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REVIEW

Drosophila Male Meiosis as a Model System for the Study of Cytokinesis in Animal Cells

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ABSTRACT. ***Drosophila*** **male meiosis offers unique opportunities for mutational dissection of cytokinesis. This system allows easy and unambiguos identification of mutants defective in cytokinesis through the examination of spermatid morphology. Moreover, cytokinesis defects and protein immunostaining can be analyzed with exquisite cytological resolution because of the large size of meiotic spindles. In the past few years several mutations have been isolated that disrupt meiotic cytokinesis in *Drosophila* males. These mutations specify genes required for the assembly, proper constriction or disassembly of the contractile ring. Molecular characterization of these genes has identified essential components of the cytokinetic machinery, highlighting the role of the central spindle during cytokinesis. This structure appears to be both necessary and sufficient for signaling cytokinesis. In addition, many data indicate that the central spindle microtubules cooperatively interact with elements of the actomyosin contractile ring, so that impairment of either of these structures prevents the formation of the other.**

Key words: cytokinesis/central spindle/contractile ring/meiosis/*Drosophila*

Cytokinesis is the complex process by which two cells separate at the end of cell division. While in plant cells this process is mediated by the deposition of cell wall material between the two daughter cells, in animal cells cytokinesis is accomplished by the contraction of a ring-shaped cellular structure containing actin and myosin II filaments. This structure is anchored to the plasma membrane at the equator of the dividing cell and constricts in purse string fashion, leading to the separation of the two daughter cells. The cytokinetic process can be subdivided into at least four subprocesses that must be tightly coordinated to ensure the fidelity of chromosome segregation (reviewed by Glotzer, 1997; Straight and Field, 2000). First, interactions between the spindle and the cortex determine the site of cleavage furrow formation. Second, an actomyosin-based contractile ring assembles at this cortical site. Third, the actomyosin ring constricts, leading to furrow ingression. Fourth, during both furrow ingression and the completion of cytokinesis new membrane is added to allow separation of the daughter cells.

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Although genetic, biochemical and cell biological approaches have provided some insight into the cellular structures that orchestrate the final step of cell division, still the mechanisms underlying the assembly and functioning of the cytokinetic apparatus remain largely unknown. A powerful approach for molecular dissection of cytokinesis is the identification and molecular characterization of genes that control this process. This approach has been exploited in both budding and fission yeast, leading to the discovery of several proteins involved in cytokinesis (reviewed by Goldberg *et al*., 1998, Field *et al*., 1999, Robinson and Spudich, 2000). More recently, a genetic approach has also been employed in *Drosophila melanogaster* and *Caenorhabditis elegans*, and many additional gene products required for cytokinesis have been identified and characterized (reviewed by Field *et al*., 1999; Gatti *et al*., 2000; Robinson and Spudich, 2000).

The advantages offered by *Drosophila* for genetic analysis are well known. Moreover, this organism offers unique opportunities for cytological examination of mutant phenotypes and immunolocalization of gene products involved in cytokinesis (reviewed by Goldberg *et al*. 1998; Gatti *et al*., 2000). *Drosophila* cytokinesis can be examined in different cell types, including cells undergoing embryonic divisions after cellularization (Adams *et al*., 1998; Prokopenko *et al*., 1999), larval neuroblasts (Gatti and Baker, 1989; Karess *et al*., 1991; Castrillon and Wasserman 1994; Gunsalus *et al*.,

1995; Giansanti *et al*., 2001; Wakefield *et al*., 2001), and male meiotic cells (Gunsalus *et al*., 1995; Williams *et al*., 1995; Hime *et al*., 1996; Basu *et al*., 1998; Bonaccorsi *et al*., 1998; Carmena *et al*., 1998; Giansanti *et al*., 1998; Herrmann *et al*., 1998; Giansanti *et al*., 1999; Brill *et al*., 2000; Wakefield *et al*., 2001). Thus, phenotypic analysis of mutations affecting this process is likely to provide information on whether different cells utilize different mechanisms to accomplish cytokinesis.

Here we focus on cytokinesis in *Drosophila* male meiosis, describing the advantages of this systems for mutational and phenotypical analysis of cytokinesis. In addition, we describe the main results obtained from the study of meiotic cytokinesis of *Drosophila* males, highlighting the role of central spindle during this process.

