Regulation and Execution of Meiosis in Drosophila Males

Jean Maines and Steven Wasserman
Department of Molecular Biology and Oncology
University of Texas Southwestern Medical Center
Dallas, Texas 75235–9148

I. Introduction
   A. Overview of Spermatogenesis
   B. Morphological Description of Meiosis
   C. Identification of Male Meiotic Mutants
II. Regulation of the Meiotic Cell Divisions
   A. Entry into Meiosis
   B. Regulation of the Cell Cycle Machinery
   C. Coordination of Meiosis with Differentiation
   D. Regulation of the Second Meiotic Division
III. Spindle Formation and Function in the Meiotic Cell Divisions
   A. Meiosis-Specific Spindle Architecture
   B. Regulation of Spindle Formation
IV. Cytokinesis
   A. Contractile Ring Assembly and Function
   B. Cytokinesis in the Drosophila Germ Line and in Budding Yeast
V. Conclusions and Perspectives

In this chapter we review the regulation and execution of the meiotic cell divisions in the context of the developmental program that comprises Drosophila spermatogenesis. Male germ line cells undergoing meiosis are readily identifiable and are of a size and abundance that makes this system well suited for morphological characterizations of cell division. Furthermore, a wide range of molecular genetic techniques are available, facilitating mechanistic investigations. We present an overview of key stages in spermatogenesis and, in particular, meiosis. We consider the pathways controlling entry into the meiotic divisions in the context of established cell cycle regulators as well as newly identified loci required for meiotic entry. We then review the assembly and function of both the meiotic spindle and the contractile ring. We conclude with a consideration of questions and problems that await further investigation.

Copyright © 1998 by Academic Press.

I. Introduction

In this chapter we review the regulation and execution of the meiotic cell divisions in Drosophila males. In the past 5 yrs a number of new meiotic mutants
have been identified and the gene products of several loci required for male meiosis have been identified. These genetic and molecular analyses have revealed substantial similarities between meiotic regulation in *Drosophila* and vertebrates and between cytokinesis in *Drosophila* and the budding yeast.

As a model system for the study of meiosis, *Drosophila* spermatogenesis is particularly valuable. The isolation and identification of mutations, ordering of gene activities, and molecular characterization of loci of interest are all readily accomplished. *Drosophila* meiosis occurs in the context of a complex developmental program and is therefore likely to provide insights into the regulation of meiosis in higher eukaryotes, including vertebrates, distinct from those gathered by studying the corresponding process in yeast. Furthermore, *Drosophila* germ line cells undergoing meiosis can be identified easily and are of a size and abundance that makes them well suited to the investigation of mechanisms for the segregation of chromosomes and the partitioning of cytoplasmic contents during cell division. Together, the availability of a range of molecular genetic techniques, the developmental setting in which meiosis occurs, and the accessibility and dimensions of dividing cells make this system a unique and valuable subject of study for probing the regulation and execution of meiosis.

We begin our review by presenting the key morphological characteristics of the stages of spermatogenesis and, in particular, of the meiotic divisions. We turn next to the regulation of entry into the meiotic divisions, then to the assembly and function of the meiotic spindle and to the execution of cytokinesis. We conclude with a consideration of questions and problems that await further investigation.

### A. Overview of Spermatogenesis

In presenting our overview of spermatogenesis, we draw heavily on several excellent reviews available in the published literature (Cooper, 1965; Lindsley and Tokuyasu, 1980; Fuller, 1993), as well as on the elegant characterization of the spermatogenic stem cell populations by Gönczy and DiNardo (1996).

In adults, spermatogenesis takes place within each of two coiled testis tubules. The coordinate activities of two types of stem cells, anchored at the apical tip of each tubule, initiate spermatogenesis (Fig. 1). A germ line stem cell divides asymmetrically, generating a blast cell, the spermatogonium, and the regenerated stem cell. Similarly, the asymmetrical division of two somatic cyst progenitor cells produces a pair of cyst cells and a pair of regenerated stem cells. The cyst cells, which do not divide further, encyst the spermatogonium and its progeny until the completion of sperm differentiation.

Four mitotic divisions of the spermatogonium result in a cyst of 16 primary spermatocytes. These divisions occur with incomplete cytokinesis, such that all 16 spermatocytes are interconnected by cytoplasmic bridges or ring canals. Following the fourth gonial division, spermatocytes carry out premeiotic DNA synthesis and enter an extended G2 or growth phase.
During the growth phase, transcription occurs at a high level as spermatocyte volume increases 25-fold. A wide variety of genes active in many aspects of spermatogenesis are transcribed during this time (Olivieri and Olivieri, 1965; Hoyle and Raff, 1990; Schafer et al., 1990; Eberhart and Wasserman, 1995).
contrast, transcription is nearly undetectable during meiosis and postmeiotic differentiation (Brink, 1968). Furthermore, analysis of 70 male-sterile and 4200 fertile enhancer trap insertions revealed no examples in which the onset of reporter gene expression occurred after the growth phase (Gönčzy et al., 1992; P. Gönčzy, personal communication). It is therefore believed that both meiosis and postmeiotic differentiation rely on the RNAs transcribed during this spermatocyte growth phase.

The first and second meiotic divisions (MI and MII) occur in rapid succession upon completion of the growth phase. The MI division is reductional, segregating the XY pair as well as the three autosomal pairs. The MII division is equational and in general closely resembles a mitotic division.

No meiotic recombination occurs in *Drosophila* males. Classic meiotic stages such as leptotene, zygotene, and pachytene are therefore absent. As in the spermatogonial mitotic divisions, cytokinesis in both meiotic divisions is incomplete. The product of meiosis is thus a cyst of 64 interconnected haploid spermatids encysted by two somatic cells.

Following completion of meiosis, the haploid germ cells carry out an elaborate program of differentiation. The nucleus in each spermatid reforms and the mitochondria fuse to form the mitochondrial derivative, or nebenkern. As each spermatid bundle migrates toward the base of the testis, the nuclei are reduced in volume and transformed in shape from spheres to long, thin cylinders. At the same time, the developing axoneme provides the foundation for generation of the sperm tail and is flanked by the elongating nebenkern. Sperm individualization and coiling ensue, followed by the mature spermatozoa exiting the basal end of the testis and entering the seminal vesicle for storage.

Morphologically, the overall process of spermatogenesis has been highly conserved during evolution (Tokuyasu et al., 1972a,b). Moreover, there are many similarities in the regulation and execution of male germ line development among a wide range of species. Common features of spermatogenesis in flies and mammals include, but are not limited to, the maintenance of a germ line stem cell population, a proliferative spermatogonial stage, a functional interaction between germ line and somatic cells in the testis, the packaging of spermatocyte RNA for later translation, the ordering of the reductional and equational meiotic divisions, and the presence of cytoplasmic bridges among clonally related groups of spermatogonia, spermatocytes, and spermatids. Furthermore, evidence has begun to accumulate that the underlying molecular mechanisms are also conserved.

