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Regulation and Execution of Meiosis in *Drosophila* Males

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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.

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

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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 G_2 or growth phase.

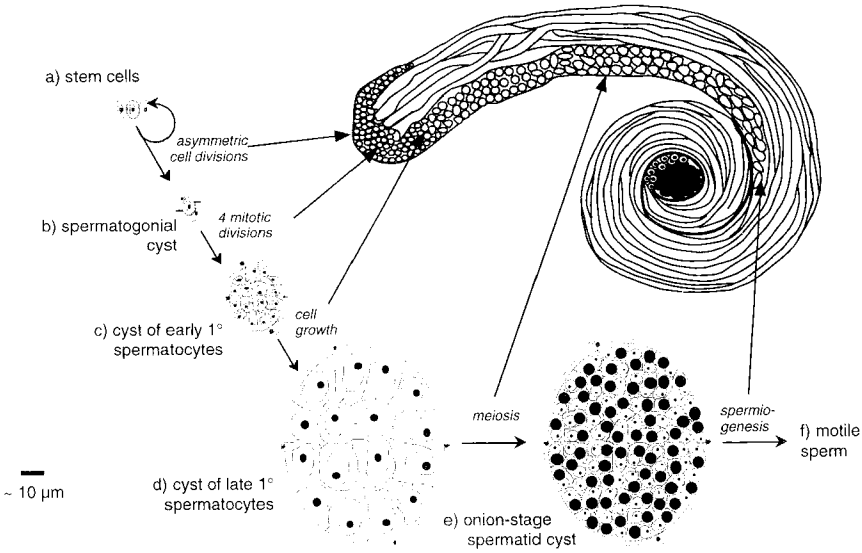


Fig. 1 Schematic representation of landmark stages of spermatogenesis in *Drosophila melanogaster*. Lettered drawings are intended to convey the appearance of the germ line cells as they appear in phase-contrast microscopy of unfixed testis contents. The two somatic cyst cells that surround each group of germ cells are also shown but are not readily visualized by phase contrast. Open circles represent nuclei. Germ cell cytoplasm is shown in gray; somatic cells are white. Nucleoli are shown as black spots in (a–d); the black spots in nuclei in (e) represent protein bodies.

The accompanying arrows and line drawing of a testis tubule indicate the approximate location within the testis where each stage begins. The apical tip is to the left; the basal end, which *in vivo* connects to the seminal vesicle, is shown at the center of the coil. Fully elongated spermatids have their nuclei at the basal end; their tails stretch nearly to the apical tip, a distance of about 2 mm.

(a) Germ line stem cells and somatic cyst progenitor cells are attached to a specialized structure of somatic origin, the hub (Hardy *et al.*, 1979). The germ line stem cell is closely associated with two cyst progenitor cells. These stem cells divide asymmetrically. (b) The single spermatogonium and the two cyst cells are released, while the parental stem cells (one germ line, two somatic) remain attached to the hub. (c) Four rounds of mitotic division with incomplete cytokinesis result in a cyst of 16 early primary spermatocytes. These spermatocytes enter a growth phase, during which time they increase in volume 25-fold. (d) The late primary spermatocytes undergo two meiotic divisions to yield a cyst of 64 haploid spermatids. (e) The nuclei reform (open circles) and the mitochondria fuse to form the nebenkern (solid circles). At the onion stage, so called because of the multilamellate appearance of the nebenkern in electron micrographs, both of these structures are closely associated and highly uniform in size and shape. (f) Each spermatid is transformed into a mature sperm through a complex process of cytodifferentiation (Tokuyasu *et al.*, 1972a,b). This process involves nuclear condensation as well as the dramatic elongation of the axoneme and the mitochondrial derivative to form the sperm tail. The final steps of spermatogenesis are individualization and coiling. (Adapted with permission from Castrillon *et al.*, 1993.)

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). In

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 (Gönczy *et al.*, 1992; P. Gönczy, 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

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).

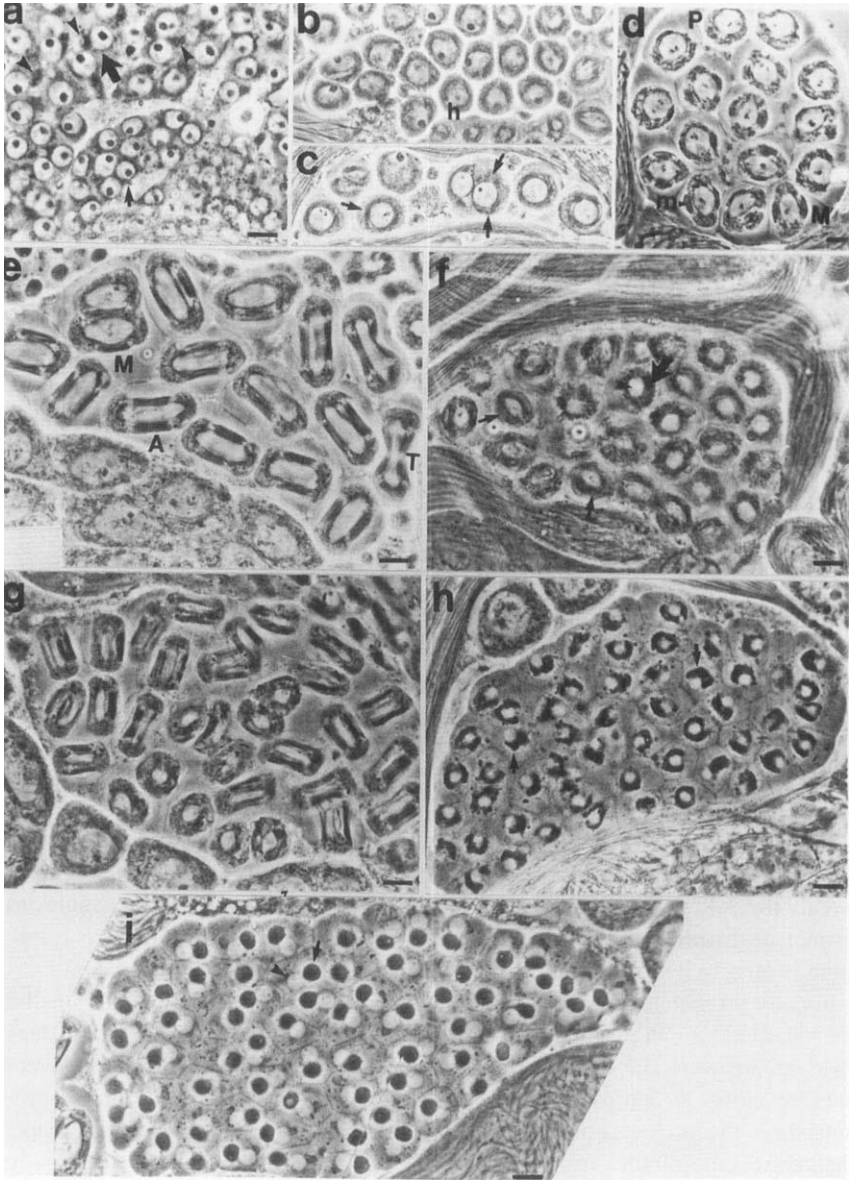
The study of meiosis in *Drosophila* males depends largely on the stereotypical morphology of germ cells before, during, and just after the two meiotic divisions (Tates, 1971; reviewed by Fuller, 1993). No systems are currently available for analyzing meiosis in isolated germ cells or in testis culture. 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; Gönczy *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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the nuclear lamins disperse (White-Cooper *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 *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 ¼ hrs, counting from early prometaphase to the end of telophase (Cenci *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 *et al.*, 1989), variable nuclear size is indicative of a defect in chromosome segregation. Similarly, since inhibition of cytokinesis