Male meiosis as a model system for the study of cytokinesis

Male meiosis occurs in the context of a complex developmental process, called spermatogenesis, that leads to the formation of 64 spermatozoa, starting from a single gonial cell generated by the asymmetric division of a germ line stem cell. Concomitantly with the formation of this founder cell, the asymmetric division of two cyst progenitor cells generates two cyst cells which engulf the primary gonium and its progeny till completion of spermatogenesis. The primary gonial cell undergoes four rounds of mitotic divisions, giving rise to 16 primary spermatocytes. These cells, after a dramatic growth phase, that results in a 25-fold increase in nuclear volume, enter meiotic division, producing in turn 32 secondary spermatocytes and 64 spermatids (reviewed by Lindsley and Tokuyasu, 1980; Fuller, 1993; Cenci *et al*., 1994; Maines and Wasserman, 1998).

In both gonial and meiotic divisions the execution of cytokinesis does not lead to complete separation of the daughter cells, which remain connected by cytoplasmic bridges, called the ring canals (Lindsley and Tokuyasu, 1980; Hime *et al*., 1996). These ring canals, whose function during spermatogenesis is still poorly understood, develop from arrested contractile rings and contain components of the cytokinetic apparatus, such as anillin, the Peanut septin and myosin II (Hime *et al*., 1996, Giansanti *et al*., 1999; our unpublished results).

Both meiotic divisions occur within a double nuclear membrane which is invaginated and possibly fenestrated at positions underlying the asters, but does not otherwise appear to disassemble (Tates, 1971; Church and Lin, 1982). In addition, spermatocytes undergoing meiotic divisions are surrounded by three to five layers of parafusorial membranes which lie parallel to the spindle axis (Tates, 1971; Fuller, 1993). During meiosis I and meiosis II, mitochondria line up along the parafusorial membranes and are equally partitioned between the two daughter cells at each division. At the end of meiotic division the mitochondria inherited by each cell fuse to form a complex interlaced structure, called the Nebenkern. Thus, immediately following meiosis, at the so-called onion-stage, a cyst is composed of 64 spermatids, each containing a single nucleus associated with a single Nebenkern.

This peculiar structure of *Drosophila* spermatids provides one of the main advantages of male meiosis for the study of cytokinesis. Mutants affecting cytokinesis can be easily and unambiguously identified by examining the onion-stage spermatids. In wild type, each onion-stage spermatid consists of a round, phase-light nucleus associated with a single phase-dark Nebenkern of similar size (reviewed by Fuller, 1993). Failures in cytokinesis abrogate proper mitochondrial partition between the daughter cells and result in aberrant spermatids composed of an abnormally large Nebenkern associated with multiple nuclei (Fuller, 1993). Thus, spermatids composed of a large Nebenkern associated with two or four nuclei of regular size, are diagnostic of failures in meiotic cytokinesis (Fuller, 1993; Castrillon and Wasserman, 1994; Gunsalus *et al*., 1995; Williams *et al*., 1995; Giansanti *et al*., 1998). On the other hand, since the volume of an onion-stage nucleus is proportional to its chromatin content (Gonzalez *et al*., 1989), spermatids containing a large Nebenkern associated with nuclei of different dimensions are indicative of errors in both chromosome segregation and cytokinesis (Herrmann *et al*., 1998; Carmena *et al*., 1998; Wakefield *et al*., 2001). In this context, it should be noted that meiosis occurs in testes of third instar larvae, thus also allowing examination of the meiotic phenotype of mutants that die at the larval-pupal boundary (see, for example, Gunsalus *et al*., 1995, Wakefield *et al*., 2001).

A further advantage of male meiosis for the phenotypical analysis of cytokinesis is provided by the weak spindle checkpoint that characterizes spermatocyte divisions. While in larval brain cells the presence of disorganized spindles activates the spindle integrity checkpoint, precluding the observation of cell division subsequent to arrested metaphases (Carmena *et al*., 1998; Avides and Glover, 1999; Wakefield *et al*., 2001), in spermatocytes the spindle checkpoint is not stringent and causes only a small delay in progression through meiosis (Basu *et al*., 1999, Rebollo and Gonzalez, 2000). For example, mutations in the *polo* and *abnormal spindle (asp)* genes, which cause metaphase arrest in larval brain cells, do not block meiotic division in males but produce frequent failures in cytokinesis (Herrmann *et al*., 1998; Carmena *et al*., 1998; Wakefield *et al*, 2001). Thus, by analyzing meiotic division, it is possible to ask whether genes involved in spindle formation also play a role during later stages of cell division, including cytokinesis.