**B. Morphological Description of Meiosis**

The analysis of meiosis in *Drosophila* males is facilitated by a regular progression in time and space of the stages of gametogenesis. The number of stem cells, the frequency of stem cell division, and the length of each stage are such that all
9. Regulation and Execution of Meiosis in *Drosophila* Males

Stages of spermatogenesis are often present in a single testis. Furthermore, the stages of spermatogenesis are arrayed in a fixed progression along the length of the testis. Within each cyst, developmental events including meiosis are nearly, but not totally, synchronous, occurring as a wave sweeping across the cyst (Lifschytz and Hareven, 1977; see Fig. 2).

The study of meiosis in *Drosophila* males depends largely on the stereotypical morphology of germ cells before, during, and just after the two meiotic divisions (Tates, 1971; reviewed by Fuller, 1993). No systems are currently available for analyzing meiosis in isolated germ cells or in testis culture. However, both the course and outcome of the meiotic divisions can be readily observed in live samples prepared from dissected testes (Kemphues et al., 1980; M. Fuller, personal communication). The meiotic divisions occur along the inside of the first coil of the testis, about one third of the length from the apical end (see Fig. 1). By making an incision near this point in the testis and allowing the contents to spill out, one can release cysts in meiosis, as well as those in slightly earlier or later stages. The preparation is then gently squashed under the weight of a coverslip as liquid is blotted from the edges of the glass.

Much of spermatogenesis can be characterized directly in unfixed and unstained cysts under phase-contrast optics, although detailed analysis of chromosome behavior requires staining with aceto-orcein or Hoechst dye (reviewed by Gatti and Goldberg, 1991). The chronology of spermatogenesis presented in the subsequent discussion is based on the morphological characteristics of unfixed germ cells, as well as on the results of DNA staining and of immunohistochemical labeling of tubulin, lamin, and cyclin A (Cenci et al., 1994; Gönçzy et al., 1994; Lin et al., 1996). Representative phase-contrast micrographs are shown in Fig. 2; micrographs of immunostained samples are presented in Fig. 3.

In the testis, late primary spermatocytes at the end of the growth phase have a large nucleus with a prominent nucleolus (Fig. 2b). DNA staining at this stage reveals the XY pair and the paired bivalent second and third chromosomes as distinct structures at the nuclear periphery (Fig. 3a); the bivalent fourth chromosome is largely heterochromatic and is too small to be readily observed.

In early prometaphase, the nucleus rounds up, the nucleolus becomes smaller and paler, and a cytoplasmic aster-like array appears at one side of the nucleus (Fig. 2c, arrows). The chromosomes begin to condense, frequently at different rates, resulting in one round and one oblong pair of autosomes. Later in prometaphase, the nucleolus breaks down as the nucleus develops an irregular shape. The centrosome divides in two and moves along the outer nuclear envelope to form the two poles of the meiotic spindle (Fig. 3b). The mitochondria are arrayed along the long axis of the nascent meiotic spindle (Fig. 2d). Chromosomes continue to condense and begin to move from the nuclear periphery toward the metaphase plate (Fig. 3c). Cyclin A, previously exclusively cytoplasmic, becomes nuclear.

At metaphase, the distinction between the nucleus and the cytoplasm blurs as
the nuclear lamins disperse (White-Cooper \textit{et al}., 1993; Eberhart and Wasserman, 1995). The bivalent chromosomes condense completely and become attached to spindle fibers emanating from asters located at opposite sides of the nucleus (Fig. 3d, upper cell). Cyclin A degrades (Gönczy \textit{et al}., 1994) and homologous chromosomes segregate.

Chromosomes move rapidly toward the poles in anaphase of MI (Fig. 2e; Fig. 3d, lower cell). The central spindle, composed of interdigitated microtubules, becomes prominent late in anaphase (Fig. 3e). At telophase, the spindle is squeezed into an hourglass shape. Daughter nuclei are again distinct from the cytoplasm, and mitochondria aligned along the spindle are evenly divided into the daughter cells during cytokinesis. The very brief interphase between MI and MII is visualized by the decondensation of the chromosomes and the enlargement of the nuclei (Fig. 2f, arrowhead). Beginning with recondensation of the chromosomes, MII proceeds largely as MI (Fig. 2g), with the significant difference that sister chromatids, rather than homologs, segregate. Each of the two meiotic divisions lasts about 1 \( \frac{1}{4} \) hrs. counting from early prometaphase to the end of telophase (Cenci \textit{et al}., 1994).

Following MII, chromosomes decondense and spermiogenesis, a process encompassing the postmeiotic stages of spermatogenesis, begins (reviewed by Tates, 1971; Lindsley and Tokuyasu, 1980; Fuller, 1993). The individual mitochondria in each spermatid condense into a dark, spherical nebenkern (Figs. 2h,i). Next to each nebenkern, and roughly equal in size and shape, lies a pale nucleus. Because nuclear diameter in early spermatids correlates with chromosome content (Gonzalez \textit{et al}., 1989), variable nuclear size is indicative of a defect in chromosome segregation. Similarly, since inhibition of cytokinesis

---

**Fig. 2** Meiosis in the male germ line: phase-contrast light micrographs. The contents of dissected testes were gently squashed under a coverslip and viewed by phase-contrast microscopy. (a) Young spermatocytes (small arrow) and polar primary spermatocytes (large arrow) from the apical tip of the adult testes. (b) Mature primary spermatocytes. (c) Primary spermatocytes entering the first meiotic division (MI). (d) Cyst of 16 spermatocytes in prometaphase (P) to metaphase (M) of MI. The spindle and chromosomes are in the clear region in the center of each cell and are outlined by phase-dense layers of membrane and mitochondria (m) aligned along the periphery of the spindle. (e) Cyst of 16 cells in metaphase (M), anaphase (A), and telophase (T) of MI. Note the characteristic gradient of meiotic stages within a cyst. (f) Cyst of secondary spermatocytes in interphase between MI and MII. The nuclei (arrowhead) have reformed, and cytoplasmic components (large arrow) are clustered around them. Some cells have begun the second meiotic division (small arrows). (g) Cyst of 32 cells in MII. As in MI, membranes and mitochondria surround the clear region that contains the spindle. The two meiotic divisions are distinguishable from one another by the size and number of cells in a cyst. (h) Cyst of early spermatids at the coalescence or agglomeration stage immediately after the completion of MII. Mitochondria (arrow) have begun to aggregate to one side of each nucleus. (i) Cyst of onion-stage early spermatids. The mitochondria in each cell have aggregated to form the mitochondrial derivative or nebenkern, a structure consisting of wrapped layers of membrane. The nebenkerns (arrow) appear as dark circles adjacent to the white nuclei (arrowhead). Scale bars = 10 \( \mu \)m. (Reproduced with permission from Fuller, 1993.)
Fig. 3  Meiosis in the male germ line: Indirect immunofluorescence, immunohistochemical labeling, and phase-contrast micrographs. Testes were dissected and fixed, then stained with antitubulin antibodies and the DNA-binding dye Hoechst 33258 prior to examination. (a) A mature primary spermatocyte; the nucleolus (arrow) is surrounded by the sex chromosome chromatin. (b) Cell in late meiotic prophase of MI. (c) Cell in prometaphase of MI. The nuclear–cytoplasmic demarcation is no longer visible. (d) Cells in metaphase (top) and early anaphase (bottom) of MI. (e) Cells in late anaphase of MI. In the tubulin panel, note the prominent central spindle; in the phase-contrast and Hoechst panels note the characteristic arrangement of mitochondria along the central spindle. Scale bar = 10 μm. (Adapted with permission of the Company of Biologists Ltd. from Cenci et al., 1994.)
leads to the aggregation of the four nebenkerne into a single mass (Liebrich, 1982), a cytokinesis defect results in early spermatid cells with two, or frequently four, nuclei associated with a single nebenkern. Thus the composition of early spermatid cysts serves as an excellent indicator of any lack of fidelity in the meiotic divisions.