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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 nebenkerne (arrow) appear as dark circles adjacent to the white nuclei (arrowhead). Scale bars = 10 μm. (Reproduced with permission from Fuller, 1993.)

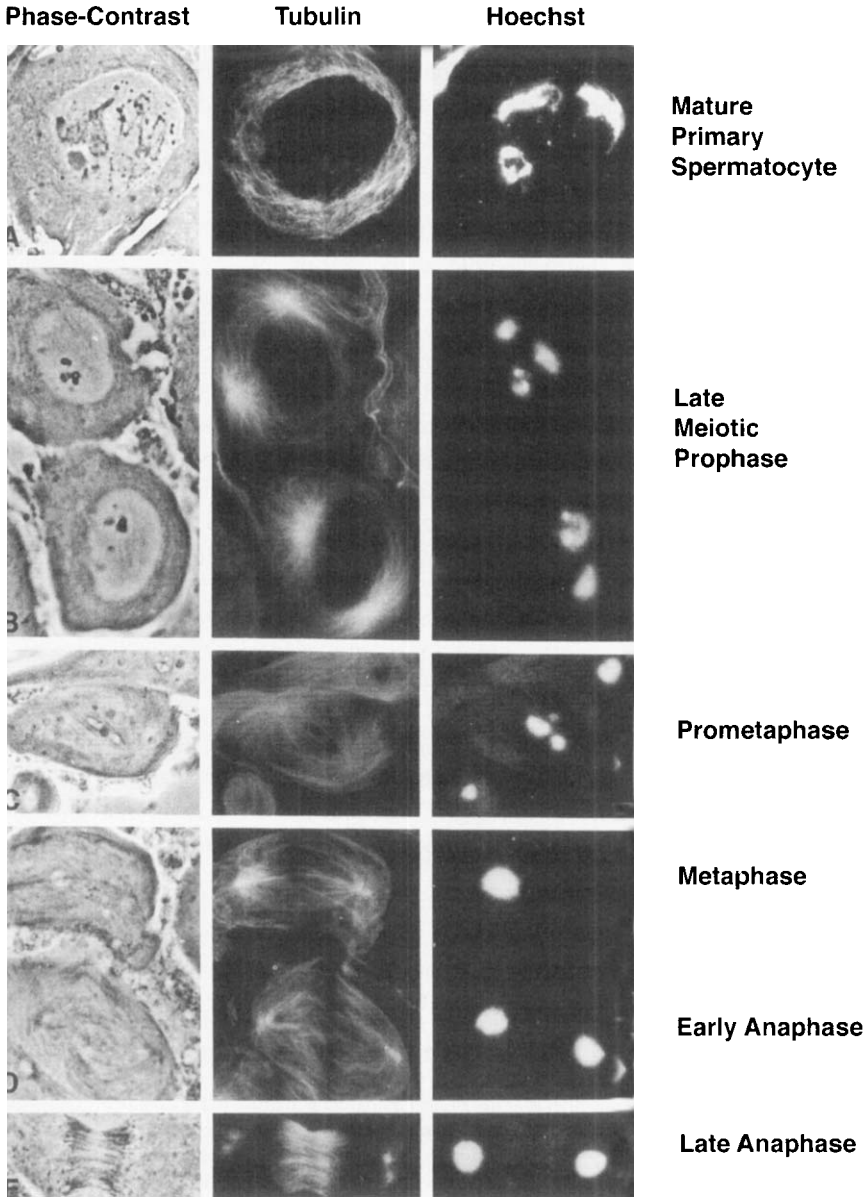


Fig. 3 Meiosis in the male germ line: Indirect immunofluorescence, immunochemical labeling, and phase-contrast micrographs. Testes were dissected and fixed, then stained with antitubulin antibodies and the DNA-binding dye Hoechst 33258 prior to examination. (a) A mature primary spermatocyte; the nucleolus (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 *Dmcdc2* 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 *twine* 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 *twine* are sterile; no meiotic figures are observed in *twine* males, and no products of meiosis are detected.

Premeiotic stages of spermatogenesis, including the mitotic divisions, appear phenotypically normal in *twine* 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 $Dmcdc2^{ts}$ at the nonpermissive temperature closely resembled those in the *twine* 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 *twine* and *Dmcdc2* phenotypes indicate a block in the execution of metaphase of the first meiotic division.

