Spermatogenesis 2:3, 185-196; July/August/September 2012; © 2012 Landes Bioscience

Cytokinesis in Drosophila male meiosis

Maria Grazia Giansanti,* Stefano Sechi, Anna Frappaolo, Giorgio Belloni and Roberto Piergentili

Istituto di Biologia e Patologia Molecolari del CNR; Dipartimento di Biologia e Biotecnologie Università Sapienza di Roma; Rome, Italy

Keywords: *C. elegans*, *D. melanogaster*, humans, invertebrates, rodents, spermatogenesis

Cytokinesis separates the cytoplasm and the duplicated genome into two daughter cells at the end of cell division. This process must be finely regulated to maintain ploidy and prevent tumor formation. Drosophila male meiosis provides an excellent cell system for investigating cytokinesis. Mutants affecting this process can be easily identified and spermatocytes are large cells particularly suitable for cytological analysis of cytokinetic structures. Over the past decade, the powerful tools of Drosophila genetics and the unique characteristics of this cell system have led researchers to identify molecular players of the cell cleavage machinery and to address important open questions. Although spermatocyte cytokinesis is incomplete, resulting in formation of stable intercellular bridges, the molecular mechanisms are largely conserved in somatic cells. Thus, studies of Drosophila male meiosis will shed new light on the complex cell circuits regulating furrow ingression and substantially further our knowledge of cancer and other human diseases.

Introduction

Cytokinesis is the final event of cell division, when the cytoplasm and the segregated genome are physically partitioned into two daughter cells. Execution accuracy of this process is crucial for maintaining ploidy and preventing tumor formation.[1] Although the details of cytokinesis differ between organisms, a common molecular model can be drawn. In animal cells, cytokinesis is mediated by contraction of the contractile ring, a transient organelle containing F-actin filaments and active Myosin II that forms just beneath the plasma membrane around the equator of the dividing cell.[1] Actomyosin ring constriction draws in the plasma membrane and leads to the formation of a cleavage furrow that invaginates until the two daughter cells remain connected by a thin cytoplasmic bridge. In most models, the intercellular bridge is ultimately severed during abscission, resulting in the complete separation of daughter cells.[2,3] In some tissues of Drosophila and other organisms, cells do not complete abscission and incomplete cytokinesis leads to the formation of stable intercellular bridges that interconnect mitotically or meiotically related cells.[4-6]

Contractile ring assembly is directed by the Rho guanosine triphoshatase (GTPase) module, which controls actin nucleation and activates Myosin (reviewed in refs. 7 and 8). The initial events involve an interplay between the prominent microtubule (MT) bundle that forms in ana-telophase between the segregating chromosomes, dubbed the central spindle, and the actomyosin ring.[2,7,9] The central spindle dictates the assembly and the position of the cleavage furrow by controlling the local concentration of Rho regulators on the cortex (for a review, see refs. 2, 7, 8 and 10).

A large number of proteins, including kinesins, the chromosomal passenger complex (CPC), kinases, actin and tubulin regulatory factors, and proteins required for membrane insertion, travel along the central spindle microtubules to the cell equator, where they act on cytokinesis (for a review, see refs. 2 and 10). Studies in a variety of organisms indicate that membrane trafficking from internal membrane stores plays a critical role during cytokinesis.[11,12] Membrane remodeling during cytokinesis relies on components of the secretory and endocytic/recycling trafficking pathways, as well as the membrane fusion machinery.[11-15] Secretion involves vesicle transport from the endoplasmic reticulum (ER) to the Golgi apparatus and then to the plasma membrane. In the endocytic pathway, plasma membrane-derived vesicles proceed through the early endosome and the recycling endosome (RE), which directs them back to the plasma membrane.[15]

Since the first description of this process more than a century ago, at least one hundred proteins have been identified that are involved in cytokinesis.[16] Progress in their identification has been hampered by difficulties in applying biochemical strategies that have been particularly successful in other studies. A major limitation in proteomic analysis of cytokinesis has been the isolation of the transient structures involved in this process that assemble and disassemble during a limited cell cycle window. Skop and coworkers isolated midbodies from Chinese hamster ovary cells (CHO) and analyzed the proteins enriched in these structures by tandem liquid chromatography and mass spectrometry.[17] However midbodies characterize only very late stages of cytokinesis. A fruitful approach, that allowed to identify several molecular players, consists of the genetic dissection of this process in suitable model organisms such as *Drosophila melanogaster*.[18]

Drosophila Male Meiotic Cytokinesis: A Suitable Cell System for Exploring Cytokinesis

In *D. melanogaster*, spermatogenesis starts with the asymmetric mitotic division of a germ-line stem cell that generates another stem cell and a primary spermatogonium. Each primary spermatogonial cell represents the mitotic founder of a cluster of

*Correspondence to: Maria Grazia Giansanti;
Email: Mariagrazia.Giansanti@uniroma1.it
Submitted: 04/27/12; Revised: 08/02/12; Accepted: 08/02/12
http://dx.doi.org/10.4161/spmg.21711

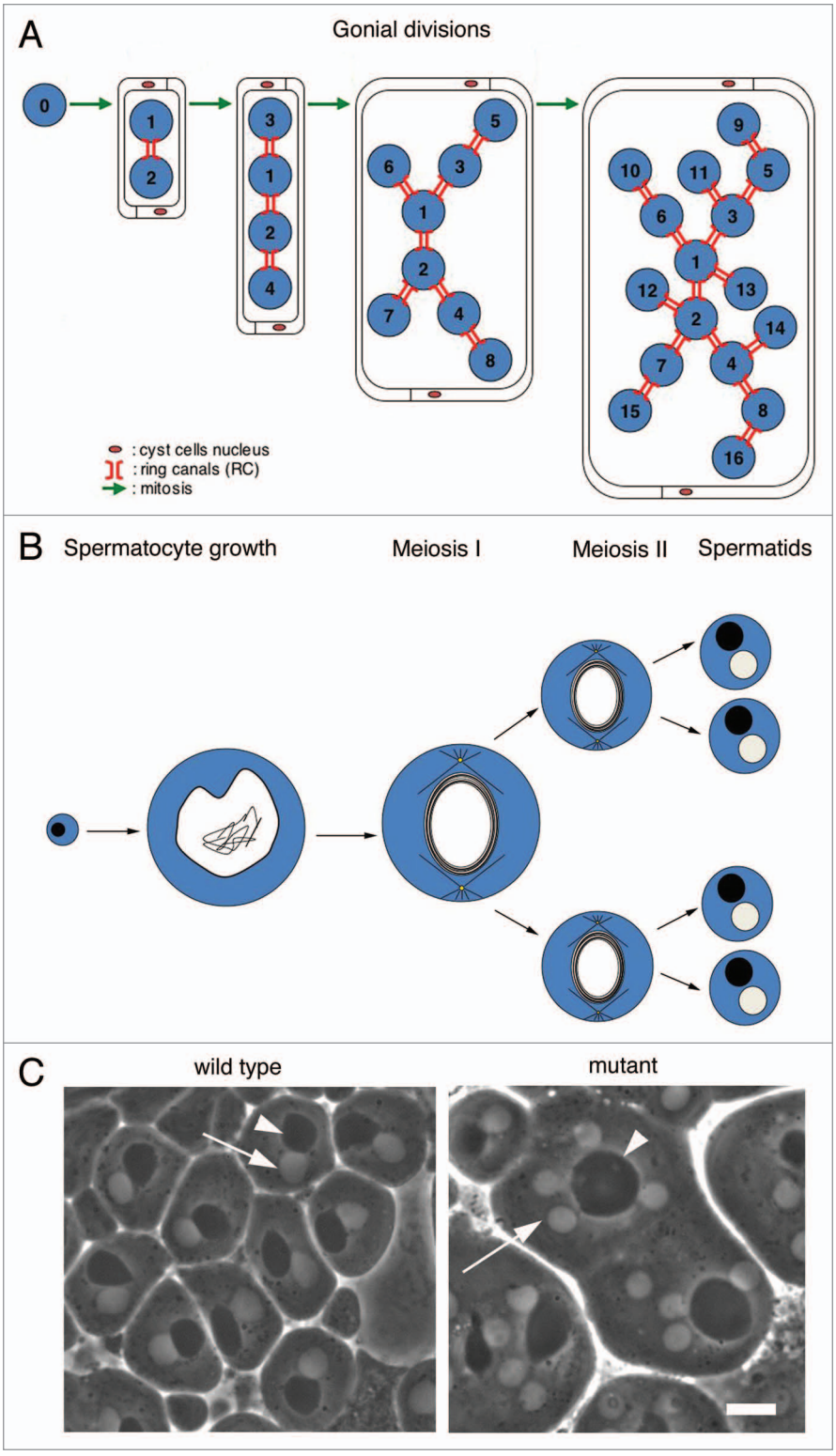

Figure 1. Spermatogenesis in *Drosophila melanogaster*. (A and B) Schematic representation of spermatogenesis in *D. melanogaster*. (A) A single primary spermatogonium undergoes four mitotic divisions. Numbers indicate gonial cells, number 0 indicates the primary spermatogonium, the mitotic founder of a cluster of dividing secondary spermatogonia connected by ring canals. Two cyst cells engulf the progeny of the primary spermatogonium throughout spermatogenesis. Blue, spermatogonia. (B) Each primary spermatocyte undergoes a growth phase, which lasts 90 h before undergoing two meiotic divisions. A wild type spermatid cyst at the so called onion stage contains 64 spermatids connected by 63 ring canals (not shown). Each spermatid contains a single nucleus (white) associated with a nebenkern (black) of similar size. Only four spermatids are represented. Blue, cytoplasm. (C) Spermatids at the onion-stage viewed by phase-contrast microscopy. Each wild type spermatid contains a single light nucleus (arrow) associated with a dark nebenkern (arrowhead). Spermatids from a mutants defective in male meiotic cytokinesis, contain large nebenkerne (arrowhead) associated with 2 or 4 nuclei of similar size (arrow). Bar, 10μm.