C. Identification of Male Meiotic Mutants

Male meiotic mutants can be identified in several ways. Mutations that affect the execution of meiosis are identified by using genetic markers to monitor defects in chromosome disjunction (reviewed by Hawley, 1993). Although such mutants are very useful for identifying genes involved in karyokinesis (see Chap. 8, by D. P. Moore and T. L. Orr-Weaver, this volume), this approach only identifies viable and fertile mutants. In another approach, a primary screen identifies male sterile mutants. Mutations that affect the regulation of meiosis are then selected from the male-sterile set by dissecting testes from each mutant line and examining the germ line cells under the light microscope (e.g., Castrillon et al., 1993).

Traditionally, mutations have been generated with ethyl methane sulfate (EMS), an alkylating agent that generates point mutations. Efficient and random mutagens such as EMS provide the ability to conduct screens to saturation and to create alleles of differing strengths, including null mutations. More recently, insertional mutagenesis with the P transposable element has been used to generate physically tagged, genetically marked mutations that are easily mapped and analyzed molecularly (Cooley et al., 1988). Although this mutagen shows site selectivity, it allows for straightforward cloning and the ability to induce null mutations by imprecise excision.

II. Regulation of the Meiotic Cell Divisions

A. Entry into Meiosis

Genetic evidence has demonstrated that meiotic cell division in Drosophila males is governed by the well-established regulators of the mitotic cell cycle identified in yeast, frogs, and other species (reviewed by Nurse, 1990). Studies by Nurse, Hartwell, and others identified members of the p34/cdc2 kinase family and the cdc25 phosphatase family as being essential in yeast for initiation of mitosis (Hereford and Hartwell, 1974; Nurse and Thuriaux, 1980; Nurse and Bissett, 1981). Similarly, function of Dmc1c2 and the cdc25 homolog twine is essential in Drosophila males for the onset of the meiotic cell divisions (Jimenez et al., 1990; Lehner and O’Farrell, 1990; Alphey et al., 1992; Courtot et al., 1992; Stern et al., 1993).

The role of the conserved cell cycle oscillator in regulating the spermatogenic meiotic divisions is most readily evident for the twine locus; because this locus is
not required for mitosis, mutations in *twin* do not affect the somatic development or viability of the fly (Alphey *et al.*, 1992; Courtot *et al.*, 1992; White-Cooper *et al.*, 1993). Flies mutant for *twin* are sterile; no meiotic figures are observed in *twin* males, and no products of meiosis are detected.

Premeiotic stages of spermatogenesis, including the mitotic divisions, appear phenotypically normal in *twin* mutants. Chromosome condensation begins, centrosomes duplicate, cyclin A moves into the nucleus, and the nucleolus breaks down. However, chromosome condensation is incomplete, cyclin A fails to degrade, and chromosomes remain at the nuclear periphery. In addition, centrosomes do not separate and the spindle does not form.

*Dmcdc2* is required for mitosis; mutations result in developmental defects and larval lethality (Stern *et al.*, 1993). Lehner and colleagues therefore generated a conditional *Dmcdc2* allele to investigate its role in meiosis (Sigrist *et al.*, 1995). Using information from yeast studies, they engineered a temperature-sensitive *Dmcdc2* allele and introduced this transgene construct into a genetic background mutant for *Dmcdc2*. The meiotic defects in spermatocytes expressing *Dmcdc2* at the nonpermissive temperature closely resembled those in the *twin* mutant. Chromosomes partially condensed, but did not congress at the metaphase plate or attach to asters. Cyclin A persisted until well after the onset of spermatid differentiation, indicating an arrest prior to the activation of the cyclin A degradation pathway. Collectively and individually, the *twin* and *Dmcdc2* phenotypes indicate a block in the execution of metaphase of the first meiotic division.

The biochemical function of both *Dmcdc2* and *twin* in the onset of the meiotic cell divisions has been inferred from studies of mitotic homologs in other systems. The activity of cdc2 kinases is controlled by their association with a positive regulatory subunit, cyclin, and by a specific pattern of phosphorylation (reviewed by Nurse, 1990; Solomon, 1993). Phosphorylation of threonine 167 (*Schizosaccharomyces pombe* numbering) is essential for kinase activity (Gould *et al.*, 1990; Solomon *et al.*, 1990; Krek and Nigg, 1991a,b). Furthermore, cdc2 kinase activity is inhibited by phosphorylation of a residue that lies in the ATP-binding site; tyrosine 15 in *S. pombe* (Gould *et al.*, 1990), and threonine 14 in higher eukaryotes (Krek and Nigg, 1991a,b; Norbury *et al.*, 1991). Removal of these inhibitory phosphates is required for activation of cdc2 kinase and execution of M-phase (Dunphy and Newport, 1989; Gautier *et al.*, 1989; Labbe *et al.*, 1989; Morla *et al.*, 1989; Solomon *et al.*, 1990). Exit from M-phase requires inactivation of the cyclin/cdc2 complex, as catalyzed by the ubiquitin-mediated targeting of cyclin for degradation (Glotzer *et al.*, 1991).

Phosphatases belonging to the cdc25 family remove the inhibitory phosphates on tyrosine 15 and threonine 14 of cdc2 (Dunphy and Kumagai, 1991; Gautier *et al.*, 1991; Strausfeld *et al.*, 1991). In *S. pombe* there is a single phosphatase of this class, cdc25; cdc25 mutants arrest in G2 with no active cdc2 kinase (Russell and Nurse, 1986; Moreno *et al.*, 1990). In higher organisms there are two or more cdc25 loci, with a particular isoform often dedicated to the germ line (Sadhu *et
Based on the structural and functional conservation in cdc25 and cdc2 genes, we and others refer to the point at which twine and Dmdc2 are required in spermatogenesis as a G$_2$/M transition (White-Cooper et al., 1993; Eberhart and Wasserman, 1995). We note, however, that although cdc25 and cdc2 activity is thought to be necessary for chromosome condensation at the G$_2$/M transition in yeast and frogs, this does not appear to be the case in Drosophila male meiosis, because chromosomes partially condense in twine and Dmdc2 mutants. Cdc2/cdc25-independent regulation of the condensation state of chromosomes has also been observed in female meiosis (for discussion, see Murray and Hunt, 1993).