The biochemical function of both *Dmcdc2* and *twine* 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 G_2 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*

al., 1990; Alphey et al., 1992; Wu and Wolgenmuth, 1995). In *Drosophila* two cdc25 phosphatases have been identified. The String cdc25 is active primarily in mitosis, whereas Twine is active primarily in meiosis (Edgar and O'Farrell, 1989, 1990; Jimenez *et al.*, 1990; Alphey *et al.*, 1992; Courtot *et al.*, 1992).

Based on the structural and functional conservation in cdc25 and cdc2 genes, we and others refer to the point at which *twine* and *Dmcdc2* 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 *Dmcdc2*^{ts} 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 *Dmcdc2*^{ts} 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 *Dmcdc2* Activity

The phenotypic similarities between *Dmcdc2*^{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*^{ts} (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).

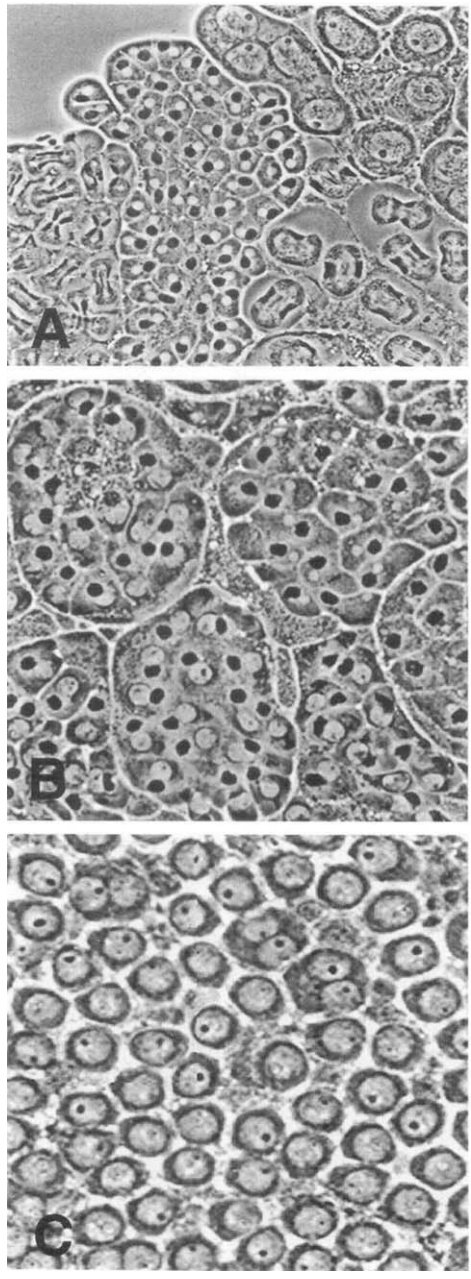
The phenotypes shared by *pelota*, *boule*, *twine* and *Dmcdc2*^{ts} define a class of genes required for entry into the meiotic cell division. We term this class the Twine class and believe that these four genes act coordinately to regulate cell cycle progression during male meiosis. Based on the known 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;

Fig. 4 Phenotypes of Twine and Spermatocyte Arrest class mutants. Photographs are of unfixed testis contents viewed by phase-contrast microscopy. (A) Wild-type testes contents. Spermatocytes, cells in meiosis I, and onion-stage spermatids with dark nebenkerne and pale nuclei can be seen. (B) *pelo*^{1} homozygote 16-cell cysts. The tetraploid spermatids each contain a nucleus and nebenkern. Because of their 4N chromosomal content, the spermatid nuclei are abnormally large. (C) Testes from *sa*^{1}/Df(sa) [*Df(3L)Pc-cp1*] males contain plentiful mature primary spermatocytes but lack postmeiotic stages. Photographs are approximately the same scale. (Adapted with permission of the Company of Biologists Ltd. from Eberhart and Wasserman, 1995, and Lin *et al.*, 1996.)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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 G_2/M transition in *Drosophila* males. Mutations in this second group, the Spermatocyte Arrest class, block both the meiotic cell division and postmeiotic spermatid diferentiation, resulting in the accumulation of cysts of 16 spematocytes. Genes in this class appear to act upstream of the Twine class in the G_2/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 I 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 $cdc2^{ts}$ 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*^{ts} 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*^{ts} 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*^{ts} transgene restored fertility at 18°C but did not alter the phenotype at the nonpermissive 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*^{ts} 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. Together, 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, *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 *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 *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 *eq* mutation has little or no effect on disjunction in females (Valentin, 1984) suggests that the *eq* locus may provide a starting point for dissecting the differences in the regulation of MII in males and females. Another X-linked mutation, *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

can therefore frequently be characterized by examining germ line divisions in the larval testis.

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 β_2-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, β_2-tubulin, is synthesized just prior to meiosis (Kemphues *et al.*, 1982). Levels of the β_1-tubulin present during the germ line mitoses decrease at the same time, such that during meiosis, levels of β_2-tubulin protein are an order of magnitude higher than those of β_1-tubulin (Kemphues *et al.*, 1980).

A number of mutations in the β_2-tubulin gene have been identified that disrupt both meiosis and spermatid differentiation (reviewed by Raff and Fuller, 1984). Recessive alleles that produce an unstable β_2-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 β_2-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 β_2-tubulin are significantly different from those of the other isoforms. Taking advantage of the thorough characterization of the β_2 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 β_3-tubulin under

control of the β_2-tubulin regulatory elements fails to complement the meiotic defect in a β_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, *plo1*, 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