synchronously dividing secondary spermatogonia. Two cyst cells, derived from the asymmetric division of two cyst progenitor cells, associate with the primary spermatogonium soon after its birth and engulf the progeny of that cell throughout spermatogenesis.[4,19] The primary spermatogonium undergoes four gonial mitotic divisions giving rise to 16 primary spermatocytes which in turn undergo two meiotic divisions (as depicted in **Fig. 1**). During both the gonial mitoses and the spermatocyte meiotic divisions, cytokinesis is incomplete[4,19] and daughter cells remain interconnected by cytoplasmic intercellular bridges called ring canals. Each primary spermatocyte undergoes an impressive growth phase before entering meiosis, which results in a 25-fold increase of its cell volume (**Fig. 1B**). During the growth phase, which lasts approximately 90 h, spermatocytes also transcribe most of the genes required in stages of spermatogenesis that follow meiosis.[4]

At metaphase I, spermatocyte nuclei become spindle shaped and maintain a double-membrane that encircles the meiotic chromosomes during meiosis in addition 5 to 7 double parafusorial membranes surrounding the nuclei of dividing spermatocytes.[20,21] At each cell pole a system of 13 umbrella-shaped layers comprise the so-called astral membranes.[20] Both the parafusorial membranes and the astral membranes derive from the ER[4,20] and are in fact enriched with the ER marker protein disulfide isomerase.[22,23] During each meiotic division mitochondria line up along the parafusorial membranes; this arrangement ensures that each daughter cell receives an equal number of these organelles upon cytokinesis. Following meiosis II, all the mitochondria contained in each spermatid aggregate to one side of the nucleus and fuse to form a complex

interlaced structure resembling an onion, named the nebenkern[20] (**Fig. 1B**). When examined by phase-contrast optics, spermatid cysts at the so called onion-stage contain 64 spermatids, each displaying a phase-dark nebenkern paired with a single phase-light nucleus of similar size (**Fig. 1B-C**). This characteristic arrangement of nuclei and nebenkerne in spermatid cysts allows easy scoring of mutants defective in meiotic cytokinesis. Cytokinesis failures during one or both meiotic divisions are expected to disrupt partitioning of mitochondria between daughter cells resulting in spermatids containing respectively two or four nuclei associated with an enlarged nebenkern (**Fig. 1C**). Thus, the presence of multinucleate spermatids is diagnostic of defects in cytokinesis in the meiotic divisions.[4] In addition, since the volume of each nucleus in the onion-stage spermatids is proportional to its chromatin content, variations in nuclear size are the consequence of errors in chromosome segregation during meiosis.[4] In this context it should be noted that the spindle assembly checkpoint, which monitors proper chromosome association to spindle microtubules and blocks cells in metaphase in the presence of unattached chromosomes or malformed spindles, is not stringent in spermatocytes, resulting only in a small delay in both anaphase onset and meiotic progression.[24-26] This particular characteristic of spermatocytes offers the advantage to investigate whether gene products required for chromosome segregation play also functions during cytokinesis. Mutants affecting both chromosome segregation and cytokinesis exhibit onion-stage spermatids containing one large nebenkern associated with multiple nuclei of different size.

Besides allowing a rapid identification of cytokinesis mutants, Drosophila spermatogenesis offers other clear advantages when exploring cytokinesis. Although meiotic cytokinesis is incomplete in spermatocytes, the mechanism of furrowing in these cells is largely conserved when compared with other animal cells (see ref. 16 and this review). Moreover Drosophila male meiotic cells are considerably larger than most somatic cells, providing a suitable cell system for immunocytological and in vivo analysis of the cytokinetic structures (**Fig. 2**).[18] Finally, several antibodies and GFP-labeled proteins are available to visualize the Golgi membranes, the ER and vesicle traffic components in Drosophila spermatocytes (for examples, see refs. 22, 23).

F-actin Ring Assembly: The Role of the Spindle Microtubules

Since the pioneering studies of Rappaport[27] it has been clearly established that the mitotic spindle plays an essential role in cleavage furrow formation. The powerful tools of genetic analysis in Drosophila were used to examine the contribution of different spindle components in signaling cytokinesis. Mutational analysis of male meiosis has indicated that chromosomes and centrosomes are dispensable for cytokinesis. Mutations in the genes *asterless* and *spd2* impair centrosome assembly and aster formation,[28,29] but enable the assembly of regular central spindles and contractile rings that mediate a successful cytokinesis. The phenotypes displayed by *fusolo* (*fsl)* and *solo fuso (suo)* mutants are also consistent with an essential role for the central spindle microtubules in signaling cytokinesis. A large percentage of secondary spermatocytes from *fsl* and *suo* mutants are devoid of chromosomes as a consequence of errors in chromosome segregation during the first meiotic division. Strikingly, these cells assemble regular central spindles and contractile rings and undergo cytokinesis even in the absence of chromosomes.[30]

Several proteins are enriched in the central spindle during cytokinesis. Among the microtubule-interacting proteins, major components required for central spindle morphogenesis are kinesins and microtubule bundling factors, (**Table 1**; reviewed in ref. 16). The conserved PRC1 protein has an in vitro microbule bundling activity and is required for central spindle assembly in mammalian tissue culture cells.[31] The Drosophila ortholog of PRC1, Fascetto (Feo), is one of the first markers to appear at the cell equator of dividing spermatocytes and decorates the central spindle midzone during anaphase and telophase.[32,33] The effects of *feo* mutations in spermatocyte cytokinesis have not been determined. However, loss of Feo leads to cytokinesis defects and affects central spindle organization in both larval neuroblasts and S2 cells.[32]

Two conserved, plus-end directed, microtubule kinesins play essential roles in central spindle assembly, the kinesin 6 family member MKLP1/Pavarotti (Pav) and the chromokinesin KIF4 (for reviews, see refs. 7, 34). The plus-end directed motor of MKLP1 can cross-link microtubules and promote sliding of one microtubule over another.[35] Thus, the activity of this kinesin is essential to mediate interactions between overlapping microbule bundles during central spindle formation. Consistent with this function, the ortholog of MKLP1, Pavarotti (Pav), accumulates at the central spindle midzone where the microtubule plus-ends overlap during anaphase of Drosophila spermatocytes.[36] The Drosophila ortholog of KIF4, KLP3A, is also concentrated at central spindle mid-zone and is required for central spindle assembly in spermatocytes.[37] The involvement of KIF4 and KLP3A in central spindle formation could be due to their ability to form a complex with the microtubule bundling protein PRC1/Feo.[38,39]

In Drosophila spermatocytes, central spindle microtubules are not only required for the initial formation of the cleavage furrow but are also essential for the maintenance of contractile structures. Phenotypic analysis of several mutants defective in male meiotic cytokinesis has suggested a mutually dependent interaction between the central spindle microtubules and elements of the actin ring during cytokinesis.[9,40,41] Indeed mutations that affect genes encoding central spindle components (**Table 1**) such as the chromokinesin Klp3A,[9,37] or the CLASP ortholog Orbit[42] lead to a secondary defect in contractile ring assembly and stability. An identical phenotype is caused by mutations in *chickadee (chic)*, *diaphanous (dia)* and *spaghetti squash* (*sqh*) which encode the actin regulators profilin, formin and Myosin II, respectively.[9,18] In *chic*, *dia* and *sqh*, a primary defect in the contractile actomyosin ring is accompanied by a secondary lesion in the central spindle.

Inoue and coworkers[42] provided the first characterization of central spindle formation in live Drosophila spermatocytes. Time-lapse analysis of spermatocytes expressing β-tubulin-EGFP and undergoing ana-telophase revealed that two different populations of MTs comprise central spindle bundles (depicted