As a result of their failure to initiate the meiotic cell divisions, twine and Dmdc2 mutant testes accumulate cysts containing 16 tetraploid cells. However, many aspects of spermiogenesis, particularly the programs for sperm head and tail shaping, still occur (White-Cooper et al., 1993). Consequently, tetraploid spermatids, but no motile sperm, are produced. Insofar as abrogation of the meiotic divisions does not prevent the wild-type postmeiotic program of differentiation, meiosis and spermiogenesis must be under separate regulation.

**B. Regulation of the Cell Cycle Machinery**

1. **Control of twine and Dmdc2 Activity**

The phenotypic similarities between Dmdc2$^{ts}$ and twine mutants suggest that Twine is the phosphatase responsible for the dephosphorylation and activation of cdc2 at meiotic entry. However, the mechanism by which the meiotic G$_2$/M transition is triggered is unclear. The twine gene is transcribed relatively early during the long G$_2$ phase, well before the onset of meiosis (Alphey et al., 1992; Courtot et al., 1992). Thus, twine RNA accumulation is not sufficient to promote entry into the meiotic divisions. This situation differs from that observed in early embryos, where the transcription of the string cdc25 phosphatase gene appears to be both necessary and sufficient to drive the G$_2$/M transition in some embryonic cell cycles (Edgar and O'Farrell, 1989, 1990). It is possible that Twine activity is sufficient to drive the G$_2$/M transition in meiosis, but that this activity is regulated at the level of protein accumulation. Alternatively, other factors, such as cyclin accumulation or the activity of a cdc2-activating kinase, may trigger meiotic entry. Upstream factors that regulate the trigger are not known, but candidates have been identified and are described in the next section.
2. Candidate Regulators of the Cell Cycle Machinery

Two genes, *pelota* and *boule*, share the loss-of-function phenotype observed with *twine* and *Dmcdc2* (Eberhart and Wasserman, 1995; Eberhart et al., 1996). Spermatocytes in *boule* and *pelota* mutants carry out chromosome condensation and centrosome duplication but not spindle formation, nuclear lamina breakdown, or chromosome congression at the metaphase plate. Despite their failure to execute meiosis, mutants in either locus still exhibit many aspects of postmeiotic differentiation (Fig. 4B).

The phenotypes shared by *pelota*, *boule*, *twine* and *Dmcdc2* define a class of genes required for entry into the meiotic cell division. We term this class the Twine class and believe that these four genes act coordinately to regulate cell cycle progression during male meiosis. Based on the known biochemical functions of proteins in the cdc25 and cdc2 families, we speculate that *twine* and *Dmcdc2* act together to trigger entry into the meiotic cell divisions and that *pelota* and *boule* are part of a pathway controlling the expression or activity of *twine* and *Dmcdc2*.

Sequence motifs in Boule and Pelota indicate that both proteins may associate with RNA molecules. The Boule protein contains a conserved RNA-binding domain, the ribonucleoprotein (RNP)-type RNA-binding domain. A 52-amino acid domain in Pelota has substantial sequence similarity to a domain common to a family of eukaryotic polypeptide chain-release factors (Frolova et al., 1994; Koonin et al., 1994). These release factors are thought to interact directly with RNA, perhaps through this conserved domain.

Although *boule* and *pelota* have a common meiotic phenotype and may have related biochemical activities, the two loci differ markedly in their patterns of expression and function. The *pelota* gene is broadly expressed and appears to act in mitosis as well as meiosis. Strong *pelota* alleles have a rough eye phenotype, indicative of a disruption in the precise pattern of cell divisions in the developing eye disc (Eberhart and Wasserman, 1995). In addition, *pelota* mutations reduce fecundity in females and dissection of ovaries reveals defects prior to the meiotic divisions. In contrast, *boule* is expressed only in the testis, and *boule* mutations apparently affect only meiosis (Eberhart et al., 1996).

Homologs for both *pelota* and *boule* have been identified and have, in some cases, been subjected to genetic analysis. Counterparts of *pelota* have been identified in humans, plants, worms, yeast, and archaeons (Lalo et al., 1994;
Verhasselt et al., 1994; Wilson et al., 1994; Eberhart and Wasserman, 1995; Bult et al., 1996; Ragan et al., 1996). Mutations inactivating the *S. cerevisiae* pelota homolog, *dom34*, result in defects in both mitosis and meiosis. Mutants in *dom34* execute mitosis slowly, while the meiotic divisions are too rapid, producing fewer spores than wild type. These growth defects can be rescued by expression of *pelota* in a *dom34* mutant yeast (Eberhart and Wasserman, 1995). Together, the molecular and genetic analyses suggest that *pelota* represents an evolutionarily conserved function broadly required for cell division.

A human locus has been identified that has similarities in sequence, expression pattern, and male sterile loss-of-function phenotype to *boule*. The Y-linked *Deleted in Azoospermia* (DAZ) locus, a candidate gene for the Azoospermia Factor (AZF), is deleted in one of eight men producing little or no sperm (Reijo et al., 1995). Boule and DAZ share similarity with one another and with autosomal vertebrate DAZ homologs in both the RNA-binding domain and a second domain termed the DAZ repeat. The positions of the RNP domain and the first DAZ repeat are conserved in all DAZ family members, including Boule. All of the DAZ-related loci are expressed predominantly or exclusively in the testis. These results indicate that Boule is likely to be a functional homolog of members of the DAZ family and suggest a conservation in the regulation of male germ line development from flies to humans.

C. Coordination of Meiosis with Differentiation

A set of loci distinct from the Twine class has been found to be required for the G2/M transition in *Drosophila* males. Mutations in this second group, the Spermatocyte Arrest class, block both the meiotic cell division and postmeiotic spermatid differentiation, resulting in the accumulation of cysts of 16 spermatocytes. Genes in this class appear to act upstream of the Twine class in the G2/M transition and may play a role in the coordination of cell division with postmeiotic differentiation.

Members of the Spermatocyte Arrest class of loci include *meiosis 1 arrest* (*mia*), *cannonball* (*can*), *spermatocyte arrest* (*sa*), and *always early* (*aly*) (Lin et al., 1996). For each gene, except *mia*, multiple alleles have been isolated, all of which seem to affect male fertility specifically. No meiotic figures or products are observed in the mutants, and there is no evidence of spermatid formation. The cells that accumulate are thus spermatocytes arrested at the end of the growth phase (Fig. 4C).