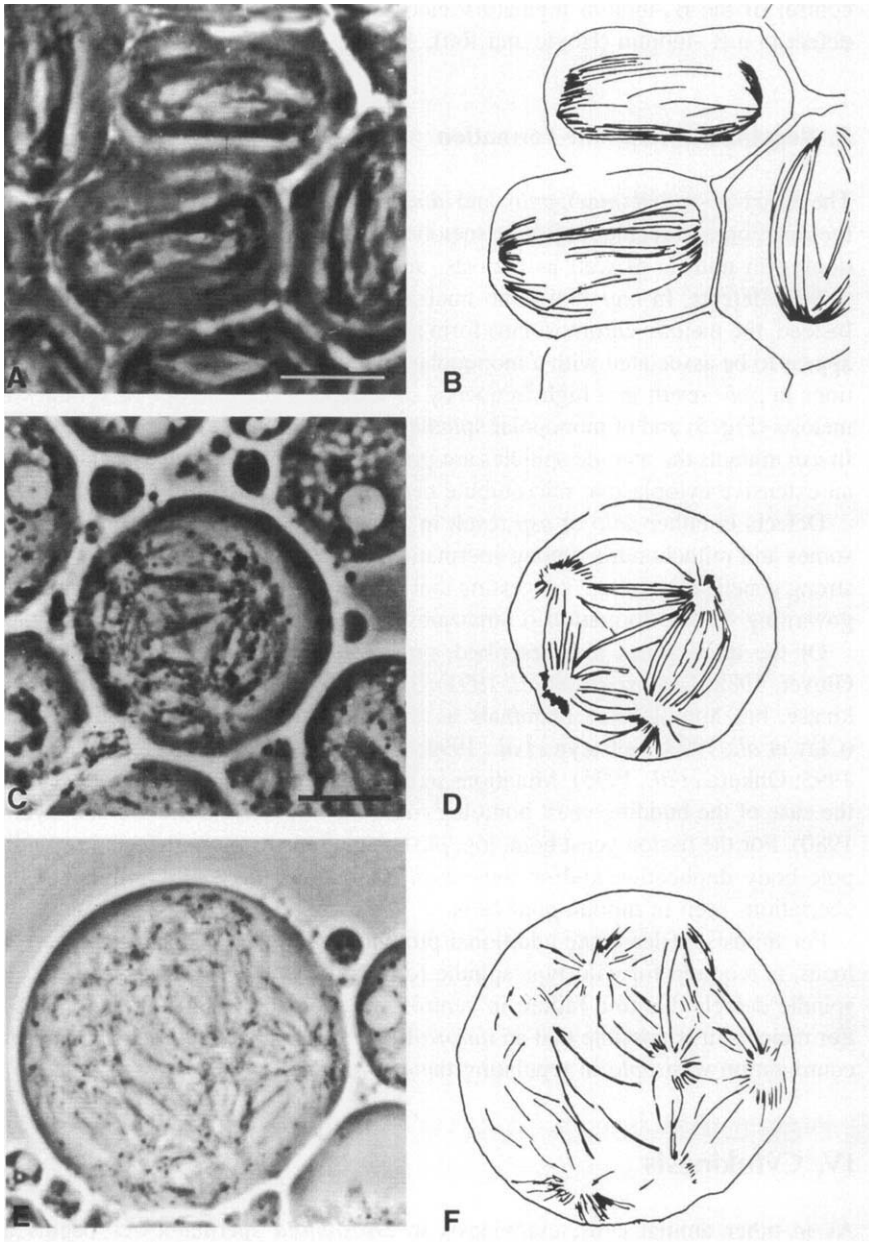


Fig. 5 Meiotic spindles in live cells. (A, B) A cyst of wild-type cells undergoing the second meiotic division. (C, D) A tetrapolar spindle in a homozygous *polo*^{1} cell undergoing meiosis. (E, F) A multipolar meiotic spindle in a homozygous *polo*^{1} cell. Scale bars = 10 μm. (Reproduced with permission 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

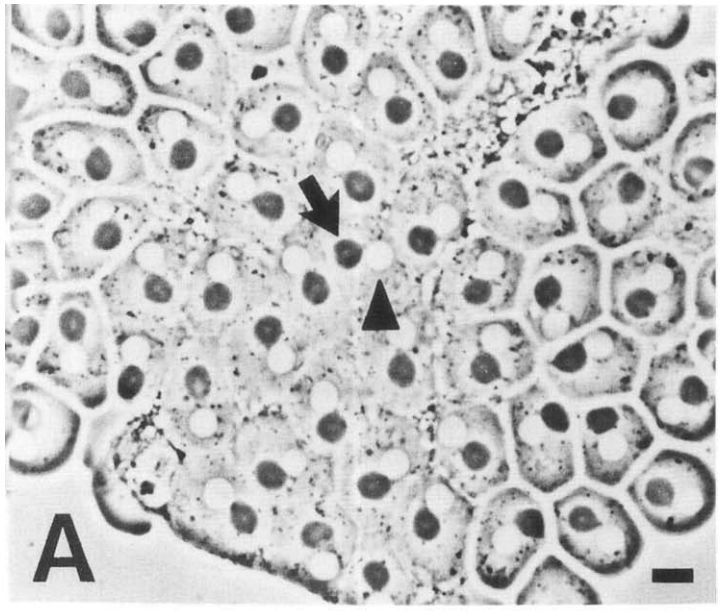
Whereas cytokinesis in *Drosophila* male meiosis involves a contractile stage followed by stabilization of an intercellular gap, cytokinesis in *S. cerevisiae* has no contractile stage, but rather comprises a membrane fusion event and septum formation at the neck between mother cell and bud (Sanders 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 a one of a group of proteins termed the formin homology (FH) family, which includes the *S. cerevisiae* protein BNI1 (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* septims—*CDC3*, *CDC10*, *CDC11*, and *CDC12*—have been shown to be necessary for cytokinesis (Hartwell, 1971; Byers and Goetsch, 1976; Longtine *et al.*, 1996). Intriguingly, *BNI1* 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.)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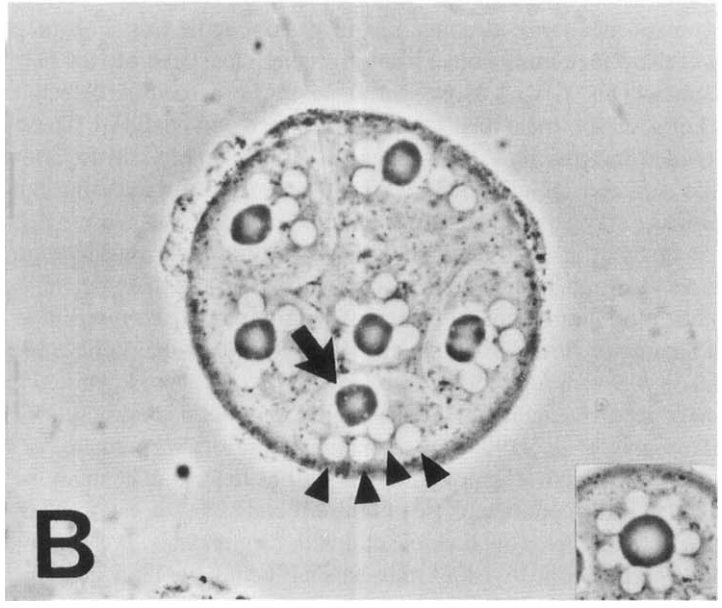
B