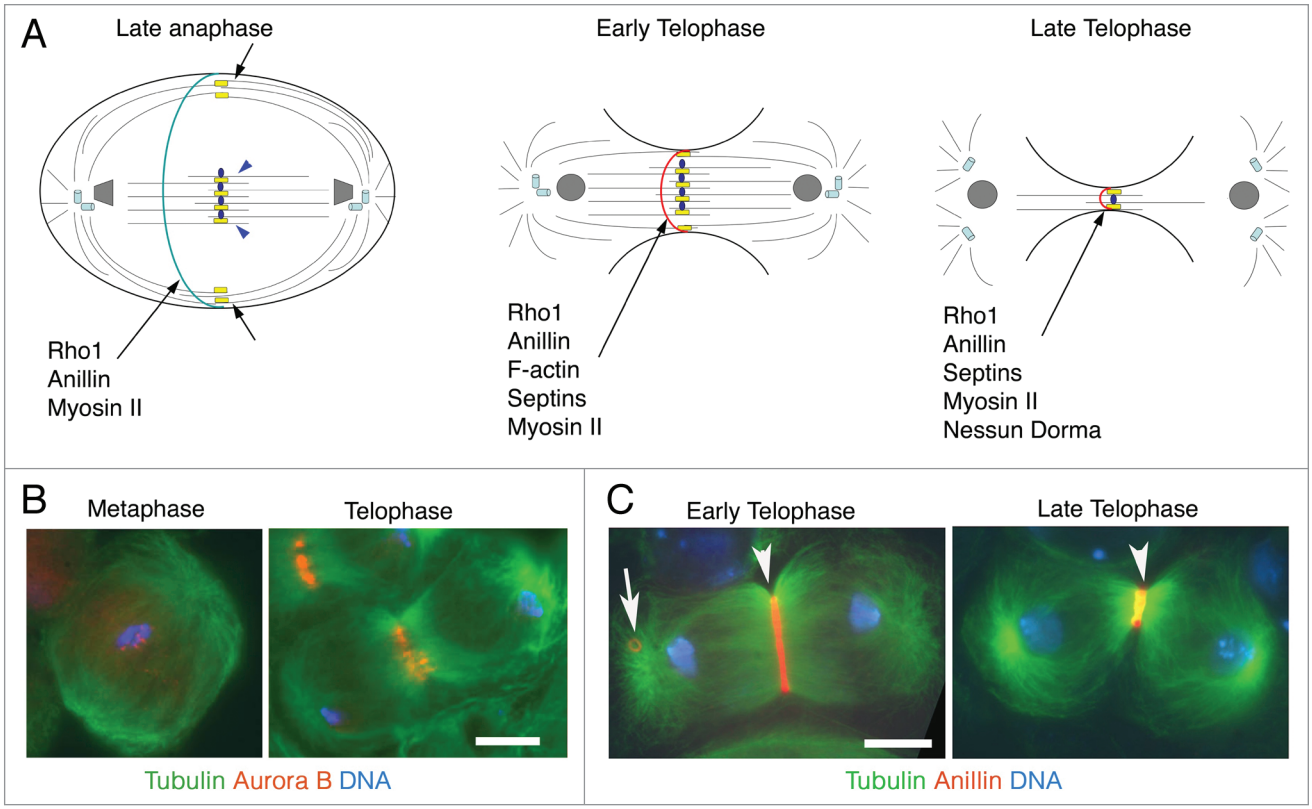

Figure 2. Cytokinesis in Drosophila spermatocytes. (A) Schematic representation of different stages of male meiotic cytokinesis. During late anaphase the central spindle is made up of two distinct populations of microtubule bundles, the "peripheral" astral microtubules (short black arrows) and the "interior" central spindle microtubules (blue arrowheads). The kinesin Pavarotti (Pav) is enriched at the central spindle midzone and associates with both populations of microtubule bundles (yellow rectangles). The microtubule plus-end stabilizing protein Orbit specifically localizes to the interior central spindle microtubules but does not associate with the peripheral astral microtubules (blue ovals). Other microtubule associated proteins, required for central spindle formation, such as Fascetto, KLP3A, KLP67A and the Chromosomal Passenger Complex (CPC) are not depicted. Rho1, Anillin and Myosin II concentrate in a narrow cortical ring during late anaphase, before the recruitment of F-actin. During early- and mid-telophase, peripheral astral microtubule bundles merge with interior microtubule bundles. At this stage Rho1, Anillin, F-actin, Septins and Myosin II colocalize in the contractile rings. Nessun Dorma is recruited to the cleavage furrow during late telophase; this protein, like Septins, Anillin and Myosin II, is a ring canal component after the completion of cytokinesis. (B) Localization of the CPC protein Aurora B in dividing spermatocytes. Primary spermatocytes were stained for tubulin (green), Aurora B (red) and DNA (blue). Note that Aurora B is enriched at metaphase centromeres and concentrates at the central spindle midzone during telophase. (C) Localization of Anillin in early telophase spermatocytes. Spermatocytes were stained for tubulin (green), Anillin (red) and DNA (blue). Arrowhead points to the contractile ring, arrow points to a ring canal. Bars, 10 μm.

in **Fig. 2A**). A set of "peripheral" astral microtubules contacts the cortex at the future cleavage site and bundles together to promote furrow ingression. A distinct set of microtubule bundles named "interior" MTs, confined inside the nuclear envelope, merges with peripheral astral microtubules to complete furrow ingression and cytokinesis. Mutations in the gene encoding the microtubule-associated protein Orbit specifically disrupt the interior central spindle bundles but do not affect the peripheral astral microtubules that can promote initial furrow ingression. The interior central spindle microtubules are essential for cytokinesis progression, as cleavage furrows are unstable and rapidly regress in *orbit* mutant spermatocytes. Defective central spindles and actomyosin rings have been also observed in mutants lacking the plus-end directed kinesin Klp67A, a member of the Kip3 subfamily of microtubule destabilizing kinesins.[43,44] Nonetheless, the central spindles of *klp67A* mutant spermatocytes appear strikingly different from those of *orbit* mutants.[44] Both peripheral and interior microtubule bundles appear severely disorganized and diminished during ana-telophase. Although in these cells cleavage furrows preferentially form in association with the few remaining peripheral microtubules, ectopic furrows can also form when the interior central spindle buckles and contacts the cortex. Thus, both populations of central spindle microtubules are able to induce furrowing, but in wild type spermatocytes the interior central spindle is not suffiiciently close to the cortex to perform this task.

Early Steps of Male Meiotic Cytokinesis: Rho1 Activation and Cleavage Site Determination

In Drosophila, as in all animal cells, contractile ring assembly and furrowing are orchestrated by the small GTPase Rho1 (the Drosophila homolog of RhoA) at the cortex. Cycling between the GDP-bound inactive form and the GTP-bound active form of Rho1 depends on the activity of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Consistent with a role of the Rho-GTPase module as a master switch in

Table 1. Proteins/Genes involved in cytokinesis of Drosophila spermatocytes, localization in dividing spermatocytes and mutant phenotypes

Protein/gene	Protein family	Predicted protein function	Localization*	Mutant phenotype†	Refs.
Rho GTPase module					
Rho1	RhoA	Rho GTPase Cleavage site determination Contractile ring assembly	Cleavage furrow, Cortical ring	ND	33, 74
Pebble	ECT2	RhoGEF	ND	Absence of both CS and CR	41
Contractile ring components					
Actin 5C	Actin	F-Actin	Contractile ring	ND	88
Zipper	Myosin II	Myosin II heavy chains	Contractile ring	ND	5, 18
Spaghetti squash	MRLC	Myosin II regulatory light chain	Contractile ring	Absence of both CS and CR	18, 33, 74
Anillin	Anillin	Anillin, Actin binding Scaffolding Protein	Contractile ring	Septins fail to localize Defects in maintenance of Rho1, F-actin and Myosin II	5, 73, 74
Peanut, Sep1, Sep2	Septin	Septins, Scaffolding Protein	Contractile ring	No defects in peanut mutants	5, 18
Actin filaments formation					
Diaphanous	Formin	Actin nucleator	ND	Absence of both CS and CR	9, 41, 104
Chickadee	Profilin	Actin-binding protein	Equatorial cortex	Absence of both CS and CR	9, 73
Associated with central spindle microtubules					
Pavarotti	MKLP1	Kinesin motor 6, microtubule motor Centralspindlin component	CS midzone	ND	33, 36
Fascetto	PRC1	Microtuble bundling	CS midzone	ND	32, 33, 51
Klp3A	Kif4A/B	Kinesin motor 4, microtubule motor	CS midzone	Absence of both CS and CR	9, 37, 73
Klp67A	KIF18	Kinesin motor 8, microtubule motor	Cell equator	Defective CS and CR	43, 44
Abnormal spindle	ASPM	Microtubule binding protein	Minus ends of CS	Defective CS and CR	25, 26
Cytokinesis regulation					
Aurora B	Aurora B	CPC subunit	CS midzone	ND	30, 33, 51
INCENP	Incenp	CPC subunit	CS midzone	Defective CS Failure to localize Pav	33, 51, 61
Deterin /Scapolo	Survivin	CPC subunit	CS midzone	Absence of both CS and CR Defects in Feo maintenance Defects in CPC targeting at CS Failure to localize Pav, Rho1 Polo	33
Australin	Borealin	CPC subunit	CS midzone	Absence of both CS and CR Defects in CPC localization Failure to recruit Pav, Feo Anillin	51
Polo	Polo	Polo kinase	CS midzone	Absence of both CS and CR	36, 67, 105
Cdc37	Cdc37	Required for the activty and stability of protein kinases, It forms a complex with Aurora B and Hsp90	ND	Absence of CS	106

*The localization of some proteins has not been studied in dividing spermatocytes. †Mutants in some genes are either early lethal or not available and could not be examined during male meiosis. ND, not determined; CS, central spindle; CR, contractile ring; CF, cleavage furrow; CPC, chromosomal passenger complex; ER, endoplasmic reticulum; PI4Kβ, phosphatidylinositol 4-kinase β, VLCFAs, very-long-chain-fatty acids.

Table 1. Proteins/Genes involved in cytokinesis of Drosophila spermatocytes, localization in dividing spermatocytes and mutant phenotypes

Protein/gene	Protein family	Predicted protein function	Localization*	Mutant phenotype†	Refs.
Membrane remodeling and vesicle traffic					
Syntaxin 5	Syntaxin 5	Golgi traffic, Vesicle fusion	Golgi	Multinucleate spermatids	79
Four way stop (Cog5), Cog7	COG complex	Golgi integrity, vesicle trafficking and glycosylation	Golgi	Defects in CR constriction and CS stability	77, 78
Brunelleschi	TRAPPII subunit	Golgi traffic	Golgi	Defects in CR constriction and CS stability	76
Giotto/vib	PITP	Phosphatidylinositol transfer vesicle traffic	ER, CF	Defects in CR constriction Defects to localize Rab11 at CF	22, 23, 87
Arf6	Arf6	Endocytic traffic	Recycling endosomes	Defects in furrow ingression	82
Four wheel drive	PI4Kβ	Phosphatidylinosytol 4-kinase	Golgi	Defects in CR constriction Defects in Rab11 localization at CF	23, 80, 81
Rab11	Rab11	Rab11GTPase Golgi and endocytic traffic	Golgi, vesicles, CF	Defects in CR constriction Defects in CS stability	23, 76, 81, 82
Bond	Elov1	Biosynthesis of VLCFAs	ND	Defects in furrow ingression Absence of CS Defects in CR stability	83
Pex2, Pex10	Peroxin proteins	Metabolism of VLCFAs	ND	Multinucleate spermatids Accumulation of VLCFAs	84
Cytokinesis completion, Ring canal formation					
Twinstar	Cofilin	Actin severing	ND	Failure of CR disassembly	88
Sticky/Dck	Citron kinase	Serine-Threonine kinase	CF	Irregular CR in late Telophase Over-constricted Anillin rings Small ring canals	91
Nessun Dorma	Nessun Dorma	Centralspindlin Partner High affinity for β-galactoside	CF and ring canal	Defects in furrowing completion Defects in ring canal formation	93
Other					
Larp	La type RNA-binding	RNA-binding	Associated with mitochondria Cell Equator	Absence of CS	107
Mitoshell	Novel, Bromodomain-Related Protein	ND	ND	Improper mitochondrial localization Defective CS and CR	108
Merlin	Neurofibromatosis 2 (NF2)	Regulation of actin cytoskeleton Microtubule binding Regulation of microtubule cytoskeleton	CS microtubules	Multipolar Telophases II	109

*The localization of some proteins has not been studied in dividing spermatocytes. †Mutants in some genes are either early lethal or not available and could not be examined during male meiosis. ND, not determined; CS, central spindle; CR, contractile ring; CF, cleavage furrow; CPC, chromosomal passenger complex; ER, endoplasmic reticulum; PI4Kβ, phosphatidylinositol 4-kinase β, VLCFAs, very-long-chain-fatty acids.

cleavage furrow formation, loss of the RhoGEF Pebble disrupts contractile ring assembly and central spindle formation in Drosophila spermatocytes.[41] Several studies have demonstrated the key role in Rho activation of an evolutionarily conserved two-protein complex termed centralspindlin. Centralspindlin is an heterotetramer consisting of the Rho family GAP MgRacGAP/Tumbleweed and the plus-directed kinesin MKLP1/Pav. This complex translocates to the plus ends of equatorial and central spindle microtubules through the activity of its motor component. Based on studies in Drosophila and mammalian cells, it has been proposed that the association between the centralspindlin

component RacGAP and RhoGEF/Pebble leads to local activation of RhoA/Rho1 at the cleavage site.[8,45-47]

Two serine/threonine kinases, Polo like kinase 1 (PLK1) and Aurora B, have been implicated in the regulation of the centralspindlin activity during central spindle formation and early steps of cytokinesis (for a review, see ref. 7).