A third feature that makes male meiosis a highly suitable system for the analysis of cytokinesis is the large size of the meiotic spindles. For example, in telophases I and telophases II the pole-to-pole distances are respectively 45 and 30 μm (Cenci *et al*., 1994). In addition, telophases of both

meiotic divisions display a prominent central spindle–the bundle of antiparallel, interdigitating microtubules between the segregating daughter nuclei–which is pinched in the middle during cytokinesis (Cenci *et al*., 1994). The favourable cytological features of *Drosophila* spermatocytes have allowed precise localization of many components of the cytokinetic apparatus. These components include contractile ring-associated proteins, such as actin, myosin II, anillin and septins; proteins that regulate F-actin polymerization, like profilin; microtubule-associated proteins, such as KLP3A, Pavarotti (Pav) and Abnormal spindle (Asp); and the Polo and Aurora B kinases. Each of these components exhibits a specific temporal and spatial pattern of accumulation in the cleavage region, which is summarized in Table I and Fig. 1.

Finally it should be noted that recent studies have shown that meiotic cytokinesis can be successfully examined in living testes, allowing visualization of of the dynamic behavior of GFP-labelled proteins involved in cytokinesis (Rebollo and Gonzalez, 2000; Sampaio *et al*., 2001).

The central spindle is the only source of signals that stimulate meiotic cytokinesis in Drosophila males

An open question about cell cleavage in animal systems is the source of signals that stimulate contractile ring formation and cytokinesis. It has been suggested that these signals may be provided either by the metaphase chromosomes (Earnshaw *et al*., 1991), or the asters (Rappaport, 1961, Hiramoto, 1971, Rappaport, 1986) or the central spindle (Rappaport and Rappaport, 1974, Cao and Wang, 1996, Fishkind *et al*., 1996). To address this question we have genetically micromanipulated *Drosophil*a male meiosis by

Table I. PROTEINS INVOLVED IN MEIOTIC CYTOKINESIS OF *Drosophila* MALES

PROTEIN/ GENE NAME	PROTEIN FAMILY	PROTEIN LOCALIZATION IN MALE MEIOSIS	REQUIRED FOR CYTOKINESIS IN:			CYTOLOGICAL DEFECTS IN MALE MEIOTIC CELLS	REFS.
			Embryonic cells (a)	*Larval neuroblasts*	*Male meiosis*		
Abnormal spindle	Abnormal spindle	CS extremities	ND	ND	Yes	Disorganization of both CS and CR	1, 2
Actin (b)	Actin	CR	ND	ND	ND	ND	3
Anillin (b)	Anillin	Equatorial cortex, CR	ND	ND	ND	ND	4, 5
Aurora B (b)	Aurora kinase	CS midzone	ND	ND	ND	ND	6, 7, 8
Chickadee	Profilin	Enriched at equatorial cortex	ND	No	Yes	Absence of both CS and CR	9, 10
Diaphanous	Formin	ND	ND	Yes	Yes	Absence of both CS and CR	10, 11
Dsup35	Translation factor	Intranuclear foci	ND	ND	Yes	Absence of both CS and CR	12
Four wheel drive	Phospholipid kinase	ND	ND	No	Yes	Failure of CR constriction	13
KLP3A	Kinesin	CS midzone	ND	No	Yes	Absence of both CS and CR	10, 14
Klp38	Kinesin	ND	ND	Yes	Yes	ND	15, 16
Myosin II (b)	Myosin II	Equatorial cortex, CR	ND	ND	ND	ND	17
Pavarotti	Kinesin	CS midzone	Yes	ND	ND	ND	18, 19
Polo	Polo kinase	CS midzone	ND	ND	Yes	Absence of both CS and CR	19, 20, 21
Peanut	Septin	CR	ND	Yes	No	No defects	22, 23, 24
Spaghetti squash	Myosin regulatory light chain	Cleavage furrow	ND	Yes	Yes	Absence of both CS and CR	25, 26, 27
Twinstar	Cofilin	ND	ND	Yes	Yes	Failure of CR disassembly	3, 5