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 BNI1 (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 cofilin, an actin-binding protein, localizes to cleavage furrows (Nagaoka *et al.*, 1995). A member of the actin depolymerization family, cofilin 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* cofilin 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 β_2-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. Lifschtyz 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 β_2-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 (BNI1p 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.

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References

Alphey, L., Jimenez, J., White-Cooper, H., Dawson, I., Nurse, P., and Glover, D. M. (1992). *twine*, a cdc25 homolog that functions in the male and female germline of *Drosophila*. *Cell* **69,** 977–988.

Brink, N. G. (1968). Protein synthesis during spermatogenesis in *Drosophila melanogaster*. *Mutation* **5,** 192–194.

Bult, C. J., *et al.* (1996). Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science* **273,** 1058–1073.

Byers, B., and Goetsch, L. (1976). A highly ordered ring of membrane-associated filaments in budding yeast. *J. Cell Biol.* **69,** 717–721.

Castrillon, D. H., Gönczy, P., Alexander, S., Rawson, R., Eberhart, C. G., Viswanathan, S., DiNardo, S., and Wasserman, S. A. (1993). Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: Characterization of male-sterile mutants generated by single P element mutagenesis. *Genetics* **135,** 489–505.

Castrillon, D. H., and Wasserman, S. A. (1994). *Diaphanous* is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformity* gene. *Development*, **120,** 3367–3377.

Cenci, G., Bonaccorsi, S., Pisano, C., Verni, F., and Gatti, M. (1994). Chromatin and microtubule organization during premeiotic, meiotic and early postmeiotic stages of *Drosophila melanogaster* spermatogenesis. *J. Cell Sci.* **107,** 3521–3534.

Chang, F., Hajibagheri, N., and Nurse, P. (1996). Fission yeast contractile ring protein cdc12p: Initiation of ring formation from a single point. *J. Cell Biol.*, in press.

Clay, F. J., McEwen, S. J., Bertoncello, I., Wilks, A. F., and Dunn, A. R. (1993). Identification and cloning of a protein kinase-encoding mouse gene, Plk, related to the *polo* gene of *Drosophila*. *Proc. Natl. Acad. Sci USA* **90,** 4882–4886.

Cooley, L., Kelley, R., and Spradling, A. (1988). Insertional mutagenesis of the *Drosophila* genome with single P elements. *Science* **239,** 1121–1128.

Cooper, K. W. (1965). Normal spermatogenesis in *Drosophila*. *In* "Biology" (M. Demerecs, Ed.), pp. 1–61. Hafner, New York.

Courtot, C., Fankhauser, C., Simanis, V., and Lehner, C. F. (1992). The *Drosophila* cdc25 homolog *twine* is required for meiosis. *Development* **116,** 405–416.

Dunphy, W. G. and Kumagai, A. (1991). The cdc25 protein contains an intrinsic phosphatase activity. *Cell* **67,** 189–196.

Dunphy, W. G., and Newport, J. W. (1989). Fission yeast p13 blocks mitotic activation and tyrosine dephosphorylation of the *Xenopus* cdc2 protein kinase. *Cell* **58,** 181–191.

Eberhart, C. G., Maines, J. Z., and Wasserman, S. A. (1996). Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. *Nature* **381,** 783–785.

Eberhart, C. G., and Wasserman, S. A. (1995). The *pelota* locus encodes a protein required for meiotic cell division: An analysis of G2/M arrest in *Drosophila* spermatogenesis. *Development* **121,** 3477–3486.

Edgar, B. A., and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* **57,** 177–187.

Edgar, B. A., and O'Farrell, P. H. (1990). The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by *string*. *Cell* **62,** 469–480.

Emmons, S., Phan, H., Calley, J., Chen, W., James, B., and Manseau, L. (1995). *Cappuccino*, a *Drosophila* maternal effect gene required for polarity of the egg and embryo, is related to the vertebrate *limb deformity* locus. *Genes Dev.* **9,** 2482–2494.

Erickson, J. (1965). Meiotic drive in *Drosophila* involving chromosome breakage. *Genetics* **51,** 557–571.

Field, C. M., and Alberts, B. M. (1995). Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. *J. Cell Biol.* **131,** 165–178.

Fishkind, D. J., and Wang, Y. L. (1995). New horizons for cytokinesis. *Curr. Opin. Cell Biol.* **7,** 23–31.

Ford, S. K., and Pringle, J. R. (1991). Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: Localization of the CDC11 gene product and the timing of events at the budding site. *Dev. Genet.* **12,** 281–292.

Frolova, L., Le Goff, X., Rasmussen, H. H., Cheperegin, S., Drugeon, G., Kress, M., Arman, I., Haenni, A. L., Celis, J. E., Philippe, M., *et al.* (1994). A highly conserved eukaryotic protein family possessing properties of polypeptide chain release factor. *Nature* **372,** 702–703.

Fuller, M. (1993). Spermatogenesis. *In* "Development of *Drosophila*" (A. Martinez-Arias and M. Bates, Eds.), pp. 61–147. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Gatti, M., and Baker, B. S. (1989). Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. *Genes Devel.* **3,** 438–453.

Gatti, M., and Goldberg, M. L. (1991). Mutations affecting cell division in *Drosophila*. *Methods Cell Biol.* **35,** 543–586.

Gautier, J., Matsukawa, T., Nurse, P., and Maller, J. (1989). Dephosphorylation and activation of *Xenopus* p34cdc2 protein kinase during the cell cycle. *Nature* **339,** 626–629.