Aurora B kinase is the catalytic subunit of the evolutionarily conserved Chromosomal Passenger complex (CPC) that also contains other three subunits, Inner Centromere Protein (INCENP), Survivin and Borealin. The CPC plays several essential roles during mitosis and meiosis showing a dynamic, cell cycle dependent localization. During interphase, it associates with chromatin and regulates chromosome condensation, then it concentrates at the inner centromeres from prometaphase until anaphase onset and monitors the kinetochore attachment to spindle microtubules. In anaphase, the CPC transfers to the spindle midzone and equatorial cortex[33,48-51]and it is involved in central spindle formation through phosphorylation of the centralspindlin component MKLP1/Pav.[52-54] Several papers have indicated that the association of the RhoGEF/Pebble with centralspindlin, critical for Pebble localization at the cortex and RhoA/Rho1, depends instead on the kinase PLK1 and its phosphorylation of MgRacGAP/RacGAP50.[55-59]

The minute dissection of the function of the CPC proteins during anaphase and cytokinesis has been hindered by the multiplicity of localizations and dynamic redistribution of CPC components during cell division, and the strict mitotic arrest (via spindle checkpoint activation) caused by CPC failure.[49,60] Drosophila male meiosis, with its lax checkpoint control, has enabled analysis of cytokinetic phenotypes caused by mutations affecting the CPC proteins, INCENP, Survivin and the Borealin-related paralog Australin (Aust)[33,51,61] (**Table 1**). The protein Aust replaces Borr only in male meiotic cells. Strikingly, although Aust lacks a region corresponding to the the central 140 amino acids, it can replace Borr during mitosis when expressed in S2 cells and can rescue the defects in chromosome alignment and cytokinesis.[51] The reason for the existence in *D. melanogaster* of a male-meiotic-specific Borealin-like subunit (and hence a male-meiotic specific CPC), is still unclear. However, a recent study has demonstrated that the Borr central region interacts with the Shrb component of ESCRT-III, a complex involved in membrane fission at the end of cytokinesis.[62] This interaction appears necessary to regulate abscission. Thus we might speculate that flies have evolved Australin to impair a potentially dangerous association with the ESCRT III machinery, allowing incomplete cytokinesis. Mammalian cells have also evolved a male-meiotic-specific CPC characterized by a novel Aurora kinase termed Aurora C.[63,64] Interestingly, Aurora C knockout mice are viable but males are sterile and exhibit polyploid spermatids.[63]

Spermatocytes of CPC mutants share similar early cytokinesis defects. Analysis of male meiosis in *INCENP (dmINCENP)* mutants carrying a hypomorphic allele has revealed a low density of central spindle microtubules.[61] *australin (aust)* null mutants display a stronger phenotype, as they do not assemble central spindles.[51] Both *dmINCENP* and *aust* mutants fail to recruit Pav at the cell equator, indicating a requirement for the CPC in centralspindlin localization.[51,61]

A recent study has elucidated the role of both Drosophila Survivin/Deterin and the CPC specifically during anaphase and cytokinesis.[33] *scapolo* (*scpo*), is a *deterin* allele, containing a missense mutation that substitutes a Serine for the wild type Proline at position 86 in the dSurvivin BIR domain. Cytological analysis has revealed that *scpo* is a "separation-of-function" allele: it allows the recruitment and function of the CPC until anaphase onset but impairs its activity in later stages. Just like dmINCENP and Aust, Survivin is essential for central spindle formation and to target the CPC and Pav to the central spindle and equatorial cortex. In spermatocytes, Survivin is also essential to localize Polo and Rho1 at the equatorial cortex. Based on these results, a possible model is that failure to localize Polo to the central spindle of *scpo* spermatocytes would prevent localization of RhoGEF by the centralspindlin complex and impair Rho1 activation and actomyosin ring assembly, resulting in early cytokinesis failures.

Like Aurora B, PLK1 is a multifunctional kinase that regulates several critical events of cell division beyond cytokinesis, including bipolar spindle formation and chromosome segregation (for a review see ref. 65). Loss of the Drosophila PLK1 homolog Polo results in metaphase arrest in Drosophila larval neuroblasts.[66] However, due to the weak spindle checkpoint of spermatocytes, mutations in *polo* do not block male meiotic progression, allowing investigation of the cytokinetic function of the Polo kinase.[36] In Drosophila spermatocytes at anaphase, Polo kinase, just like Aurora B, is enriched at the spindle midzone and enables Pav localization, central spindle formation and F-actin ring assembly.[36,67]

Scaffolding Proteins in the Contractile Ring: The Role of Anillin in Drosophila Spermatocytes

Based on current models, furrow ingression is driven by a ring-shaped structure composed of F-actin and nonmuscle Myosin II.[68] Sliding of bipolar Myosin filaments draws F-actin together in a purse-string like fashion. In order to achieve successful cytokinesis the actomyosin ring must be tightly anchored to the plasma membrane by a network of cytoskeletal proteins that act as a scaffold at the cell equator. Among scaffolding proteins (for a review see ref. 69) the GTP-binding septin proteins form filaments at the cleavage site of several organisms including Drosophila.[70] In *Drosophila melanogaster* five septins have been identified so far named Sep1, Sep2, Sep3, Sep4 and Sep5.[70] In Drosophila spermatocytes Peanut, Sep1 and Sep2 have been localized to contractile rings and in the ring canals that interconnect male germ cells (**Table 1 and Fig. 2**).[5,18] However, it has not been fully clarified whether septin proteins are required for cytokinesis in all Drosophila somatic cell types or in primary spermatocytes.[18,71]

A pivotal role in the organization of the scaffold in the spermatocyte furrow is played by the evolutionarily conserved protein Anillin. Anillin, is a polypeptide of 190kDa, originally identified as an actin-binding protein in Drosophila embryo extracts.[72] Studies in several systems showed that Anillin binds Myosin II and Septins making it a suitable candidate for the proper organization

of the actomyosin contractile structures (reviewed in ref. 69). In Drosophila spermatocytes anillin is one of the first markers of cleavage furrows. This protein starts to localize at the cell equator of dividing spermatocytes during anaphase, before the assembly of the F-actin ring (**Fig. 2**). Localization of Anillin to the nascent cleavage furrow of male meiotic cells does not require the assembly of an F-actin ring. Cytological analysis of mutants in *klp3A* and *chic* indicated that the initial formation of the Anillin cortical band does not depend on the presence of an F-actin ring.[73] The function of Anillin in Drosophila male germ cell development has been recently addressed by Goldbach and coauthors.[74] This study demonstrated that Anillin is required for the recruitment of Septins to the cleavage furrow and for the maintenance of Rho, F-actin and Myosin II in the contractile ring during later stages of cytokinesis. These authors also used FRAP experiments to test the association of GFP fusions to Sep2, Anillin and the Myosin II regulatory light chain Spaghetti Squash (Sqh; see ref. 75) with the cleavage furrow. Septins, Anillin and Sqh do not turn over in the cleavage furrow and exist in stable rather than dynamic structures during Drosophila cytokinesis. Remarkably, FRAP experiments revealed that Sqh completely loses its association with the cleavage furrow in cells depleted of Anillin. Based on these results, it has been hypothesized that Anillin might stabilize the cleavage furrow by linking the actomyosin ring to Septin filaments on the furrow membrane.[74]

Proteins Required for Membrane Remodeling and Membrane Traffic in Male Meiotic Cytokinesis

Studies in animal cells indicate that membrane traffic to the cleavage furrow is an essential facet of cytokinesis (see refs. 11–15), involving components of both the secretory and endocytic/recycling trafficking pathways, as well as the membrane fusion machinery. A mass spectrometry screen aimed at the characterization of proteins from purified mammalian midbodies, revealed that 33% of these proteins are vesicle traffic components, which is consistent with a role for membrane addition and remodeling during cytokinesis.[17] Evidence has implicated secretory traffic in cytokinesis of Drosophila spermatocytes. Cytokinesis of these cells is sensitive to Brefeldin A, a fungal metabolite that interferes with anterograde transport from the ER to the Golgi.[76] In addition it requires the wild type function of the Golgi proteins Cog5,[77] Cog7,[78] Syntaxin 5,[79] Four wheel drive (Fwd)[80,81] and Brunelleschi (Bru),[76] implicating Golgi traffic in this process (**Table 1**). Endocytic traffic also contributes to spermatocyte cytokinesis (**Table 1**).

The endosomal GTPase ARF6 is enriched in the plasma membrane and in a population of early and recycling endosomes. During cytokinesis, ARF6 is specifically enriched on recycling endosomes at the central spindle.[82] In the absence of ARF6, Rab4 and Rab11 recycling endosomes are still targeted to the spindle midzone. However ARF6 is required to boost the recycling rate required for fast cleavage furrow ingression. Remarkably ARF6 physically interacts with the centralspindlin component Pav suggesting that this protein might contribute to ARF6 recruitment to central spindle endosomes.[82]

The small GTPase Rab11, involved in both the secretory and the endocytic traffic, is also essential for cytokinesis in Drosophila male meiotic cells.[23] Time-lapse analysis of *arf6* and *rab11* mutant spermatocytes undergoing cytokinesis indicated similar defects in furrow ingression.[23,82] In spermatocytes expressing β-tubulin-EGFP, central spindles transiently formed and furrows initially ingressed at rates similar to wild type cells for almost 15 min. However, in most cells from *arf6* and *rab11* mutants, cytokinesis halted and cleavage furrows slowly regressed.