The meiotic entry arrest phenotypes of *mia*, *can*, *sa*, and *aly* are quite similar to one another and suggest in each case an arrest late in meiotic prophase. Chromosomes begin to condense but remain at the nuclear periphery, and no spindles are formed. The arrest phenotype of *aly* mutants is somewhat different from that of
the other mutants of the Spermatocyte Arrest class in that the partially condensed chromosomes are less well defined.

Two lines of evidence suggest that Spermatocyte Arrest mutants are blocked at an earlier point in the cell cycle than those in the Twine class. First, distinct dark nucleoli persist in *mia*, *can*, *sa*, and *aly* spermatocytes, whereas the nucleoli break down in *twine*, *pelota*, *boule*, and *cdc28* mutants (Sigrist et al., 1995; Lin et al., 1996). Second, whereas cyclin A enters nuclei in mutants of the Twine class, it remains predominantly cytoplasmic in mutants of the Spermatocyte Arrest class (Lin et al., 1996; Gönczy et al., 1994; Sigrist et al., 1995; H. White-Cooper and M. Fuller, personal communication).

Although *mia*, *can*, *sa* and *aly* have not been characterized at the molecular level, recent data suggest a basis for the Spermatocyte Arrest phenotype. White-Cooper and Fuller have found that RNA transcripts for several genes active in postmeiotic differentiation, including *fuzzy onions* and *janB* (Yanicostas et al., 1989; Fuller, 1993), are absent, or greatly reduced in abundance, in *mia*, *can*, *sa*, and *aly*, but not *twine*, mutants (H. White-Cooper and M. Fuller, personal communication). The Spermatocyte Arrest loci also appear to be required for expression of meiotic regulators, although in this case the mode of action is different for *aly* than for *can*, *sa*, or *mia*. In *aly* mutants the RNAs for *cyclin B*, *twine*, and *boule* fail to accumulate. In the other Spermatocyte Arrest mutants, these RNAs are present, but there are defects at the level of protein expression for Twine and Boule. Specifically, White-Cooper and Fuller find that expression of a chimeric *twine-lacZ* reporter transgene, as monitored by β-galactosidase activity, is abolished in *mia*, *can*, and *sa* mutants. In addition, we have found that at least *mia* and *sa* are required for Boule protein accumulation (J. Maines, M. Cheng, and S. Wasserman, unpublished data).

The expression data described in the preceding paragraphs indicate that the Spermatocyte Arrest loci are needed during the spermatocyte growth phase for the expression of genes required for either meiosis or differentiation. The *aly* gene is likely to act at the level of transcription or message stability. Mutations in *mia*, *can*, and *sa* alter protein levels for some genes (meiotic regulators) and RNA levels for others (spermatid differentiation factors). These Spermatocyte Arrest loci may therefore be required for transcription, and thereby regulate accumulation of translational factors, or for translation, and thereby control levels of transcriptional factors.

**D. Regulation of the Second Meiotic Division**

Although MI requires a special mechanism for reductional division, the means for equatorial division must still be available for MII. Furthermore, although MII resembles a mitotic division, the checkpoint mechanism that ensures that DNA replication has occurred prior to nuclear division in mitosis must be absent or
inactivated to allow MII. The basis for these differences and, more generally, the
regulation of MII is not well understood. No genes have been identified for which
loss-of-function mutations specifically block MII. There is evidence, however,
that three loci—Dmcdc2, twine, and roughex—play a critical role in control of
MII.

Experiments with the previously described Dmcdc2 transgene indicate that
Dmcdc2 is required for both MI and MII in males (Sigrist et al., 1995). Dmcdc2
mutant males that carry two copies of the Dmcdc2 transgene were sterile at all
temperatures, with defects in meiosis. At 18°C they executed MI but failed in
MII, producing 32-cell spermatid cysts. When shifted as adults to 27°C, they
failed to execute either meiotic division. Two additional copies of the Dmcdc2 transgene restored fertility at 18°C but did not alter the phenotype at the nonper-
missive temperature.

Two lines of evidence suggest that twine is also important in the regulation of
MII. First, in the wild-type, late primary spermatocytes express twine, but no
detectable levels of string. In a twine mutant, background expression of string
from a heat-shock promoter is sufficient to rescue entry into MI, but not MII
(Sigrist et al., 1995). Second, in females, where both string and twine are present
at meiosis, twine mutants execute MI but not MII, further suggesting that twine
plays a unique role in MII (Courtot et al., 1992; White-Cooper et al., 1993).

Since flies with two copies of the Dmcdc2 transgene execute MI but fail in
MII, the level of kinase activity may be particularly important for entry into MII.
The Twine phosphatase may be important for maintaining this level of cdc2
kinase activity. Alternatively, it is possible that the second meiotic division
depends on a product of cdc2 kinase activity formed during the first meiotic
division. Support for this idea comes from an analysis of the gene roughex (rux).

Experimental evidence suggests that roughex acts as a negative regulator of
MII (Gönczy et al., 1994). An increase in roughex gene dosage blocks MII while
allowing normal execution of MI, resulting in 32-cell cysts of postmeiotic germ
cells. The X and Y chromosomes disjoin in these cells, indicating that MI occurs
while MII is blocked. Reciprocally, germ cells from rux mutant flies attempt to
execute an extra MII-like division. After normal MI and MII divisions, the
haploid nuclei of rux mutant germ cells become less discrete, chromosomes
recondense, a monopolar meiotic spindle assembles, and chromosomes randomly
distribute into two daughter nuclei. This extra division resembles MII in that
there is no preceding DNA synthesis or apparent centrosomal duplication. To-
gether, these data strongly suggest that rux negatively regulates MII, with excess
rux preventing MII and insufficient rux permitting an additional MII.

Although the rux mutant phenotype is observed after a normal MII, there are
increases in cyclin A levels prior to MI. Furthermore, lowering the dose of either
twine or cyclin A suppresses the extra division, consistent with the hypothesis that
excess cdc2 activity is responsible for the extra meiotic division in the rux mutant.
Because cyclin A is synthesized prior to MI, degraded at metaphase of MI, and not resynthesized for either MII or the aberrant MII-like division, \textit{rux} must be acting at or before the first meiotic division to influence the second division, presumably by limiting the amount of cdc2 kinase activity available for MII.

**III. Spindle Formation and Function in the Meiotic Cell Divisions**

As discussed in the introduction to this chapter, meiosis in \textit{Drosophila} males offers the opportunity for a detailed analysis of cell division through the combined use of genetics and cell biology. Among genes required for wild-type chromosome pairing and segregation, a number appear to act specifically in males. For example, the X-linked \textit{equational producer (eq)} mutation, discovered by Schultz in 1934, causes rare equational nondisjunction of the X chromosome, resulting in sperm carrying either two X chromosomes or no sex chromosome (Morgan et al., 1934). The fact that the \textit{eq} mutation has little or no effect on disjunction in females (Valentin, 1984) suggests that the \textit{eq} locus may provide a starting point for dissecting the differences in the regulation of MII in males and females. Another X-linked mutation, \textit{Recovery Disrupter (RD)}, results in a 50% reduction in the number of male progeny through an unprecedented mechanism: fragmentation of the Y chromosome during meiosis (Novitski and Hanks, 1961; Erickson, 1965). However, the subjects of chromosome pairing and segregation are reviewed elsewhere in this volume (see Chap. 3, by B. D. McKee, and Chap. 8, by D. P. Moore and T. L. Orr-Weaver), and we will therefore restrict our attention in this section to spindle structure and function.