(a) embryonic cells of cycles 14-16; (b) mutants in genes encoding these proteins are either not available, or have not been examined for defects in cytokinesis. CS: central spindle; CR: contractile ring; ND not determined. References: 1 Saunders *et al.*, 1997. 2 Wakefield *et al.*, 2001. 3 Gunsalus *et al.*, 1995. 4 Field and Alberts, 1995. 5 Giansanti *et al.*, 1999. 6 Adams *et al*., 2001. 7 Giet and Glover, 2001. 8 Our unpublished results: using an antibody kindly provided by D. Glover, we have found that Aurora B concentrates in the CS midzone of *Drosophila* spermatocytes. 9 Cooley *et al.*, 1992. 10 Giansanti *et al.*, 1998. 11 Castrillon and Wasserman, 1994. 12 Basu *et al.*, 1998. 13 Brill *et al*., 2000. 14 Williams *et al.*, 1995. 15 Molina *et al.*, 1997. 16 Ohkura *et al.*, 1997. 17 Our unpublished results: using an antibody kindly provided by C. Field, we have found that Myosin II concentrates in the cleavage furrow of *Drosophila* spermatocytes. 18 Adams *et al.*, 1998. 19 Carmena *et al.*, 1998. 20 Herrmann *et al.*, 1998. 21 Sampaio *et al.*, 2001. 22 Neufeld and Rubin 1994. 23 Hime *et al.*, 1996. 24 Our unpublished experiments : we have observed that in testes of larvae homozygous for *peanut* null mutations meiotic cyotokinesis is normal. 25 Karess *et al.*, 1991. 26 R. Karess, personal communication: recent experiments have shown that Spaghetti squash-GFP accumulates in the cleavage furrow. 27 Our unpublished results: spermatocytes of *spaghetti squash* mutants are defective in both the CS and the CR.

