanced into meiotic prophase I, the function of this locus has become clearer. The nuclei of *am1-praI* meiocytes display a typical leptotene stage configuration of thin, very long, unpaired chromosomes. The chromosomes congregate into a knot on one side of the nucleus, as is typical of the normal zygotene stage, but under light microscopic examination they do not appear to undergo synapsis. However, electron microscopic analysis revealed that some chromosome synapsis was initiated as evidenced by the presence of short pieces of synaptonemal complex. It is apparent therefore that the *am1-praI* allele does not act to preserve a mitotic sequence. Rather, it appears to allow for the initiation of at least some of the crucial chromosomal changes that mark the initiation of meiosis at the level of cytological observation. The timing and extent of molecular events preceding or accompanying these cytological events remains unknown. However, it is worth noting in this regard that, when *cdc4* and *cdc5* homozygous meiotic mutants of *S. cerevisiae* were grown under nonpermissive temperature, reversible pachytene arrest was observed and during maintenance of the elevated temperature normal synaptonemal complexes persisted in the pachytene cells; as a result an elevated meiotic recombination was observed (BYERS and GOETSCH 1982; SIMCHEN *et al.* 1981).

If the *am1* locus normally functions to simply stop the mitotic cell cycle in the archesporial tissue and does not have a role in the initiation of meiosis, then mutations at this locus might be expected to result in the end product of archesporial development, the microsporocytes and the megasporocytes, continuing with the mitotic cell cycle, as is observed in *am1* mutant meiocytes. In this case then the degeneration and death of the meiocytes following their mitotic

division might reflect the occurrence of other independent events appropriate for meiosis in these cells that result in their programmed cell death (MURRAY 1992).

Now this comparative study of the effects of the two mutant alleles of the *ameiotic1* gene in meiosis suggest that the role of the *ameiotic1* gene is essential for the transition from the mitotic cell division cycle into the meiotic cell division cycle. The normal expression of the *ameiotic1* gene appears to be necessary for the irreversible commitment of sporocyte cells to undergo normal meiotic divisions.

Homozygosity for the *am1* mutant allele prevents the switching over of the cell division cycle program and is therefore responsible for omitting the meiotic divisions. It appears that the effect of this allele is in premeiotic interphase. But homozygosity of the *am1-pra1* allele permits cells to enter into the first meiotic prophase and progress until zygotene but they unable to progress further in meiosis. This observation indicates that the effect of the *am1-pra1* allele is at the leptotene-early zygotene stage. Our results indicate that the pathway of initiation of meiotic prophase in maize is controlled by the *am1* gene and it appears that the period of *am1* gene function in meiosis at a minimum covers the interval from premeiotic interphase until the zygotene stage of meiosis. The work of STAIGER and CANDE (1992) indicates that this gene acts during G_2, but whether it acts during the premeiotic G_1 or S phase remains to be determined. Possibly it acts throughout all three phases.

Experiments with transplantation of lily meiocytes into culture defined the leptotene stage (STERN and HOTTA 1967) as the first critical stage for irreversible commitment of meiocytes to meiosis. Experimental inhibition of zyg-DNA synthesis with deoxyadenosine resulted in both the arrest of meiosis and the blocking of pairing of homologous chromosomes, a response similar to the phenotype of the *am1-pra1* mutation. Based upon these similarities the function of the *am1* gene may possibly be directly or indirectly connected with either the metabolism of the zyg-DNA or the L-protein and the *am1-pra1* allele may be a "leaky" mutation (hypomorph) at this locus.

The second matter meriting comment is the path or pathways of genes regulating the initiation and progress of the meiotic sequence. In maize there are two different loci, *am1* (PALMER 1971) and *am2* (CURTIS and DOYLE 1991), that result in the *am1* mutant phenotype. The newly isolated *am*-485* mutation described in this paper may comprise a third locus or it may prove to be allelic to either *am1* or *am2*. It is not known whether *am1* and *am2* are in the same or separate gene pathways. Because of its unique meiotic phenotype, the *lar*487* mutation may represent an additional locus essential for initiation of meiosis or it may prove to be an allele to one of the *am1* loci. However, given the complexity of the biochemical and cytological events required for the normal meiotic sequence, the occurrence of parallel gene pathways seems both reasonable and likely.

Because of this likelihood, the interpretation of the double mutant analysis requires caution. It is evident that *am1* is epistatic over *afd1*, *ms43*, *dv1* and *ms28* and it is also evident that *afd1* is epistatic over *dsy1*, *as1* and *dv1*. However, these results are of limited usefulness for developing a model for the sequence of gene action in meiosis. This is so because both of the epistatic genes block meiosis either at the beginning (*am1*) or during certain crucial early events (*afd1*) so that the later events controlled by the other genes have no opportunity to occur. Whether this gene is similar in function to the *CDC14* gene of *S. cerevisiae* that is responsible for the commitment of cells to meiosis and is also required in the coordination of late meiotic events including the completion of chromosome segregation and spore formation (HONINGBERG, Conicella and R. E. ESPOSITO 1992) remains to be determined. However, the interaction of *afd1* and *dv1* deserves further comment. In the double homozygous recessive (*afd1/afd1*, *dv1/dv1*) mutant plants the divergent spindle phenotype would be expected in the microsporocytes if there is independence of gene action. These cells underwent an abnormal prophase I with a failure of chromosome synapsis, univalent chromosomes congregated at the metaphase plate, and the centromeres of sister chromatids precociously separated in an equational division in accordance with their *afd1/afd1* genotype. Yet, these cells contained a normal-appearing spindle at metaphase. None of the 27 plants exhibiting the *afd* phenotype also exhibited the *dv1* phenotype. It is our interpretation that *afd1* causes an abnormal meiosis rather than substitution of a mitotic division for a meiotic division (a situation where *dv1* would not be expected as is the case with *am1* mutant cells). To the degree that this is true, then the expression of the normal allele of the *afd1* gene is needed for the expression of the *dv1* mutant allele.

The results showing independent relationships in the expression of *ms43* with *ms28* and *dv1* and of *ms28* with *dv1* and with *ms4* are more straightforward to interpret. Because the double recessive mutant plants contained meiocytes displaying both mutant phenotypes, it is evident that these genes occupy separate and independent pathways.

Isolation of the *am*-485* and *lar*-487* mutations opens up new possibilities for molecular studies of the functions of the genes responsible for the control of meiosis initiation. They were isolated from highly active Robertson's *Mutator* stocks so it is likely that they are transposon tagged with a *Mutator* element.

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