The alignment of the phase-dense mitochondria along the length of the meiotic spindle renders the spindle readily visible by phase-contrast examination during MI and MII. For both meiotic divisions the spindle resembles that of a mitotically dividing cell but is much larger than that in a somatic nucleus. The meiotic spindles are thus especially well suited for immunofluorescence studies (Gatti and Goldberg, 1991).

For many loci required for cell division, hypomorphic mutations are sterile and have readily apparent defects in germ line mitoses and meioses. Furthermore, the phenotypic effects of null mutations in essential cell division functions can often be examined in the male germ line. Although mitotic proliferation is necessary for embryogenesis, the maternal contribution of most genes required for cell division is sufficient for this stage of development (Gatti and Baker, 1989). As a result, the larva is typically the first developmental stage in which cell division defects arise, with death at pupation resulting from failure in imaginal disc division. The consequences of a loss-of-function mutation in a cell division gene
A. Meiosis-Specific Spindle Architecture

The composition of the spindles in male meioses makes them particularly amenable to genetic analysis. Although comparable in large part to mitotic spindles, male meiotic spindles differ from their counterparts in both mitoses and female meioses in at least one major component. Among the four-β-tubulin isoforms in *Drosophila*, one, the β₂-tubulin, is present only in postmitotic spermatocytes and spermatids and is the predominant β-tubulin in the meiotic spindle. Because the four β-tubulins are encoded by distinct loci, it is therefore possible to specifically disrupt the meiotic spindle by mutation.

The testis-specific tubulin subunit, β₂-tubulin, is synthesized just prior to meiosis (Kemphues et al., 1982). Levels of the β₁-tubulin present during the germ line mitoses decrease at the same time, such that during meiosis, levels of β₂-tubulin protein are an order of magnitude higher than those of β₁-tubulin (Kemphues et al., 1980).

A number of mutations in the β₂-tubulin gene have been identified that disrupt both meiosis and spermatid differentiation (reviewed by Raff and Fuller, 1984). Recessive alleles that produce an unstable β₂-protein (class I alleles) fail to accumulate any tubulin, since the α subunit is destabilized in the absence of a β subunit with which to dimerize (Kemphues et al., 1982). Chromosomes condense normally in such mutants but do not align at metaphase and fail to migrate to opposite poles. The nuclear envelope thickens and the nucleolus breaks down, but spindles are absent and no cytokinesis occurs. These defects can be phenocopied by administration of colchicine (Kemphues et al., 1982), consistent with an absolute requirement for microtubules in spindle formation, chromosome segregation, and cytokinesis.

Chromosomes in class I β₂-tubulin mutants, while not undergoing segregation, nonetheless decondense and then recondense as if a normal interphase had occurred. Metaphase of the equational MII division thus lacks the checkpoint mechanism apparent in metaphase of the equational mitotic divisions (reviewed by Murray, 1992; Sluder and Rieder, 1993). This difference may reflect the fact that MII, unlike a mitotic division, is not directly preceded by DNA synthesis.

Although the β-tubulins are highly conserved in sequence, an “isoform swap” experiment suggests that the biochemical properties of the β₂-tubulin are significantly different from those of the other isoforms. Taking advantage of the thorough characterization of the β₂ tubulin promoter in the Raff and Renkawitz-Pohl laboratories (Hoyle and Raff, 1990; Kemphuses et al., 1982; Michieks et al., 1993), Hoyle and Raff demonstrated that expression of the β₁-tubulin under
9. Regulation and Execution of Meiosis in *Drosophila* Males

control of the $\beta_2$-tubulin regulatory elements fails to complement the meiotic defect in a $\beta_2$-tubulin (Hoyle and Raff, 1990).

**B. Regulation of Spindle Formation**

The *merry-go-round* (*mgr*), *polo*, and *abnormal spindle* (*asp*) genes play a role in the function and regulation of the meiotic spindle. All are essential genes, functioning in mitosis as well as meiosis, and mutations in each result in distinct spindle defects. In *mgr* males, no nuclear or cytoplasmic divisions take place. Instead, the meiotic chromosomes form a circle around the center of the cell and appear to be associated with a monopolar spindle (Gonzalez et al., 1988). Mutations in *polo* result in a high frequency of tetrapolar and multipolar spindles in meiosis (Fig. 5) and of monopolar spindles in mitosis (Sunkel and Glover, 1988). In *asp* mutants the meiotic spindles are irregularly shaped and are associated with an extensive cytoplasmic microtubule network that is absent in wild-type cells.

Defects in either *polo* or *asp* result in an asymmetric distribution of chromosomes and mitochondria among spermatids. Mutations in these two loci show a strong genetic interaction, suggesting that *polo* and *asp* act in a common pathway governing spindle formation (Llamazares et al., 1991).

Of the three genes just described, only *polo* has been cloned (Sunkel and Glover, 1988; Llamazares et al., 1991). The encoded protein, a serine/threonine kinase, has homologs in mammals as well as both fission and budding yeast (Clay et al., 1993; Golsteyn et al., 1994; Holtrich et al., 1994; Hamanaka et al., 1995; Ohkura et al., 1995). Mutations in the yeast homologs have mitotic and, in the case of the budding yeast homolog *cdc5*, meiotic defects (Schild and Byers, 1980). For the fission yeast homolog, *plol*, mutations result in defects in spindle pole body duplication and/or separation (Ohkura et al., 1995), similar to the aberrations seen in mitotic *polo* cells.

For mitosis, at least one additional protein kinase, the product of the *aurora* locus, is required for wild-type spindle formation. Mutations in *aurora* result in spindle defects due to a failure in centrosome separation (Glover et al., 1995). For meiosis, it is possible that an *aurora*-like protein kinase activity functions in coordination with *polo* in regulating the centrosome.

**IV. Cytokinesis**

As in other animal cells, cytokinesis in *Drosophila* spermatocytes begins at anaphase and is first visible as a furrowing of the plasma membrane in a plane orthogonal to the long axis of the spindle (reviewed in Satterwhite and Pollard, 1992; Fishkind and Wang, 1995; Miller and Kiehart, 1995). Cleavage is initiated
Fig. 5  Meiotic spindles in live cells. (A, B) A cyst of wild-type cells undergoing the second meiotic division. (C, D) A tetrapolar spindle in a homozygous *polo*¹ cell undergoing meiosis. (E, F) A multipolar meiotic spindle in a homozygous *polo*¹ cell. Scale bars = 10 μm. (Reproduced with permission of the Company of Biologists Ltd. from Sunkel and Glover, 1988.)
by a contractile ring, a transient cytoskeletal structure containing a network of antiparallel actin filaments. Cross-linked bipolar myosin II filaments are believed to generate the force for contraction of the ring, constricting the cleavage furrow approximately 10-fold. The gap that remains is filled in during complete cytokinesis in the soma but persists during incomplete cytokinesis in the germ line and is transformed into an intercellular bridge termed a ring canal.