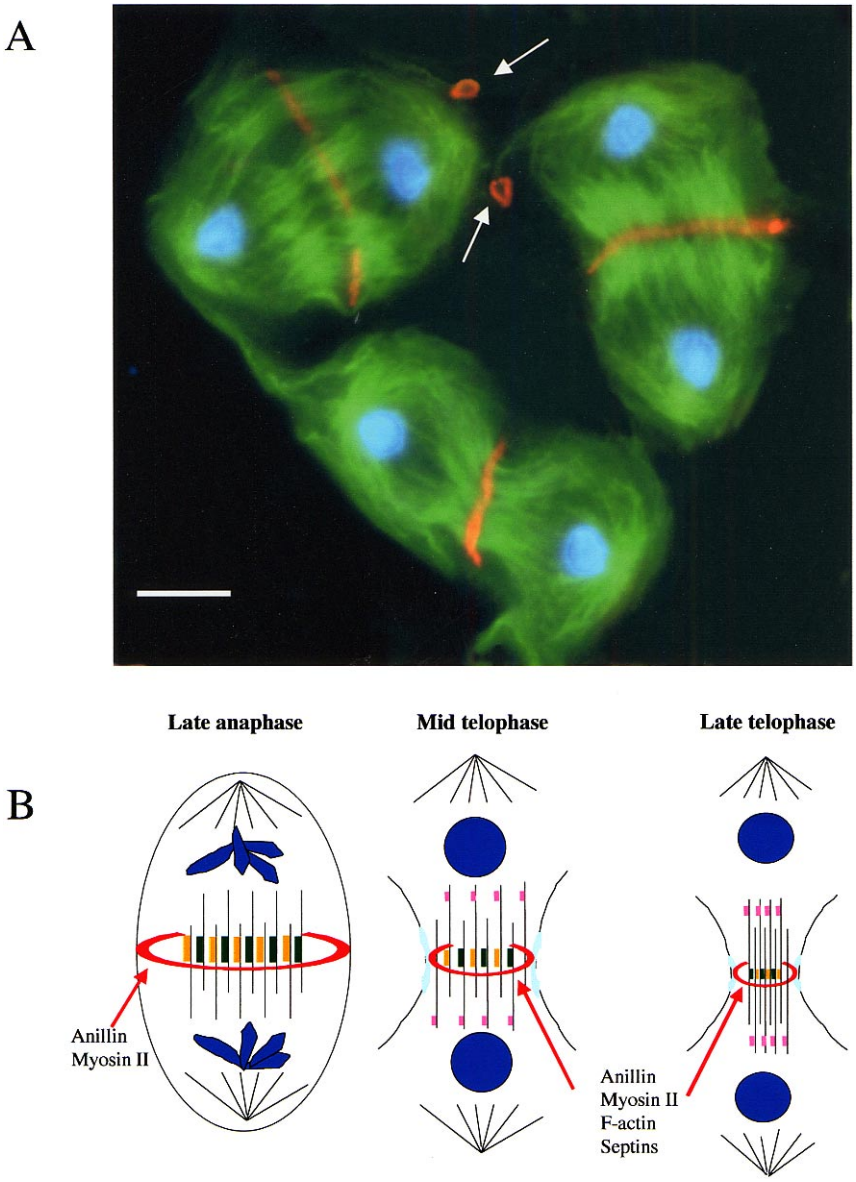

Fig. 1. Spatial and temporal localization of proteins involved in meiotic cytokinesis of *Drosophila* males. A. Anillin localization in primary spermatocytes. Cells were immunostained for tubulin (green) and anillin (orange); chromatin (blue) was detected by Hoechst 33258 staining. Anillin is a conserved protein that contains an actin binding domain and a pleckstrin homology (PH) domain (Field and Alberts, 1995; Oegema *et al*., 2000). Anillin concentrates in the equatorial cortex of *Drosophila* spermatocytes during anaphase (cell at the top left) prior to the assembly of the actin-enriched contractile ring. However, after contractile ring assembly, anillin precisely co-localizes with this structure throughout cytokinesis. In mutants devoid of both the central spindle and the contractile ring (see below) the anillin band forms but fails to contrict. Based on these observations, it has been suggested that anillin may link the contractile ring to the plasma membrane, mediating the interactions between these structures (Giansanti *et al*., 1999). Arrows point to two ring canals. Bar, 10 μm. B. Schematic localization of proteins involved in spermatocyte cytokinesis. During late anaphase, when the central spindle has just begun to assemble, anillin and myosin II concentrate in a narrow circumferential band at the cell equator, while KLP3A (yellow) and Pav (dark green) start to accumulate in the central spindle midzone. During early- and mid-telophase, when a prominent and dense central spindle has formed, F- actin, myosin II, anillin and the septins co-localize in the contractile ring. At this stage profilin (light blue) starts to accumulate at the equatorial cell cortex; KLP3A and Pav concentrate in the central spindle midzone, while Asp (pink) accumulates at the minus ends of central spindle microtubules. During late telophase all these proteins display the same subcellular localizations seen in mid-telophases. For the roles of these proteins in cytokinesis see Table I and text below.

means of mutations that cause the formation of meiotic cells devoid of either the asters or the chromosomes.

We have identified a gene we call *asterless (asl)* that is required for aster formation during male meiosis (Bonaccorsi *et al*., 1998). *asl* mutants have morphologically normal centrioles but fail to accumulate centrosomal material around these organelles. Despite the absence of asters, meiotic cells of *asl* mutants develop poorly focused anastral spindles which mediate chromosome segregation, although in a highly irregular way. Remarkably, *asl* spermatocytes eventually form a morphologically normal ana-telophase central spindle and assemble a regular actomyosin ring which undergoes normal contraction (Bonaccorsi *et al*., 1998). Thus, the central spindles of *asl* mutants appear to have full ability to induce cytokinesis. These findings strongly suggest that in *Drosophila* male meiosis the central spindle can recruit and accumulate the cytokinetic signals in the absence of both functional centrosomes and asters.

To elucidate the role of chromosomes in signaling cytokinesis we made use of *fusolo (fsl)*, a recently isolated male sterile mutation that disrupts chromosome segregation during both meiotic divisions of *Drosophila* males (E. Bucciarelli, M. G. Giansanti, S. Bonaccorsi and M. Gatti, unpublished results). During the first meiotic division, in about half of *fsl* spermatocytes all chromosomes segregate to one pole only. However, in the aberrant telophases I of *fsl* mutants cytokinesis is normal, leading to the formation of secondary spermatocytes that are completely devoid of chromosomes. In these secondary spermatocytes, centrosomes nucleate astral arrays of microtubules that move to the opposite cell poles, giving rise to bipolar spindles. These spindles, despite the absence of chromosomes, assemble morphologically regular central spindles and elongate to form telophase figures that are undistinguishable from their wild type counterparts. Moreover, *fsl* secondary spermatocytes assemble a regular contractile apparatus and undergo cytokinesis, even in the absence of chromosomes (E. Bucciarelli, M. G. Giansanti, S. Bonaccorsi and M. Gatti, unpublished results).

Taken together, the results on *asl* and *fsl* mutants indicate that neither the astral microtubules, nor the chromosomes are required for signaling cytokinesis in *Drosophila* male meiosis. Thus, at least in this system, the central spindle is both necessary and sufficient to stimulate the cytokinetic process.