Gautier, J., Solomon, M. J., Booher, R. N., Bazan, J. F., and Kirschner, M. W. (1991). cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2. *Cell* **67,** 197–211.

Glotzer, M., Murray, A. W., and Kirschner, M. W. (1991). Cyclin is degraded by the ubiquitin pathway. *Nature* **349,** 132–138.

Glover, D. M., Leibowitz, M. H., McLean, D. A. and Parry, H. (1995). Mutations in *aurora* prevent centrosome separation leading to the formation of monopolar spindles. *Cell* **81,** 95–105.

Golsteyn, R. M., Schultz, S. J., Bartek, J., Ziemiecki, A., Ried, T., and Nigg, E. A. (1994). Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila polo* and *Saccharomyces cerevisiae* Cdc5. *J. Cell Sci.* **107,** 1509–1517.

Gönczy, P., and DiNardo, S. (1996). The germ line regulates somatic cyst cell proliferation and fate during *Drosophila* spermatogenesis. *Development* **122,** 2437–2447.

Gönczy, P., Thomas, B. J., and DiNardo, S. (1994). *roughex* is a dose-dependent regulator of the second meiotic division during *Drosophila* spermatogenesis. *Cell* **77,** 1015–1025.

Gönczy, P., Viswanathan, S., and DiNardo, S. (1992). Probing spermatogenesis in *Drosophila* with P-element enhancer detectors. *Development* **114,** 89–98.

Gonzalez, C., Casal, J., and Ripoll, P. (1988). Functional monopolar spindles caused by mutation in *mgr*, a cell division gene of *Drosophila melanogaster*. *J. Cell Sci.* **89,** 39–47.

Gonzalez, C., Casal, J., and Ripoll, P. (1989). Relationship between chromosome content and nuclear diameter in early spermatids of *Drosophila melanogaster*. *Genet. Res.* **54,** 205–212.

Gould, K. L., Moreno, S., Tonks, N. K., and Nurse, P. (1990). Complementation of the mitotic activator, p80cdc25, by a human protein-tyrosine phosphatase. *Science* **250,** 1573–1576.

Gunsalus, K. C., Bonaccorsi, S., Williams, E., Verni, F., Gatti, M., and Goldberg, M. L. (1995). Mutations in *twinstar*, a *Drosophila* gene encoding a cofilin/ADF homologue, result in defects in centrosome migration and cytokinesis. *J. Cell Biol* **131,** 1243–1259.

Haarer, B. K., and Pringle, J. R. (1987). Immunofluorescence localization of the *Saccharomyces cerevisiae* CDC12 gene product to the vicinity of the 10-nm filaments in the mother-bud neck. *Mol. Cell. Biol.* **7,** 3678–3687.

Hackstein, J. H. P. (1991). Spermatogenesis in *Drosophila*: A genetic approach to cellular and subcellular differentiation. *Eur. J. Cell Biol.* **56,** 151–169.

Hamanaka, R., Smith, M. R., O'Connor, P. M., Maloid, S., Mihalic, K., Spivak, J. L., Longo, D. L., and Ferris, D. K. (1995). Polo-like kinase is a cell cycle-regulated kinase activated during mitosis. *J. Biol. Chem.* **270,** 21086–21091.

Hardy, R. W., Tokuyasu, K. T., Lindsley, D. L., and Garavito, M. (1979). The germinal proliferation center in the testis of *Drosophila melanogaster*. *J. Ultrastruct. Res.* **69,** 180–190.

Hartwell, L. H. (1971). Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Exp. Cell Res.* **69,** 265–276.

Hawley, R. S. (1993). Meiosis as an "M" thing: Twenty-five years of meiotic mutants in *Drosophila*. *Genetics* **135,** 613–618.

Hereford, L. M., and Hartwell, L. H. (1974). Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. *J. Mol. Biol.* **84,** 445–461.

Hime, G. R., Brill, J. A., and Fuller, M. T. (1996). Assembly of ring canals in the male germ line from structural components of the contractile ring. *J. Cell Sci.* in press.

Holtrich, U., Wolf, G., Brauninger, A., Karn, T., B., B., Rubsamen-Waigmann, U., and Strebhardt, K. (1994). Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors. *Proc. Natl. Acad. Sci USA* **91,** 1736–1740.

Hoyle, H. D., and Raff, E. C. (1990). Two *Drosophila* beta tubulin isoforms are not functionally equivalent. *J. Cell Biol.* **111,** 1009–1026.

Jimenez, J., Alphey, L., Nurse, P., and Glover, D. M. (1990). Complementation of fission yeast cdc2ts and cdc25ts mutants identifies two cell cycle genes from *Drosophila*: A cdc2 homologue and *string*. *EMBO J.* **9,** 3565–3571.

Karess, R. E., Chang, X. J., Edwards, K. A., Kulkarni, S., Aguilera, I., and Kiehart, D. P. (1991). The regulatory light chain of nonmuscle myosin is encoded by *spaghetti-squash*, a gene required for cytokinesis in *Drosophila*. *Cell* **65,** 1177–1189.

Kemphues, K. J., Kaufman, T. C., Raff, R. A., and Raff, E. C. (1982). The testis-specific beta-tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. *Cell* **31,** 65–670.

Kemphues, K. J., Raff, E. C., Raff, R. A., and Kaufman, T. C. (1980). Mutation in a testis-specific beta-tubulin in *Drosophila*: Analysis of its effects on meiosis and map location of the gene. *Cell* **21,** 445–451.

Kim, H. B., Haarer, B. K., and Pringle, J. R. (1991). Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: Localization of the CDC3 gene product and the timing of events at the budding site. *J. Cell Biol.* **112,** 535–544.

Koonin, E. V., Bork, P., and Sander, C. (1994). A novel RNA-binding motif in omnipotent suppressors of translation termination, ribosomal proteins and a ribosome modification enzyme? *Nucleic Acids Res.* **22,** 2166–2167.

Krek, W., and Nigg, E. A. (1991a). Differential phosphorylation of vertebrate p34cdc2 kinase at the G_1/S and G_2/M transitions of the cell cycle: Identification of major phosphorylation sites. *EMBO J.* **10,** 305–316.