Specialized membrane domains are emerging as important factors in regulating rearrangement of the cytokinetic structures and vesicle fusion to the cleavage furrow. The gene *bond* encodes a Drosophila member of the family of Elovl proteins involved in biosynthesis of very-long-chain-fatty acids (VLCFAs). Mutations in this gene disrupt both cleavage-furrow ingression and central spindle assembly during early telophase of spermatocytes suggesting an intimate relationship between membrane lipids and cytoskeletal dynamics during cytokinesis.[83] Movies from *bond* mutant spermatocytes revealed a striking difference between these cells and those from *arf6* males. In *bond* cells the rate of cleavage furrow ingression was slow from the beginning of cytokinesis, central spindles failed to assemble and the Myosin ring, marked by Sqh-GFP, detached from the cortex and collapsed to one side of the cell. These findings have suggested that VLCFAs or their derivative lipids are essential to permit the plasma membrane to deform during cleavage furrow invagination and to maintain a stable connection with contractile ring structures. Interestingly, a recent study reported that mutants in Drosophila peroxin genes (*pex*) accumulate elevated levels of VLCFAs and exhibit cytokinesis defects in male meiotic cells just like *bond*, indicating that regulation of proper VLCFA levels is crucial for cytokinesis. Consistent with this hypothesis, loss of one copy of a *pex* gene can suppress the cytokinesis defects of *bond* mutant homozygotes.[84]

The distribution of phosphatidylinositol phosphates is also strictly regulated during cytokinesis and proteins involved in the phosphoinositide cycle have been implicated in furrowing (for a review, see ref. 85). Using green fluorescent protein (GFP) fused to the PLCδ-PH domain, which specifically binds to phosphatidylinositol 4,5-bisphosphate (PIP2), Wong and coworkers found this phosphoinositide localized in the plasma membrane and the cleavage furrow of dividing spermatocytes.[86] Drugs that interfere with PIP2 hydrolysis mediated by phospholipase C affect the completion of furrow ingression suggesting the importance of PIP2 turnover during cytokinesis.[86] The Drosophila phosphatidylinositol (PI) transfer protein Giotto/Vibrator (Gio) and the small Rab11 GTPase concentrate at the cleavage furrow and are both required for male meiotic cytokinesis.[22,23,81,87] The Drosophila phosphatidylinositol 4-kinase β (PI4Kβ) Fwd is also involved in spermatocyte cytokinesis.[80,81] Recent data have shown that wild type function of Fwd is required for the synthesis of PI4P on Golgi membranes and for the formation of Rab11-and PI4P containing organelles at the cell equator.[81] Genetic and phenotypic analyses have suggested that Rab11, Gio and Fwd might function in the same pathway controlling membrane addition to the

spermatocyte cleavage site, with Gio and Fwd acting upstream of Rab11.[23]

Proteins Required for Cytokinesis Completion in Male Meiosis

Like male germ cells in mammals, Drosophila spermatogonia and spermatocytes do not completely separate at the end of cytokinesis. After cleavage furrow constriction, abscission does not occur and daughter cells remain interconnected by cytoplasmic intercellular bridges called ring canals. During the final step of male meiotic cytokinesis the F-actin ring disassembles, while the contractile ring proteins Septins, Anillin and Myosin II persist as components of the ring canals.[5,18,73] Genetic dissection of male meiosis has allowed to identify proteins required at this stage but not in earlier steps of cytokinesis (**Table 1**).

The gene *twinstar* (*tsr*) encodes a polypeptide homologous to cofilins,[88] a family of small actin severing proteins. Mutations in *tsr* do not affect contractile ring assembly and cleavage furrow ingression, but they impair F-actin disassembly at the end of cytokinesis.[73,88] Late telophases from *tsr* males display abnormally large F-actin aggregates that fail to disassemble and are likely to interfere with completion of cytokinesis.[88]

Citron kinase is also involved in late cytokinesis. This protein belongs to an evolutionarily conserved family of serine-threonine kinases. In mammalian cells, it is enriched at the cleavage furrow, and is essential to regulate abscission.[89,90] Mutations in *Drosophila citron kinase (dck)/sticky* affect the completion of cytokinesis in different cell types including spermatocytes.[91,92] Cytological analysis of spermatocytes from *dck* mutant males did not reveal defects in cleavage furrow assembly or ingression. However very late telophases of *dck* displayed abnormally extended F-actin aggregates associated with the midbodies. In addition Anillin rings appeared over-constricted, thus resulting in smaller ring canals.[91]

A recent study characterized a novel component of Drosophila ring canals, dubbed Nessun Dorma (Nesd), identified as an evolutionarily conserved partner of the centralspindlin complex proteins in S2 tissue culture cells.[93] Nesd can directly bind both Pav and RacGAP50C through a highly conserved domain in its N-terminus which also mediates its localization to the midbody in cultured cells. Nesd is required in late cytokinesis of male meiotic cells but not in cultured cells and in neuroblasts. Time-lapse analysis of mutant spermatocytes expressing the contractile ring marker Sqh-GFP or the plasma membrane marker PLCδ-PH-GFP, indicates a requirement for Nesd in stabilization of the actomyosin ring and in maintaining the association of this structure with the plasma membrane at the end of cytokinesis.[93] Consistent with a role in late cytokinesis and in ring canal formation, Nesd is enriched in the cleavage furrow during late telophase. Interestingly, Nesd contains a pectin lyase-like domain in its C-terminal half and displays an in vitro high binding affinity for β-galactosides. These results have suggested a role for glycosylated proteins during late steps of cytokinesis in Drosophila male germline.

Drosophila Spermatocytes are More Sensitive to Mutations Affecting Cytokinesis than Mitotic Cells

Most mutations affecting Drosophila cytokinesis have been identified by screening collections of either male sterile or late lethal mutants.[18] RNAi screens in Drosophila tissue culture cells have also provided a valid approach for genetic dissection of mitotic cytokinesis.[94-96] Remarkably, a large proportion of the proteins required for mitotic cytokinesis in S2 cells or larval neuroblasts have been also implicated in male meiotic cytokinesis. Some of the proteins discovered in RNAi screens could not easily be studied in spermatocyte cytokinesis because of their lethal phenotypes (**Table 1**). However, when tested in both cell systems, most proteins required for mitotic cytokinesis were found to play a similar function in male meiosis. Borealin represents an exception to this assumption; during male meiosis this protein is replaced by its paralog Aust that lacks the Shrb-interacting region and might be suitable for incomplete cytokinesis.[51,62]

Although most cytokinesis players are conserved, there are several proteins required for meiotic cytokinesis but dispensable for mitotic cytokinesis in S2 cells or in neuroblasts. For example, mutations affecting the kinesin-like protein Klp3A and the profilin Chic cause strong cytokinesis phenotypes in male meiosis but do not impair cytokinesis in somatic cells.[9,18,37,94] The stronger effects of cytokinetic perturbations on spermatocytes are especially evident when examing mutations affecting membrane trafficking. Spermatocyte cytokinesis appers particularly dependent on vesicle trafficking pathways when compared with somatic cells. Mutations affecting *fwd, gio, fws, Arf6* disrupt cytokinesis in spermatocytes but cause little or no effect in S2 cells and larval neuroblasts.[22,23,77,78,80-82,87,94-96] Primary spermatocytes undergo a very long growth phase before entering meiosis, giving rise to quite large cells (approx. 20μm in diameter). In addition, these cells complete two consecutive divisions in less than two hours with a very short intervening interphase.[4,18] We might speculate that these characteristics of male meiotic divisions cause extra demands on secretory/endocytic pathways to provide enough membrane during cytokinesis.

Conclusions and Future Perspectives

Drosophila male meiosis proved to offer a powerful model system in the genetic and molecular dissection of cytokinesis. Moreover, because molecular machineries involved in mitotic cytokinesis are conserved in meiosis (see above), Drosophila spermatocytes appear as a good model to study cytokinesis not only in germ cells, but in all cell types.

Thanks to the powerful tools of Drosophila genetics and the unique characteristics of this cell system, much progress has been made in the identification of new molecular players of the cell cleavage machinery. However, the inventory of cytokinesis proteins is far from complete and we have yet to understand how these proteins interact and the molecular pathways involved. One particularly intriguing issue concerns the precise molecular mechanisms underlying membrane traffic during cytokinesis

and how membrane remodeling is coordinated with cytoskeletal dynamics (for a review, see ref. 11).

Remarkably, the human counterparts of cytokinesis genes/proteins have been involved in cancer pathogenesis (for reviews see refs. 1 and 97). Failures in cytokinesis lead to the formation of genetically unstable tetraploid cells with multiple centrosomes and the following mitotic division results in aneuploid cells with altered growth properties, thus promoting carcinogenesis.[97] Indeed tetraploid lines from p53 null ($p53^{-/-}$) mouse mammary epithelial cells (MMEC) exhibit an increase in the frequency of chromosomal mis-segregation with respect to the MMEC diploid lines. These tetraploid lines, but not the diploid ones, promoted malignant mammary epithelial cancers when transplanted into nude mice.[98] In agreement with an early role of tetraploidy in oncogenesis, dominant mutations in the tumor suppressor gene *adenomatous polyposis coli (APC)* suppress cytokinesis and cause tetraploidy before the early steps of colorectal cancer development.[99] Moreover, genes involved in cytokinesis are either down-regulated or upregulated in human cancers, or have been mapped to chromosomal regions that are either deleted or amplified in tumors or tumor-derived cell lines.[100-103] Hence, the identification of novel molecular players involved in cytokinesis and the elucidation of the mechanisms underlying this process can contribute to both cancer diagnosis and therapy.

In some human tissues and cells such as human hepatocytes and cardyomiocytes, polyploidization is not the pathological state; instead, in these cells, a certain degree of polyploidy is essential for proper function (reviewed in ref. 1), and pathologies can result from cytokinesis completion. Thus, the mechanisms that control cytokinesis can be different in different cell types. In this context, incomplete cytokinesis and intercellular bridges formation must be tightly regulated in the germline. It has been suggested that premature completion of cytokinesis in these cells might affect the formation and stability of intercellular bridge and lead to human infertility.[1]

For all these reasons we are confident that studies of Drosophila male meiosis will provide novel insight into the complex molecular circuits underlying furrow ingression during cytokinesis and have a substantial impact on our knowledge of cancer biology and other human diseases.