The analysis of mutations that disrupt both the central spindle and the contractile ring reveals interactions between these cytokinetic structures

Several *Drosophila* mutants have been identified that disrupt meiotic cytokinesis in males and affect specific cellular structures involved in this process. These mutants can be subdivided into two broad categories: those displaying both a central spindle and a contractile ring and those severely defective or devoid of both these structures (Table I).

The mutations that disrupt the assembly of both the central spindle and the contractile ring identify a variety of genes with diverse functions. The simultaneous absence of both these cytokinetic structures has been observed in mutants in the *chickadee* (*chic*), *diaphanous (dia)*, *spaghetti squash (sqh)*, *KLP3A* and *polo* genes, and has been phenocopied by treatment with cytochalasin B (Williams *et al.*, 1995; Carmena *et al*., 1998; Giansanti *et al*., 1998; Herrmann *et al*., 1998; M. G. Giansanti, S. Bonaccorsi and M. Gatti, unpublished results).

The *KLP3A* gene encodes a kinesin-like protein that accumulates in the central spindle midzone of spermatocytes (Williams *et al.*, 1995). Because kinesins act as microtubule-based motors and have microtubule-binding activity, *KLP3A* mutations are likely to affect primarily the formation of the central spindle, and secondarily the assembly of the contractile ring. A simultaneous absence of the central spindle and the contractile ring has been also observed in embryonic cells of mutants in the *pavarotti (pav)* locus, which encodes a kinesin-like protein related to the mammalian CHO1/MKLP1and to the *C. elegans* ZEN-4 (Adams *et al*., 1998; Powers *et al*., 1998; Raich *et al*., 1998). The role of Pav in spermatocyte cytokinesis could not be determined, as the extant mutants in this locus die during embryogenesis, preventing the cytological analysis of male meiosis. However, several findings indicate that Pav is required for central spindle formation both in embryonic and meiotic cells. Pav forms a complex with Polo kinase, and both Pav and Polo accumulate in the central spindle midzone of spermatocytes. In addition, *polo* mutant spermatocytes are severely defective in both central spindle and actomyosin ring, and display failures in cytokinesis (Adams *et al*., 1998; Carmena *et al*., 1998; Herrmann *et al*., 1998). It is thus likely that the Pav-Polo complex is primarily required for spermatocyte central spindle formation and secondarily for contractile ring assembly.

The phenotypes of *chic* and *sqh*, on the other hand, would suggest the opposite: that a primary defect in contractile ring assembly can secondarily disrupt central spindle formation. *chic* encodes a *Drosophila* profilin, a small actin binding protein that promotes actin polymerization (Cooley *et al*., 1992; Giansanti *et al*., 1998). *sqh* encodes a regulatory light chain of myosin II that is also likely to be involved in the assembly of the actomyosin contractile ring (Karess *et al*., 1991). Thus, the results on *chic*, *sqh* and *KLP3A* suggest the existence of a cooperative interaction between elements of the actin-based contractile ring and the central spindle microtubules: when either of these structures is perturbed, the proper assembly of the other is disrupted (Giansanti *et al*., 1996, 1998).

The finding that *dia* mutants lack both the central spindle and the contractile ring is consistent with the idea that these cytokinetic structures are interdepedent, and suggests the hypothesis that Dia may mediate the underlying micro-

tubule-F actin interactions. Dia is a member of the FH (formin homology) protein family. These proteins are highly conserved and play an essential role in cytokinesis also in fungi, worms and mammals. In all these organisms FH proteins interact with both Rho GTPases and profilin, and thus appear to be involved in the organization of the actin cytoskeleton (reviewed by Wasserman, 1998). However, recent studies have identified mDia as a downstream Rho effector that associates with mouse fibrobast microtubules, promoting their stabilization (Palazzo *et al*., 2001). Thus, it is conceivable that in *Drosophila* spermatocytes Dia contributes both to the stabilization of central spindle microtubules and to the assembly of the contractile ring.

Additional insight into the relationships between the central spindle and the contractile ring is provided by the cytological analysis of a number of newly isolated mutants defective in spermatocyte cytokinesis. By screening a large collection of male sterile mutants isolated by B. Wakimoto, D. Lindsley, E. Koundakjian and C. Zuker (unpublished work), we have isolated mutants in 19 genes required for cytokinesis. The cytological analysis of representative mutant alleles in 18 loci revealed that 7 of them are severely defective in both the central spindle and the contractile ring, while 11 of them allow the assembly of both these structures but are defective either in ring constriction or disassembly (Giansanti *et al*., 1999b). These results establish a strong correlation between the presence of the central spindle and the contractile ring, giving further support to the hypothesis that these structures are interdependent.