Krek, W., and Nigg, E. A. (1991b). Mutations of p34cdc2 phosphorylation sites induce premature mitotic events in HeLa cells: Evidence for a double block to p34cdc2 kinase activation in vertebrates. *EMBO J.* **10,** 3331–3341.

Labbe, J. C., Capony, J. P., Caput, D., Cavadore, J. C., Derancourt, J., Kaghad, M., Leliss, J. M., Picard, A., and Doree, M. (1989). MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO J.* **8,** 3053–3058.

Lalo, D., Stettler, S., Mariotte, S., Gendreau, E., and Thuriaux, P. (1994). Organization of the centromeric region of chromosome XIV in *Saccharomyces cerevisiae*. *Yeast* **10,** 523–533.

Lehner, C. F., and O'Farrell, P. H. (1990). *Drosophila* cdc2 homologs: A functional homolog is coexpressed with a cognate variant. *EMBO J.* **9,** 3573–3581.

Liebrich, W. (1982). The effects of cytochalasin B and colchicine on the morphogenesis of mitochondria in *Drosophila hydei* during meiosis and early spermiogenesis: An in vitro study. *Cell Tissue Res.* **224,** 161–168.

Lifschytz, E., and Hareven, D. (1977). Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. *Dev. Biol.* **58,** 276–294.

Lifschytz, E., and Meyer, G. F. (1977). Characterisation of male meiotic-sterile mutations in *Drosophila melanogaster*: The genetic control of meiotic divisions and gametogenesis. *Chromosoma* **64,** 371–392.

Lin, T. Y., Viswanathan, S., Wood, C., Wilson, P. G., Wolf, N., and Fuller, M. T. (1996). Coordinate developmental control of the meiotic cell cycle and spermatid differentiation in *Drosophila* males. *Development* **122,** 1331–1341.

Lindsley, D., and Tokuyasu, K. T. (1980). Spermatogenesis. *In* "Genetics and Biology of *Drosophila*" (A. M. and W. T. R.s, Eds.), pp. 225–294. Academic Press, New York.

Llamazares, S., Moreira, A., Tavares, A., Girdham, C., Spruce, B. A., Gonzalez, C., Karess, R. E., Glover, D. M., and Sunkel, C. E. (1991). *polo* encodes a protein kinase homolog required for mitosis in *Drosophila*. *Genes Dev.* **5,** 2153–2165.

Longtine, M. S., DeMarini, D. J., Valencik, M. L., Al-Awar, O. S., Fares, H., De Virgilio, C., and Pringle, J. R. (1996). The septins: Roles in cytokinesis and other processes. *Curr. Opin. Cell Biol.* **8,** 106–119.

Michiels, F., Buttgereit, D., and Renkawitz-Pohl, R. (1993). An 18-bp element in the 5′ untranslated region of the *Drosophila* beta 2 tubulin mRNA regulates the mRNA level during postmeiotic stages of spermatogenesis. *Eur. J. Cell Biol.* **62,** 66–74.

Miller, K. G., Field, C. M., and Alberts, B. M. (1989). Actin-binding proteins from *Drosophila* embryos: A complex network of interacting proteins detected by F-actin affinity chromatography. *J. Cell Biol.* **109,** 2963–2975.

Miller, K. G., and Kiehart, D. P. (1995). Fly division. *J. Cell Biol.* **131,** 1–5.

Moreno, S., Nurse, P., and Russell, P. (1990). Regulation of mitosis by cyclic accumulation of p80cdc25 mitotic inducer in fission yeast. *Nature* **344,** 549–552.

Morgan, T. H., Bridges, A. H., Schultz, J., and Schultz, J. (1934). Constitution of the germinal material in relation to heredity. Carnegie Institute, Washington, D.C. *In* "Yearbook of the Carnegie Institute," volume 33, pp. 274–280.

Morla, A. O., Draetta, G., Beach, D., and Wang, J. Y. (1989). Reversible tyrosine phosphorylation of cdc2: Dephosphorylation accompanies activation during entry into mitosis. *Cell* **58,** 193–203.

Murray, A. W. (1992). Creative blocks: Cell-cycle checkpoints and feedback controls. *Nature* **359,** 599–604.

Murray, A. W., and Hunt, T. (1993). "The Cell Cycle." Freeman, New York.

Nagaoka, R., Abe, H., Kusano, K., and Obinata, T. (1995). Concentration of cofilin, a small actin-binding protein, at the cleavage furrow during cytokinesis. *Cell Mot. Cytoskel.* **30,** 1–7.

Neufeld, T. P., and Rubin, G. M. (1994). The *Drosophila peanut* gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell* **77,** 371–379.

Nishida, E. (1985). Opposite effects of cofilin and profilin from porcine brain on rate of exchange of actin-bound adenosine 5′-triphosphate. *Biochemistry* **24,** 1160–1164.

Nishida, E., Maekawa, S., and Sakai, H. (1984). Cofilin, a protein in porcine brain that binds to actin filaments and inhibits their interactions with myosin and tropomyosin. *Biochemistry* **23,** 5307–5313.

Norbury, C., Blow, J., and Nurse, P. (1991). Regulatory phosphorylation of the p34cdc2 protein kinase in vertebrates. *EMBO J.* **10,** 3321–3329.

Novitski, E., and Hanks, G. D. (1961). Analysis of irradiated *Drosophila* populations for meiotic drive. *Nature* **190,** 989–990.

Nurse, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* **344,** 503–508.

Nurse, P., and Bissett, Y. (1981). Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. *Nature* **292,** 558–560.

Nurse, P., and Thuriaux, P. (1980). Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. *Genetics* **96,** 627–637.

Nurse, P., Thuriaux, P., and Nasmyth, K. (1976). Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **146,** 167–178.

Ohkura, H., Hagan, I. M., and Glover, D. M. (1995). The conserved *Schizosaccharomyces pombe* kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells. *Genes Dev.* **9,** 1059–1073.

Olivieri, G., and Olivieri, A. (1965). Autoradiographic study of nucleic acid synthesis during spermatogenesis in *Drosophila melanogaster*. *Mutat. Res.* **2,** 366–380.