Little is known about how contractile rings are assembled, how contraction is regulated, or how the molecular pathways for complete and incomplete cytokinesis differ. Recent experiments in the Drosophila germ line have started to provide answers to these questions. Genetic studies have identified a number of loci encoding components of contractile rings or ring canals (Neufeld and Rubin, 1994; Robinson et al., 1994; Hime et al., 1996). Sequencing of cDNAs from these loci has revealed surprising molecular parallels between incomplete cytokinesis in Drosophila gametogenesis and the variant form of cytokinesis observed in the division of the budding yeast (Castrillon and Wasserman, 1994; Sanders and Field, 1995; Longtine et al., 1996). Finally, antibodies raised against the products of the Drosophila genes have enabled cell biologists to begin to dissect the organization of these cytoskeletal structures.

A. Contractile Ring Assembly and Function

Two critical issues with regard to assembly of the contractile ring are how the position of the cleavage furrow is determined and how components of the contractile ring are recruited into a functional apparatus. There is substantial evidence that positioning of the cleavage furrow is mediated by the spindle asters (Rappaport, 1986). Characterization of germ line cell divisions in wild-type and mutant males indicates that the spindle may also play a role in ring assembly.

During wild-type meioses and mitoses the protein product of the KLP3A (Kinesin-Like-Protein-at-3A) gene is highly concentrated in the midbody, a structure surrounding the interdigitated microtubules that constitute the central spindle (see Fig. 3e). Dissection of males carrying a null mutation in KLP3A mutant males revealed defects late in anaphase of meiosis I: the central spindle and midbody were absent (Williams et al., 1995). Cytokinesis was also affected. KLP3A spermatids frequently contained an abnormally large nebenkern associated with two equally sized nuclei; rarer spermatids had a single nebenkern and four nuclei. Since in both cases the nuclei were always of equal size, there appeared to be a defect in cytokinesis but not in chromosome segregation.

Together, the molecular and phenotypic characterizations of KLP3A indicate that this gene plays a key role in assembly of the midbody and central spindle, and that these structures are in turn critical for proper assembly or function of the contractile ring.
B. Cytokinesis in the *Drosophila* Germ Line and in Budding Yeast

Whereas cytokinesis in *Drosophila* male meiosis involves a contractile stage followed by stabilization of an intercellular gap, cytokinesis in *S. cerevisiae* has no contractile stage, but rather comprises a membrane fusion event and septum formation at the neck between mother cell and bud (Sanders and Field, 1995). It was therefore quite unexpected that two *Drosophila* loci active in meiotic and mitotic cytokinesis would have homologs in the budding yeast.

The *diaphanous* (*dia*) and *peanut* (*pnut*) genes are apparently required only for cytokinesis (Castrillon and Wasserman, 1994; Neufeld and Rubin, 1994). Null alleles give rise to polyploidy in homozygous larvae; imaginal discs are severely reduced, with many multinucleate cells. Cleavage furrows are absent. Despite the polyploidy resulting from the cytokinesis defect, chromosome segregation appears to be relatively normal. In many hyperploid *dia* cells, anaphases involve bipolar spindles and chromosomes are segregated equally, with no lagging chromosomes or other abnormalities. In other, more extremely hyperploid cells, spindles are multipolar, as has been observed in other mutants that produce hyperploid cells (Gatti and Baker, 1989; Karess et al., 1991).

Defects in cytokinesis during meiosis are readily apparent in males homozygous for a hypomorphic *dia* mutation (Fig. 6). Most *dia*~1~ spermatids contain two or four nuclei of normal size associated with a single large nebenkern, the size of which correlates with the number of nuclei. The presence of rare spermatids containing eight nuclei (Fig. 6, inset) indicates that cytokinesis in *dia*~1~ males can also fail in the mitotic divisions preceding meiosis.

Diaphanous is one of a group of proteins termed the formin homology (FH) family, which includes the *S. cerevisiae* protein BNII (Bud Neck Involved 1) (Castrillon and Wasserman, 1994). Peanut belongs to the highly conserved septin protein family, which has four members in the budding yeast and at least three in *Drosophila*. Although *pnut* is the only *Drosophila* septin gene for which mutations have been identified, all four *S. cerevisiae* septins—*CDC3*, *CDC10*, *CDC11*, and *CDC12*—have been shown to be necessary for cytokinesis (Hartwell, 1971; Byers and Goetsch, 1976; Longtine et al., 1996). Intriguingly, *BNII* was first identified on the basis of a synthetic lethal interaction with a mutation in the *CDC12* septin (Longtine et al., 1996), which suggests that the two genes act in the same pathway and perhaps interact.

Fig. 6  Cytokinesis defect in *dia*~1~ testis. Photographs are of unfixed testis contents visualized by phase-contrast microscopy. (A) Part of a 64-cell cyst of wild-type spermatids. Each spermatid contains a single pale nucleus (arrowhead) and a single dark nebenkern (arrow). Although this cyst is intact, spermatid cysts typically rupture into smaller groups of cells owing to the absence of a fixation step. (B) Group of six *dia*~1~ spermatids, each containing four nuclei (arrowheads) associated with a single large nebenkern (arrow). *Inset*: Single *dia*~1~ spermatid containing eight nuclei. Scale basis = 10 μm. (Reproduced with permission of the Company of Biologists Ltd. from Castrillon and Wasserman, 1994.)
9. Regulation and Execution of Meiosis in *Drosophila* Males

![Image A](image_a)

![Image B](image_b)
Diaphanous and Peanut, as well as their yeast counterparts, are found in regions of the cell where cytokinesis occurs. Peanut and Diaphanous localize to the contractile ring and intercellular bridge of dividing cells (Neufeld and Rubin, 1994; B. S. Gish and S. Wasserman, unpublished results), while BNI (Longtine et al., 1996) and the yeast septins localize to the region of the mother-bud neck. There the septins appear to be components of the ring of 10-nm filaments (Haarer and Pringle, 1987; Ford and Pringle, 1991; Kim et al., 1991).

It is possible that Diaphanous and Peanut are not required for the contractile phase of cytokinesis but rather are necessary to maintain the cytoskeleton in the contracted state. The existence of S. cerevisiae homologs for these proteins would then be less paradoxical. Two lines of evidence support this hypothesis. First, it has recently been shown that Peanut protein persists at the cleavage furrow after arrest, localizing to the ring canals of spermatocyte and spermatid cysts (Hine et al., 1996). Second, other members of the FH family, to which Diaphanous belongs, are not limited in function to cytokinesis but participate in a variety of cytoskeletal-mediated processes in a wide range of organisms (Nurse et al., 1976; Emmons et al., 1995; Petersen et al., 1995; Chang et al., 1996).