Although the results on *Drosophila* male meiosis strongly suggest that the central spindle and the contractile ring are mutually dependent, this is not true in all animal cells. Studies on mammalian cells have shown that central spindle plays an essential role during cytokinesis but have provided limited information on whether perturbations in the actomyosin ring assembly disrupt the central spindle (Cao and Wang, 1996; Wheatley and Wang, 1996; Eckley *et al*., 1997; reviewed by Gatti *et al*., 2000). In contrast, studies on *C. elegans* have clearly shown that, at least in the early stages of embryonic cytokinesis, the actomyosin ring and the central spindle can assemble independently (Powers *et al*., 1998; Raich *et al*., 1998; Jantsch-Plunger *et al*., 2000).

Why do *Drosophila* spermatocytes, and possibly mammalian cells, differ from *C. elegans* embryos in the interactions between the central spindle and the contractile ring? To answer to this question we should bear in mind that in both *Drosophila* and mammalian cells the central spindle assembles in the proximity of the equatorial cortex, while in the large *C. elegans* embryonic cells the central spindle assembles in the center of the cell at considerable distance from the cortex. In these embryonic cells the central spindle and the actomyosin ring come into contact only after substantial furrow ingression. We thus speculate that in *C. elegans* embryos cytokinesis consists of two steps: an early step, where the central spindle and the contractile ring assemble independently in distant cellular regions, and a late step that begins when the central spindle and the contractile ring have come into contact. It is conceivable that during this late step the central spindle and the contractile ring interact cooperatively to complete cytokinesis successfully.

The role of Abnormal spindle (Asp) in central spindle assembly and cytokinesis

Asp is a microtubule binding protein of 220kD that possesses cdc2 kinase and MAP kinase phosphorylation sites, as well as putative calmodulin and actin binding sites (Saunders *et al*., 1997). Asp localizes to the polar region of the *Drosophila* mitotic and meiotic spindles and is required for spindle pole formation (Saunders *et al*., 1997; Avides and Glover, 1999; Wakefield *et al*., 2001). Besides its function at the spindle poles, Asp appears to perform an additional function during central spindle assembly. Asp exhibits a striking enrichment at the minus ends of central spindle microtubules in both mitotic cells and spermatocytes (Wakefield *et al*., 2001). In *asp* mutants, a large fraction of spermatocyte telophases display severe defects in central spindle morphology, fail to organize a regular actomyosin ring and do not complete cytokinesis. The abnormalities seen in the central spindle of *asp* spermatocytes suggest that the Asp protein may help to cross-link the minus ends of central spindle microtubules, preventing them from sliding and splaying apart (Wakefield *et al*., 2001). Taken together, these observations strongly suggest that central spindle formation not only depends on microtubule plus end-associated proteins such as KLP3A and Pav, but also on minus end-associated proteins such as Asp. These proteins are likely to work in concert to ensure proper orientation, alignment and stabilization of central spindle microtubules, allowing the correct formation of the acto-myosin contractile apparatus required for cytokinesis.

Genes required for actomyosin ring constriction or disassembly

Mutations in four genes allow the formation of both the central spindle and the actomyosin ring but affect either ring contriction or diassembly. In *four weel drive (fwd)* and *giotto (gio)* mutants the central spindle remains normal throughout meiotic division but the cytokinetic ring fails to constrict properly, thus impairing completion of cytokinesis (Brill *et al*., 2000; our unpublished observations). *fwd* encodes a phosphatydilinositol 4-kinase (PI 4-kinase), that belongs to a family of proteins involved in the synthesis of the membrane phosphilipid PIP2 (Brill *et al*., 2000). The function specified by the *gio* mutation has not yet been identified as the molecular characterization of *gio* is still under way. Although *fwd* encodes a product involved in membrane trafficking, this gene appears to be required for contraction of the actomyosin ring. This finding suggests

that proper membrane behavior is an essential requirement for actomyosin ring constriction. A precedent for this conclusion is provided by studies on *C. elegans* embryos depleted of Syntaxin-4, a cytokinesis specific t-SNARE involved in membrane-vesicle fusion processes. These embryos exhibit frequent failures in cleavage furrow ingression, suggesting an underlying defect in the contractile ring machinery (Jantsch-Plunger and Glotzer, 1999).