Petersen, J., Weilguny, D., Egel, R., and Nielsen, O. (1995). Characterization of fus1 of *Schiz-*

asaccharomyces pombe: A developmentally controlled function needed for conjugation. *Mol. Cell. Biol.* **15,** 3697–3707.

Pope, B., Way, M., Matsudaira, P. T., and Weeds, A. (1994. Characterisation of the F-actin binding domains of villin: Classification of F-actin binding proteins into two groups according to their binding sites on actin. *FEBS Lett.* **338,** 58–62.

Raff, E. C., and Fuller, M. T. (1984). Genetic analysis of microtubule function in *Drosophila*. *In* "Molecular Biology of the Cytoskeleton" (G. Borisys, Ed.), pp. 293–304. Cold Spring Harbor Laboratories, Cold Spring Harbor.

Ragan, M. A., Logsdon, J. M., Sensen, C. W., Charlebois, R. L., and Doolittle, W. F. (1996). An archaebacterial homolog of Pelota, a meiotic cell division protein in eukaryotes. *FEMS Micro. Lett.* **144,** 151–155.

Rappaport, R. (1986). Establishment of the mechanism of cytokinesis in animal cells. *Int. Rev. Cytol.* **105,** 245–281.

Reijo, R., Lee, T. Y., Salo, P., Alagappan, R., Brown, L. G., Rosenberg, M., Rozen, S., Jaffe, T., Straus, D., Hovatta, O., *et al.* (1995). Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet.* **10,** 383–393.

Robinson, D. N., Cant, K., and Cooley, L. (1994). Morphogenesis of *Drosophila* ovarian ring canals. *Development* **120,** 2015–2025.

Russell, P., and Nurse, P. (1986). *cdc25*$^{+}$ functions as an inducer in the mitotic control of fission yeast. *Cell* **45,** 145–53.

Sadhu, K., Reed, S. I., Richardson, H., and Russell, P. (1990). Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2. *Proc. Natl. Acad. Sci. USA* **87,** 5139–5143.

Sanders, S. L., and Field, C. M. (1995). Cell division: Bud-site selection is only skin deep. *Curr. Biol.* **5,** 1213–1215.

Satterwhite, L. L., and Pollard, T. D. (1992). Cytokinesis. *Curr. Opin. Cell Biol.* **4,** 43–52.

Schild, D., and Byers, B. (1980). Diploid spore formation and other meiotic effects of two cell-division-cycle mutations of *Saccharomyces cerevisiae*. *Genetics* **96,** 859–876.

Sigrist, S., Ried, G., and Lehner, C. F. (1995). Dmcdc2 kinase is required for both meiotic divisions during *Drosophila* spermatogenesis and is activated by the Twine/cdc25 phosphatase. *Mech. Dev.* **53,** 247–260.

Sluder, G., and Rieder, C. L. (1993). The events and regulation of anaphase onset. *In* "Chromosome Segregation and Aneuploidy" (B. V. Vigs, Ed.), pp. 211–224. Springer, New York.

Solomon, M. J. (1993). Activation of the various cyclin/cdc2 protein kinases. *Curr. Opin. Cell Biol.* **5,** 180–186.

Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M., and Kirschner, M. W. (1990). Cyclin activation of p34cdc2. *Cell* **63,** 1013–1024.

Stern, B., Ried, G., Clegg, N. J., Grigliatti, T. A., and Lehner, C. F. (1993). Genetic analysis of the *Drosophila* cdc2 homolog. *Development* **117,** 219–232.

Strausfeld, U., Labbe, J. C., Fesquet, D., Cavadore, J. C., Picard, A., Sadhu, K., Russell, P., and Doree, M. (1991). Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein. *Nature* **351,** 242–245.

Sunkel, C. E., and Glover, D. M. (1988). *polo*, a mitotic mutant of *Drosophila* displaying abnormal spindle poles. *J. Cell Sci.* **89,** 25–38.

Tates, A. D. (1971). Cytodifferentiation during Spermatogenesis in *Drosophila melanogaster*: An Electron Microscope Study. Ph.D. thesis, Rijksuniversiteit, Leiden.

Tokuyasu, K. T., Peacock, W. J., and Hardy, R. W. (1972a). Dynamics of spermiogenesis in *Drosophila melanogaster*. I. Individualization process. *Z. Zellforsch. Mikrosk. Anat.* **124,** 479–506.

Tokuyasu, K. T., Peacock, W. J., and Hardy, R. W. (1972b). Dynamics of spermiogenesis in *Drosophila melanogaster*. II. Coiling process. *Z. Zellforsch. Mikrosk. Anat.* **127,** 492–525.

Valentin, J. (1984). Genetic control of meiosis: The *eq* gene in *Drosophila melanogaster*. *Hereditas* **101,** 115–117.

Verhasselt, P., Aert, R., Voet, M., and Volckaert, G. (1994). Twelve open reading frames revealed in the 23.6 kb segment flanking the centromere on the *Saccharomyces cerevisiae* chromosome XIV right arm. *Yeast* **10,** 1355–1361.

White-Cooper, H., Alphey, L., and Glover, D. M. (1993). The cdc25 homologue *twine* is required for only some aspects of the entry into meiosis in *Drosophila*. *J. Cell Sci.* **106,** 1035–1044.

Williams, B. C., Riedy, M. F., Williams, E. V., Gatti, M., and Goldberg, M. L. (1995). The *Drosophila* kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis. *J. Cell Biol.* **129,** 709–723.

Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., *et al.* (1994). 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature* **368,** 32–38.

Wu, S., and Wolgemuth, D. J. (1995). The distinct and developmentally regulated patterns of expression of members of the mouse Cdc25 gene family suggest differential functions during gametogenesis. *Dev. Biol.* **170,** 195–206.

Yanicostas, C., Vincent, A., and Lepesant, J. A. (1989). Transcriptional and posttranscriptional regulation contributes to the sex-regulated expression of two sequence-related genes at the *janus* locus of *Drosophila melanogaster*. *Mol. Cell. Biol.* **9,** 2526–2535.