C. Additional Cytokinesis Factors

A number of genes in addition to diaphanous and peanut have been shown to encode components of the cleavage furrow or contractile ring. For the products of many of these loci, immunolocalization studies have been carried out in wild-type spermatocytes as well as in spermatocytes with specific defects in cytokinesis. For a subset, mutations have been isolated and analyzed. These studies have provided insights into the mechanisms for assembly and function of the contractile ring and, in addition, have contributed to the increasing number of immunological and genetic reagents available for dissecting these mechanisms.

Studies in other species have shown that coflin, an actin-binding protein, localizes to cleavage furrows (Nagaoka et al., 1995). A member of the actin depolymerization family, coflin competes with tropomyosin, myosin, and villin for actin binding in vitro (Nishida et al., 1984; Nishida, 1985; Pope et al., 1994). Mutations in a Drosophila coflin locus, twinstar (tsr), were identified among a collection of recessive lethal mutations exhibiting mitotic abnormalities in larval brains (Gunsalus et al., 1995). Examination of primary spermatocytes in tsr males revealed a failure of cytokinesis, as well as defects in centrosome migration and separation at prometaphase of both MI and MII. During prophase of MI, aggregates of actin were found associated with centrosomes. In addition, during anaphase of both meiotic divisions, misshapen F-actin-containing structures were observed at the normal site of contractile ring formation.

Gatti, Goldberg, and colleagues argue that the function of twinstar is to regulate the assembly of actin into cytoskeletal structures (Gunsalus et al., 1995). They speculate that in the absence of tsr activity, there is an uncontrolled accu-
mulation of actin at nucleation centers situated near centrosomes. In addition, they suggest that a disorganized recruitment of actin into the contractile ring precludes a wild-type disassembly of the ring.

Anillin, a component of both contractile rings and ring canals, was first identified by actin affinity chromatography (Miller et al., 1989; Field and Alberts, 1995). This Drosophila protein, which has no known homologs, bundles actin filaments in vitro and is found specifically in dividing cells. Immunofluorescence studies have demonstrated a cell cycle-dependent compartmentalization of anillin (Field and Alberts, 1995). Anillin is nuclear during interphase, whereas during anaphase and telophase it is concentrated in the cleavage furrow. In spermatocytes, anillin localizes to contractile rings and, later, to ring canals, whereas actin is lost following constriction of the contractile ring (Hime et al., 1996; M. Gatti, personal communication).

Together, the pattern of localization of anillin in vivo and the biochemical properties of the protein in vitro suggest two possible functions in meiotic cytokinesis (Field and Alberts, 1995). First, anillin may play a role in organizing the contractile apparatus, perhaps serving as part of the link between the plasma membrane and the developing actin-based ring. Second, anillin may help to stabilize the intercellular ring canals (Hime et al., 1996).

V. Conclusions and Perspectives

Although a number of genes involved in the regulation and execution of meiosis in Drosophila males have been cloned and characterized, we understand the basic biochemical function for only those few, such as twine and β2-tubulin, for which there has been extensive characterization in other systems. Furthermore, there are many loci for which no molecular studies have been conducted. This set of loci includes the Spermatocyte Arrest class of meiotic entry genes and the cytokinesis loci shank, fumble, and four wheel drive (Castrillon et al., 1993; Fuller, 1993). It is clear, therefore, that much remains to be learned about the known meiotic loci.

Genetic screens aimed at identifying additional factors required for meiosis hold considerable promise. There have been only a few large-scale screens for male-sterile mutations affecting spermatogenesis or, more specifically, meiosis (Lifschytz and Hareven, 1977; Hackstein, 1991; Castrillon et al., 1993; C. Wu, M. Fuller, and S. DiNardo, personal communications). Furthermore, none of these screens has achieved saturation, and the number of genes identified to date is likely to be small relative to the total number of relevant loci (for discussion, see Castrillon et al., 1993). Efforts directed at identifying chromosome-specific disjunctional mutations have also been quite limited (reviewed by Hawley, 1993). Further screens for mutations affecting male fertility or meiotic chromosome disjunction should therefore provide a substantially expanded basis for analyzing the molecular mechanisms governing meiosis.

Although work on the molecular foundations of Drosophila male meiosis is
still in an early stage, two general themes have become apparent. One is that the meiotic divisions constitute a program that is in large measure independent of the developmental events that either precede or follow MI and MII. For example, the onset of the meiotic cell divisions is apparently indifferent to the faithful execution of the preceding mitotic divisions. Lifschytz has found that mutations that reduce the number of mitotic gonial divisions do not block meiosis (Lifschytz and Meyer, 1977), and we and others have found that meiosis can occur following a failure in cytokinesis during the gonial mitoses (see, e.g., Fig. 6, inset). Similarly, the initial phase of the program for spermatid differentiation is unaffected by mutations that disrupt chromosome segregation (e.g., class I β2-tubulin alleles) or that block the meiotic cell divisions altogether (e.g., Twine class mutations).

Another theme emerging from the studies reviewed in this chapter is that mechanisms governing the regulation and execution of meiosis have been broadly conserved. As discussed earlier, components and regulators of the cell cycle oscillator in Drosophila male meiosis, such as twine and boule, have counterparts in the male germ line of vertebrates, (e.g., Cdc25C and DAZ), and components of the contractile apparatus in flies (Diaphanous and Peanut) have homologs in the budding yeast (BN11p and Septins).

A number of questions remain unexplored regarding the regulation and execution of the meiotic cell divisions in males: Are the waves of meiosis within cysts triggered or coordinated by a single cell? If so, is this spermatocyte, like the oocyte, one of the two cells among 16 that are directly interconnected with four other cells? What role, if any, do the somatic cyst cells play in the control of meiosis? Why is the onset of MI regulated by a testis-specific gene when the striking difference between meiosis in males and females occurs at MII? The answers to these and other questions will, we hope, become clear as we learn more about the genes, the gene products, and the pathways governing Drosophila male meiosis.

Acknowledgments

We thank Helen White-Cooper, Minx Fuller, Maurilio Gatti, Pierre Gönzy, and Stephen DiNardo for communication of unpublished results; Pierre Gönzy, Stephen DiNardo, and Dennis McKearin for critical reading of the manuscript; and Gianni Cenci, Minx Fuller, and Alisha Tizenor for assistance in preparation of figures. This work was supported by a grant to S.A.W. from the Excellence in Education Fund.

References


9. Regulation and Execution of Meiosis in *Drosophila* Males


9. Regulation and Execution of Meiosis in *Drosophila* Males


9. Regulation and Execution of Meiosis in *Drosophila* Males

*a saccharomyces pombe*: A developmentally controlled function needed for conjugation. *Mol.


Cytol.* 105, 245–281.


Biol.* 5, 1213–1215.