In *twinstar (tsr)* and *l(3)7m62* mutant spermatocytes the central spindle has a normal appearance throughout meiotic division. The contractile ring assembles normally too and undergoes a normal contraction. However, at the end of both meiotic divisions the contractile rings of these mutants fail to disassemble, overgrow and form large and persistent F-actin aggregates that interfere with completion of cytokinesis (Gatti and Baker, 1989; Gunsalus *et al*., 1995; Giansanti *et al*., 1999; S. Bonaccorsi, M. G. Giansanti and M. Gatti, unpublished results). These results indicate that *tsr* and *l(3)7m62* are primarily involved in the disassembly of the contractile ring. Consistent with this conclusion, the molecular characterization of *tsr* showed that this gene encodes a polypetide homologous to cofilins (Gunsalus *et al*., 1995), a family of low molecular mass actin-binding proteins that can sever and depolymerize actin filaments *in vitro* (Moon and Drubin, 1995). *l(3)7m62* has not been characterized at the molecular level.

The genetic control of meiotic cytokinesis

The mutations affecting *Drosophila* cytokinesis have been identified either by screening collections of male sterile mutants for failures in meiotic cytokinesis, or by examining late lethal mutants for defects in neuroblast cytokinesis (Table I and references therein). Interestingly, while some of the genes required for meiotic cytokinesis are also needed for neuroblast cytokinesis, others appear to be specifically involved in meiotic cytokinesis. Conversely, there are genes that control neuroblast cytokinesis which seem to have little or no role during meiotic cytokinesis (Table I).

Null mutations in *KLP3A*, a gene encoding a kinesin-like protein expressed both in testes and in somatic tissues, are viable, have no effects on neuroblast mitosis but disrupt meiotic cytokinesis in males (Williams *et al*., 1995). Similarly, null mutants in *fwd*, that are fully viable but male sterile, specifically affect spermatocyte cytokinesis (Brill *et al*., 2000). Another gene which seems to be involved in meiotic but not in neuroblast cytokinesis is *chic.* This gene, which encodes a *Drosophila* profilin, is identified by viable leaky alleles which cause sterility in both sexes, as well as by null alleles causing lethality during embryogenesis (Cooley *et al*., 1992; Verheyen and Cooley, 1994). Heteroallelic *chic* combinations resulting in lethality at the larval-pupal boundary exhibit severe disruptions in meiotic cytokinesis but do not affect neuroblast cell division, suggesting that the *chic* function is either unnecessary or has only a minor role during neuroblast cytokinesis (M. G. Giansanti, S. Bonaccorsi, and M. Gatti, unpublished results).

On the other hand, the *peanut (pnut)* gene, which encodes a *Drosophila* septin, is required for neuroblast cytokinesis but appears to be dispensable for spermatocyte cytokinesis. The Pnut protein co-localizes with the contractile ring in several *Drosophila* cell types, including embryonic, imaginal disk, neuroblast and male meiotic cells (Neufeld and Rubin, 1994; Hime *et al*., 1996; our unpublished results). Null mutants in the *pnut* gene die at at the larval-pupal transition and display polyploid cells in their brains, consistent with a defect in cytokinesis (Neufeld and Rubin, 1994). However, in testes of larvae homozygous for null *pnut* mutations, spermatocytes display regular central spindles and contractile rings and do not exhibit defects in cytokinesis (S. Bonaccorsi M. G. Giansanti, and M. Gatti, unpublished results). Thus, although Pnut is associated with the contractile ring, it does not appear to have a crucial role in meiotic cytokinesis of *Drosophila* males. In addition, the analysis of germline clones homozygous for *pnut* has shown that this gene is not required for cytokinesis during the cystobast divisions in females (Adam *et al*., 2000).

Taken together these results indicate that the mechanisms underlying neuroblast and spermatocyte cytokinesis are different, at least in part. This conclusion is consistent with previous studies on diverse organisms that have led to the suggestion that cytokinetic mechanisms may vary in different cell types (reviewed by Satterwhite and Pollard, 1992; Goldberg *et al*., 1998). The identification and characterization of additional genes involved in *Drosophila* cytokinesis is thus likely to provide information not only on the gene products underlying cytokinesis, but also how various combinations of these products are used by cells employing different strategies to accomplish cytokinesis.

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