Acknowledgments

The work in the lab of M.G.G. is funded by AIRC grant IG10775.

References

1. Lacroix B, Maddox AS. Cytokinesis, ploidy and aneuploidy. J Pathol 2012; 226:338-51; http://dx.doi.org/10.1002/path.3013; PMID:21984283.
2. Glotzer M. The molecular requirements for cytokinesis. Science 2005; 307:1735-9; http://dx.doi.org/10.1126/science.1096896; PMID:15774750.
3. Schweitzer JK, D'Souza-Schorey C. Finishing the job: cytoskeletal and membrane events bring cytokinesis to an end. Exp Cell Res 2004; 295:1-8; http://dx.doi.org/10.1016/j.yexcr.2003.12.023; PMID:15051485.
4. Fuller MT. Spermatogenesis. In Bate M and Martinez-Arias A., eds. The Development of *Drosophila melanogaster*. Cold Spring Harbor, NY: Cold Spring Harbor Press 1993:71-147.
5. Hime GR, Brill JA, Fuller MT. Assembly of ring canals in the male germ line from structural components of the contractile ring. J Cell Sci 1996; 109:2779-88; PMID:9013326.
6. Robinson DN, Cooley L. Stable intercellular bridges in development: the cytoskeleton lining the tunnel. Trends Cell Biol 1996; 6:474-9; http://dx.doi.org/10.1016/0962-8924(96)84945-2; PMID:15157506.
7. D'Avino PP, Savoian MS, Glover DM. Cleavage furrow formation and ingression during animal cytokinesis: a microtubule legacy. J Cell Sci 2005; 118:1549-58; PMID:15811947; http://dx.doi.org/10.1242/jcs.02335.
8. Piekny A, Werner M, Glotzer M. Cytokinesis: welcome to the Rho zone. Trends Cell Biol 2005; 15:651-8; http://dx.doi.org/10.1016/j.tcb.2005.10.006; PMID:16243528.
9. Giansanti MG, Bonaccorsi S, Williams B, Williams EV, Santolamazza C, Goldberg ML, et al. Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. Genes Dev 1998; 12:396-410; PMID:9450933; http://dx.doi.org/10.1101/gad.12.3.396.
10. Barr FA, Gruneberg U. Cytokinesis: placing and making the final cut. Cell 2007; 131:847-60; http://dx.doi.org/10.1016/j.cell.2007.11.011; PMID:18045532.
11. McKay HF, Burgess DR. 'Life is a highway': membrane trafficking during cytokinesis. Traffic 2011; 12:247-51; http://dx.doi.org/10.1111/j.1600-0854.2010.01139.x; PMID:21054718.
12. Neto H, Collins LL, Gould GW. Vesicle trafficking and membrane remodelling in cytokinesis. Biochem J 2011; 437:13-24; http://dx.doi.org/10.1042/BJ20110153; PMID:21668412.
13. Albertson R, Riggs B, Sullivan W. Membrane traffic: a driving force in cytokinesis. Trends Cell Biol 2005; 15:92-101; PMID:15695096; http://dx.doi.org/10.1016/j.tcb.2004.12.008.
14. Boucrot E, Kirchhausen T. Endosomal recycling controls plasma membrane area during mitosis. Proc Natl Acad Sci U S A 2007; 104:7939-44; http://dx.doi.org/10.1073/pnas.0702511104; PMID:17483462.
15. Prekeris R, Gould GW. Breaking up is hard to do - membrane traffic in cytokinesis. J Cell Sci 2008; 121:1569-76; http://dx.doi.org/10.1242/jcs.018770; PMID:18469013.
16. Eggert US, Mitchison TJ, Field CM. Animal cytokinesis: from parts list to mechanisms. Annu Rev Biochem 2006; 75:543-66; http://dx.doi.org/10.1146/annurev.biochem.74.082803.133425; PMID:16756502.
17. Skop AR, Liu H, Yates J 3rd, Meyer BJ, Heald R. Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. Science 2004; 305:61-6; http://dx.doi.org/10.1126/science.1097931; PMID:15166316.
18. Giansanti MG, Bonaccorsi S, Bucciarelli E, Gatti M. *Drosophila* male meiosis as a model system for the study of cytokinesis in animal cells. Cell Struct Funct 2001; 26:609-17; http://dx.doi.org/10.1247/csf.26.609; PMID:11942616.
19. Lindsley DL, Tokuyasu KT. Spermatogenesis. In: Ashburner M, Wright TRF, eds. Genetics and Biology of *Drosophila*. New York: Academic Press, 1980: 225-94.
20. Tates AD. Cytodifferentiation durng spermatogenesis in Drosophila melanogaster: an electron microscope study. Ph.D Thesis Leiden, Netherlands: Rijksuniversiteit de leiden, 1971.
21. Church K, Lin HP. Meiosis in Drosophila melanogaster. II. The prometaphase-I kinetochore microtubule bundle and kinetochore orientation in males. J Cell Biol 1982; 93:365-73; PMID:6807996; http://dx.doi.org/10.1083/jcb.93.2.365.
22. Giansanti MG, Bonaccorsi S, Kurek R, Farkas RM, Dimitri P, Fuller MT, et al. The class I PITP giotto is required for Drosophila cytokinesis. Curr Biol 2006; 16:195-201; http://dx.doi.org/10.1016/j.cub.2005.12.011; PMID:16431372.
23. Giansanti MG, Belloni G, Gatti M. Rab11 is required for membrane trafficking and actomyosin ring constriction in meiotic cytokinesis of Drosophila males. Mol Biol Cell 2007; 18:5034-47; http://dx.doi.org/10.1091/mbc.E07-05-0415; PMID:17914057.
24. Rebollo E, González C. Visualizing the spindle checkpoint in Drosophila spermatocytes. EMBO Rep 2000; 1:65-70; http://dx.doi.org/10.1093/embo-reports/kvd011; PMID:11256627.
25. Wakefield JG, Bonaccorsi S, Gatti M. The drosophila protein asp is involved in microtubule organization during spindle formation and cytokinesis. J Cell Biol 2001; 153:637-48; http://dx.doi.org/10.1083/jcb.153.4.637; PMID:11352927.
26. Riparbelli MG, Callaini G, Glover DM, Avides MdoC. A requirement for the Abnormal Spindle protein to organise microtubules of the central spindle for cytokinesis in Drosophila. J Cell Sci 2002; 115:913-22; PMID:11870210.
27. Rappaport R. Experiments concerning the cleavage stimulus in sand dollar eggs. J Exp Zool 1961; 148:81-9; PMID:14490383; http://dx.doi.org/10.1002/jez.1401480107.
28. Bonaccorsi S, Giansanti MG, Gatti M. Spindle self-organization and cytokinesis during male meiosis in asterless mutants of Drosophila melanogaster. J Cell Biol 1998; 142:751-61. http://dx.doi.org/10.1083/jcb.142.3.751; PMID:9700163
29. Giansanti MG, Bucciarelli E, Bonaccorsi S, Gatti M. Drosophila SPD-2 is an essential centriole component required for PCM recruitment and astral-microtubule nucleation. Curr Biol 2008; 18:303-9; http://dx.doi.org/10.1016/j.cub.2008.01.058; PMID:18291647.
30. Bucciarelli E, Giansanti MG, Bonaccorsi S, Gatti M. Spindle assembly and cytokinesis in the absence of chromosomes during Drosophila male meiosis. J Cell Biol 2003; 160:993-9; http://dx.doi.org/10.1083/jcb.200211029; PMID:12654903.

31. Mollinari C, Kleman JP, Jiang W, Schoehn G, Hunter T, Margolis RL. PRC1 is a microtubule binding and bundling protein essential to maintain the mitotic spindle midzone. J Cell Biol 2002; 157:1175-86; http://dx.doi.org/10.1083/jcb.200111052; PMID:12082078.
32. Vernì F, Somma MP, Gunsalus KC, Bonaccorsi S, Belloni G, Goldberg ML, et al. Feo, the Drosophila homolog of PRC1, is required for central-spindle formation and cytokinesis. Curr Biol 2004; 14:1569-75; http://dx.doi.org/10.1016/j.cub.2004.08.054; PMID:15341744.
33. Szafer-Glusman E, Fuller MT, Giansanti MG. Role of Survivin in cytokinesis revealed by a separation-of-function allele. Mol Biol Cell 2011; 22:3779-90; http://dx.doi.org/10.1091/mbc.E11-06-0569; PMID:21865602.
34. Douglas ME, Mishima M. Still entangled: assembly of the central spindle by multiple microtubule modulators. Semin Cell Dev Biol 2010; 21:899-908; http://dx.doi.org/10.1016/j.semcdb.2010.08.005; PMID:20732438.
35. Nislow C, Lombillo VA, Kuriyama R, McIntosh JR. A plus-end-directed motor enzyme that moves antiparallel microtubules in vitro localizes to the interzone of mitotic spindles. Nature 1992; 359:543-7; http://dx.doi.org/10.1038/359543a0; PMID:1406973.
36. Carmena M, Riparbelli MG, Minestrini G, Tavares AM, Adams R, Callaini G, et al. Drosophila polo kinase is required for cytokinesis. J Cell Biol 1998; 143:659-71; http://dx.doi.org/10.1083/jcb.143.3.659; PMID:9813088.
37. Williams BC, Riedy MF, Williams EV, Gatti M, Goldberg ML. The Drosophila kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis. J Cell Biol 1995; 129:709-23; PMID:7730406; http://dx.doi.org/10.1083/jcb.129.3.709.
38. Kurasawa Y, Earnshaw WC, Mochizuki Y, Dohmae N, Todokoro K. Essential roles of KIF4 and its binding partner PRC1 in organized central spindle midzone formation. EMBO J 2004; 23:3237-48; http://dx.doi.org/10.1038/sj.emboj.7600347; PMID:15297875.
39. D'Avino PP, Archambault V, Przewloka MR, Zhang W, Lilley KS, Laue E, et al. Recruitment of Polo kinase to the spindle midzone during cytokinesis requires the Feo/Klp3A complex. PLoS One 2007; 2:e572; http://dx.doi.org/10.1371/journal.pone.0000572; PMID:17593971.
40. Gatti M, Giansanti MG, Bonaccorsi S. Relationships between the central spindle and the contractile ring during cytokinesis in animal cells. Microsc Res Tech 2000; 49:202-8; http://dx.doi.org/10.1002/(SICI)1097-0029(20000415)49:2<202::AID-JEMT13>3.0.CO;2-8; PMID:10816260.
41. Giansanti MG, Farkas RM, Bonaccorsi S, Lindsley DL, Wakimoto BT, Fuller MT, et al. Genetic dissection of meiotic cytokinesis in Drosophila males. Mol Biol Cell 2004; 15:2509-22; PMID:15004238.
42. Inoue YH, Savoian MS, Suzuki T, Máthé E, Yamamoto MT, Glover DM. Mutations in orbit/mast reveal that the central spindle is comprised of two microtubule populations, those that initiate cleavage and those that propagate furrow ingression. J Cell Biol 2004; 166:49-60; http://dx.doi.org/10.1083/jcb.200402052; PMID:15240569.
43. Gandhi R, Bonaccorsi S, Wentworth D, Doxsey S, Gatti M, Pereira A. The Drosophila Kinesin-like Protein KLP67A is essential for mitotic and male meiotic spindle assembly. Mol Biol Cell 2003; 15:121-31; PMID:13679514; doi:http://www.molbiolcell.org/content/15/1/121.long - fn-5
44. Gatt MK, Savoian MS, Riparbelli MG, Massarelli C, Callaini G, Glover DM. Klp67A destabilises preanaphase microtubules but subsequently is required to stabilise the central spindle. J Cell Sci 2005; 118:2671-82; http://dx.doi.org/10.1242/jcs.02410; PMID:15928044.
45. Somers WG, Saint R. A RhoGEF and Rho family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. Dev Cell 2003; 4:29-39; http://dx.doi.org/10.1016/S1534-5807(02)00402-1; PMID:12530961.
46. Nishimura Y, Yonemura S. Centralspindlin regulates ECT2 and RhoA accumulation at the equatorial cortex during cytokinesis. J Cell Sci 2006; 119:104-14; http://dx.doi.org/10.1242/jcs.02737; PMID:16352658.
47. Chalamalasetty RB, Hümmer S, Nigg EA, Silljé HH. Influence of human Ect2 depletion and overexpression on cleavage furrow formation and abscission. J Cell Sci 2006; 119:3008-19; PMID:16803869; doi: http://jcs.biologists.org/content/119/14/3008.long - corresp-1
48. Adams RR, Wheatley SP, Gouldsworthy AM, Kandels-Lewis SE, Carmena M, Smythe C, et al. INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. Curr Biol 2000; 10:1075-8; http://dx.doi.org/10.1016/S0960-9822(00)00673-4; PMID:10996078.
49. Vagnarelli P, Earnshaw WC. Chromosomal passengers: the four-dimensional regulation of mitotic events. Chromosoma 2004; 113:211-22; http://dx.doi.org/10.1007/s00412-004-0307-3; PMID:15351889.
50. Vader G, Medema RH, Lens SM. The chromosomal passenger complex: guiding Aurora-B through mitosis. J Cell Biol 2006; 173:833-7; http://dx.doi.org/10.1083/jcb.200604032; PMID:16769825.
51. Gao S, Giansanti MG, Buttrick GJ, Ramasubramanyan S, Auton A, Gatti M, et al. Australin: a chromosomal passenger protein required specifically for Drosophila melanogaster male meiosis. J Cell Biol 2008; 180:521-35; http://dx.doi.org/10.1083/jcb.200708072; PMID:18268101.
52. Guse A, Mishima M, Glotzer M. Phosphorylation of ZEN-4/MKLP1 by aurora B regulates completion of cytokinesis. Curr Biol 2005; 15:778-86; http://dx.doi.org/10.1016/j.cub.2005.03.041; PMID:15854913.
53. Neef R, Klein UR, Kopajtich R, Barr FA. Cooperation between mitotic kinesins controls the late stages of cytokinesis. Curr Biol 2006; 16:301-7; http://dx.doi.org/10.1016/j.cub.2005.12.030; PMID:16461284.
54. Douglas ME, Davies T, Joseph N, Mishima M. Aurora B and 14-3-3 coordinately regulate clustering of centralspindlin during cytokinesis. Curr Biol 2010; 20:927-33; http://dx.doi.org/10.1016/j.cub.2010.03.055; PMID:20451386.
55. Ebrahimi S, Fraval H, Murray M, Saint R, Gregory SL. Polo kinase interacts with RacGAP50C and is required to localize the cytokinesis initiation complex. J Biol Chem 2010; 285:28667-73; http://dx.doi.org/10.1074/jbc.M110.103887; PMID:20628062.
56. Brennan IM, Peters U, Kapoor TM, Straight AF. Polo-like kinase controls vertebrate spindle elongation and cytokinesis. PLoS One 2007; 2:e409; http://dx.doi.org/10.1371/journal.pone.0000409; PMID:17476331.
57. Santamaria A, Neef R, Eberspächer U, Eis K, Husemann M, Mumberg D, et al. Use of the novel Plk1 inhibitor ZK-thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. Mol Biol Cell 2007; 18:4024-36; http://dx.doi.org/10.1091/mbc.E07-05-0517; PMID:17671160.
58. Burkard ME, Maciejowski J, Rodriguez-Bravo V, Repka M, Lowery DM, Clauser KR, et al. Plk1 self-organization and priming phosphorylation of HsCYK-4 at the spindle midzone regulate the onset of division in human cells. PLoS Biol 2009; 7:e1000111; http://dx.doi.org/10.1371/journal.pbio.1000111; PMID:19468302.
59. Wolfe BA, Takaki T, Petronczki M, Glotzer M. Polo-like kinase 1 directs assembly of the HsCyk-4 RhoGAP/Ect2 RhoGEF complex to initiate cleavage furrow formation. PLoS Biol 2009; 7:e1000110; http://dx.doi.org/10.1371/journal.pbio.1000110; PMID:19468300.
60. Carmena M. Cytokinesis: the final stop for the chromosomal passengers. Biochem Soc Trans 2008; 36:367-70; http://dx.doi.org/10.1042/BST0360367; PMID:18481960.
61. Resnick TD, Satinover DL, MacIsaac F, Stukenberg PT, Earnshaw WC, Orr-Weaver TL, et al. INCENP and Aurora B promote meiotic sister chromatid cohesion through localization of the Shugoshin MEI-S332 in Drosophila. Dev Cell 2006; 11:57-68; http://dx.doi.org/10.1016/j.devcel.2006.04.021; PMID:16824953.
62. Capalbo L, Montembault E, Takeda T, Bassi ZI, Glover DM, D'Avino PP. The chromosomal passenger complex controls the function of endosomal sorting complex required for transport-III Snf7 proteins during cytokinesis. Open Biol 2012; 2:120070; http://dx.doi.org/10.1098/rsob.120070; PMID:22724069.
63. Dieterich K, Soto Rifo R, Faure AK, Hennebicq S, Ben Amar B, Zahi M, et al. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. Nat Genet 2007; 39:661-5; http://dx.doi.org/10.1038/ng2027; PMID:17435757.
64. Kimmins S, Crosio C, Kotaja N, Hirayama J, Monaco L, Höög C, et al. Differential functions of the Aurora-B and Aurora-C kinases in mammalian spermatogenesis. Mol Endocrinol 2007; 21:726-39; http://dx.doi.org/10.1210/me.2006-0332; PMID:17192404.
65. Barr FA, Silljé HH, Nigg EA. Polo-like kinases and the orchestration of cell division. Nat Rev Mol Cell Biol 2004; 5:429-40; http://dx.doi.org/10.1038/nrm1401; PMID:15173822.
66. Llamazares S, Moreira A, Tavares A, Girdham C, Spruce BA, Gonzalez C, et al. polo encodes a protein kinase homolog required for mitosis in Drosophila. Genes Dev 1991; 5(12A):2153-65; http://dx.doi.org/10.1101/gad.5.12a.2153; PMID:1660828.
67. Herrmann S, Amorim I, Sunkel CE. The POLO kinase is required at multiple stages during spermatogenesis in Drosophila melanogaster. Chromosoma 1998; 107:440-51; http://dx.doi.org/10.1007/PL00013778; PMID:9914376.
68. Satterwhite LL, Pollard TD. Cytokinesis. Curr Opin Cell Biol 1992; 4:43-52; PMID:1313686; http://dx.doi.org/10.1016/0955-0674(92)90057-J.
69. D'Avino PP. How to scaffold the contractile ring for a safe cytokinesis - lessons from Anillin-related proteins. J Cell Sci 2009; 122:1071-9; http://dx.doi.org/10.1242/jcs.034785; PMID:19339546.
70. Field CM, al-Awar O, Rosenblatt J, Wong ML, Alberts B, Mitchison TJ. A purified Drosophila septin complex forms filaments and exhibits GTPase activity. J Cell Biol 1996; 133:605-16; PMID:8636235; http://dx.doi.org/10.1083/jcb.133.3.605.
71. Adam JC, Pringle JR, Peifer M. Evidence for functional differentiation among Drosophila septins in cytokinesis and cellularization. Mol Biol Cell 2000; 11:3123-35; PMID:10982405.
72. Miller KG, Field CM, Alberts BM. Actin-binding proteins from Drosophila embryos: a complex network of interacting proteins detected by F-actin affinity chromatography. J Cell Biol 1989; 109:2963-75; PMID:2512303; http://dx.doi.org/10.1083/jcb.109.6.2963.
73. Giansanti MG, Bonaccorsi S, Gatti M. The role of anillin in meiotic cytokinesis of Drosophila males. J Cell Sci 1999; 112:2323-34; PMID:10381388.
74. Goldbach P, Wong R, Beise N, Sarpal R, Trimble WS, Brill JA. Stabilization of the actomyosin ring enables spermatocyte cytokinesis in Drosophila. Mol Biol Cell 2010; 21:1482-93; http://dx.doi.org/10.1091/mbc.E09-08-0714; PMID:20237160.
75. Royou A, Field C, Sisson JC, Sullivan W, Karess R. Reassessing the role and dynamics of nonmuscle myosin II during furrow formation in early Drosophila embryos. Mol Biol Cell 2004; 15:838-50; http://dx.doi.org/10.1091/mbc.E03-06-0440; PMID:14657248.

76. Robinett CC, Giansanti MG, Gatti M, Fuller MT. TRAPPII is required for cleavage furrow ingression and localization of Rab11 in dividing male meiotic cells of Drosophila. J Cell Sci 2009; 122:4526-34; http://dx.doi.org/10.1242/jcs.054536; PMID:19934220.
77. Farkas RM, Giansanti MG, Gatti M, Fuller MT. The Drosophila Cog5 homologue is required for cytokinesis, cell elongation, and assembly of specialized Golgi architecture during spermatogenesis. Mol Biol Cell 2003; 14:190-200; PMID:12529436; http://dx.doi.org/10.1091/mbc.E02-06-0343.
78. Belloni G, Sechi S, Riparbelli MG, Callaini G, Giansanti MG. Mutations in *Cog7* affect Golgi structure, meiotic cytokinesis and sperm development during *Drosophila* spermatogenesis. JCS; In press.
79. Xu H, Brill JA, Hsien J, McBride R, Boulianne GL, Trimble WS. Syntaxin 5 is required for cytokinesis and spermatid differentiation in Drosophila. Dev Biol 2002; 251:294-306; http://dx.doi.org/10.1006/dbio.2002.0830; PMID:12435359.
80. Brill JA, Hime GR, Scharer-Schuksz M, Fuller MT. A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. Development 2000; 127:3855-64; PMID:10934029.
81. Polevoy G, Wei HC, Wong R, Szentpetery Z, Kim YJ, Goldbach P, et al. Dual roles for the Drosophila PI 4-kinase four wheel drive in localizing Rab11 during cytokinesis. J Cell Biol 2009; 187:847-58; http://dx.doi.org/10.1083/jcb.200908107; PMID:19995935.
82. Dyer N, Rebollo E, Domínguez P, Elkhatib N, Chavrier P, Daviet L, et al. Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. Development 2007; 134:4437-47; http://dx.doi.org/10.1242/dev.010983; PMID:18039970.
83. Szafer-Glusman E, Giansanti MG, Nishihama R, Bolival B, Pringle J, Gatti M, et al. A role for very-long-chain fatty acids in furrow ingression during cytokinesis in Drosophila spermatocytes. Curr Biol 2008; 18:1426-31; http://dx.doi.org/10.1016/j.cub.2008.08.061; PMID:18804373.
84. Chen H, Liu Z, Huang X. Drosophila models of peroxisomal biogenesis disorder: peroxins are required for spermatogenesis and very-long-chain fatty acid metabolism. Hum Mol Genet 2010; 19:494-505; http://dx.doi.org/10.1093/hmg/ddp518; PMID:19933170.
85. Brill JA, Wong R, Wilde A. Phosphoinositide function in cytokinesis. Curr Biol 2011; 21:R930-4; http://dx.doi.org/10.1016/j.cub.2011.10.001; PMID:22115464.
86. Wong R, Hadjiyanni I, Wei HC, Polevoy G, McBride R, Sem KP, et al. PIP2 hydrolysis and calcium release are required for cytokinesis in Drosophila spermatocytes. Curr Biol 2005; 15:1401-6; http://dx.doi.org/10.1016/j.cub.2005.06.060; PMID:16085493.
87. Gatt MK, Glover DM. The *Drosophila* phosphatidylinositol transfer protein encoded by vibrator is essential to maintain cleavage-furrow ingression in cytokinesis. J Cell Sci 2006; 119:2225-35; http://dx.doi.org/10.1242/jcs.02933; PMID:16684816.
88. Gunsalus KC, Bonaccorsi S, Williams E, Verni F, Gatti M, Goldberg ML. Mutations in twinstar, a Drosophila gene encoding a cofilin/ADF homologue, result in defects in centrosome migration and cytokinesis. J Cell Biol 1995; 131:1243-59; PMID:8522587; http://dx.doi.org/10.1083/jcb.131.5.1243.
89. Madaule P, Furuyashiki T, Reid T, Ishizaki T, Watanabe G, Morii N, et al. A novel partner for the GTP-bound forms of rho and rac. FEBS Lett 1995; 377:243-8; http://dx.doi.org/10.1016/0014-5793(95)01351-2; PMID:8543060.
90. Di Cunto F, Imarisio S, Hirsch E, Broccoli V, Bulfone A, Migheli A, et al. Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. Neuron 2000; 28:115-27; PMID:11086988; http://dx.doi.org/10.1016/S0896-6273(00)00090-8.
91. Naim V, Imarisio S, Di Cunto F, Gatti M, Bonaccorsi S. *Drosophila* citron kinase is required for the final steps of cytokinesis. Mol Biol Cell 2004; 15:5053-63; http://dx.doi.org/10.1091/mbc.E04-06-0536; PMID:15371536.
92. D'Avino PP, Savoian MS, Glover DM. Mutations in sticky lead to defective organization of the contractile ring during cytokinesis and are enhanced by Rho and suppressed by Rac. J Cell Biol 2004; 166:61-71; http://dx.doi.org/10.1083/jcb.200402157; PMID:15240570.
93. Montembault E, Zhang W, Przewloka MR, Archambault V, Sevin EW, Laue ED, et al. Nessun Dorma, a novel centralspindlin partner, is required for cytokinesis in Drosophila spermatocytes. J Cell Biol 2010; 191:1351-65; http://dx.doi.org/10.1083/jcb.201007060; PMID:21187330.
94. Somma MP, Fasulo B, Cenci G, Cundari E, Gatti M. Molecular dissection of cytokinesis by RNA interference in Drosophila cultured cells. Mol Biol Cell 2002; 13:2448-60; http://dx.doi.org/10.1091/mbc.01-12-0589; PMID:12134082.
95. Eggert US, Kiger AA, Richter C, Perlman ZE, Perrimon N, Mitchison TJ, et al. Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. PLoS Biol 2004; 2:e379; http://dx.doi.org/10.1371/journal.pbio.0020379; PMID:15547975.
96. Echard A, Hickson GR, Foley E, O'Farrell PH. Terminal cytokinesis events uncovered after an RNAi screen. Curr Biol 2004; 14:1685-93; http://dx.doi.org/10.1016/j.cub.2004.08.063; PMID:15380073.
97. Normand G, King RW. Understanding cytokinesis failure. Adv Exp Med Biol 2010; 676:27-55; PMID:20687468; http://dx.doi.org/10.1007/978-1-4419-6199-0_3.
98. Fujiwara T, Bandi M, Nitta M, Ivanova EV, Bronson RT, Pellman D. Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. Nature 2005; 437:1043-7; http://dx.doi.org/10.1038/nature04217; PMID:16222300.
99. Caldwell CM, Green RA, Kaplan KB. APC mutations lead to cytokinetic failures in vitro and tetraploid genotypes in Min mice. J Cell Biol 2007; 178:1109-20; http://dx.doi.org/10.1083/jcb.200703186; PMID:17893240.
100. Mountzios G, Dimopoulos MA, Bamias A, Papadopoulos G, Kastritis E, Syrigos K, et al. Abnormal bone remodeling process is due to an imbalance in the receptor activator of nuclear factor-kappaB ligand (RANKL)/osteoprotegerin (OPG) axis in patients with solid tumors metastatic to the skeleton. Acta Oncol 2007; 46:221-9; http://dx.doi.org/10.1080/02841860600635870; PMID:17453373.
101. Sinha S, Mondal G, Hwang EJ, Han W, Dutta SK, Iyer S, et al. Von Hippel-Lindau gene product directs cytokinesis: a new tumor suppressor function. J Cell Sci 2011; 124:2132-42; http://dx.doi.org/10.1242/jcs.087122; PMID:21652636.
102. Lind GE, Raiborg C, Danielsen SA, Rognum TO, Thiis-Evensen E, Hoff G, et al. SPG20, a novel biomarker for early detection of colorectal cancer, encodes a regulator of cytokinesis. Oncogene 2011; 30:3967-78; http://dx.doi.org/10.1038/onc.2011.109; PMID:21499309.
103. Roversi G, Pfundt R, Moroni RF, Magnani I, van Reijmersdal S, Pollo B, et al. Identification of novel genomic markers related to progression to glioblastoma through genomic profiling of 25 primary glioma cell lines. Oncogene 2006; 25:1571-83; http://dx.doi.org/10.1038/sj.onc.1209177; PMID:16247447.
104. Castrillon DH, Wasserman SA. Diaphanous is required for cytokinesis in Drosophila and shares domains of similarity with the products of the limb deformity gene. Development 1994; 120:3367-77; PMID:7821209.
105. Sampaio P, Rebollo E, Varmark H, Sunkel CE, González C. Organized microtubule arrays in gamma-tubulin-depleted Drosophila spermatocytes. Curr Biol 2001; 11:1788-93; http://dx.doi.org/10.1016/S0960-9822(01)00561-9; PMID:11719222.
106. Lange BM, Rebollo E, Herold A, González C. Cdc37 is essential for chromosome segregation and cytokinesis in higher eukaryotes. EMBO J 2002; 21:5364-74; http://dx.doi.org/10.1093/emboj/cdf531; PMID:12374737.
107. Ichihara K, Shimizu H, Taguchi O, Yamaguchi M, Inoue YH. A Drosophila orthologue of larp protein family is required for multiple processes in male meiosis. Cell Struct Funct 2007; 32:89-100; http://dx.doi.org/10.1247/csf.07027; PMID:17951964.
108. Bergner LM, Hickman FE, Wood KH, Wakeman CM, Stone HH, Campbell TJ, et al. A novel predicted bromodomain-related protein affects coordination between meiosis and spermiogenesis in Drosophila and is required for male meiotic cytokinesis. DNA Cell Biol 2010; 29:487-98; PMID:20491580; http://dx.doi.org/10.1089/dna.2009.0989.
109. Dorogova NV, Akhmametyeva EM, Kopyl SA, Gubanova NV, Yudina OS, Omelyanchuk LV, et al. The role of Drosophila Merlin in spermatogenesis. BMC Cell Biol 2008; 9:1; PMID:18186933; http://dx.doi.org/10.1186/1471-2121-9-1.