Cytokinesis in Drosophila male meiosis

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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. 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. Actomyosin ring constriction draws in the plasma membrane. In most models, the intercellular bridge is ultimately severed during abscission, resulting in the complete separation of daughter cells. 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.

Contractile ring assembly is directed by the Rho guanosine triphosphatase (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. 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. 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.

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. 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. 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.

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...
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 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.

At metaphase I, spermatocyte nuclei become spindle shaped and maintain a double-membrane that encircles the meiotic chromosomes during meiosis in addition to double parafusorial membranes surrounding the nuclei of dividing spermatocytes. At each cell pole a system of 13 umbrella-shaped layers comprise the so-called astral membranes. Both the parafusorial membranes and the astral membranes derive from the ER and are in fact enriched with the ER marker protein disulfide isomerase. 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.

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. The primary spermatogonium undergoes four gonial mitotic divisions giving rise to 16 primary spermatocytes that in turn undergo two meiotic divisions (as depicted in Fig. 1). During both the gonial mitoses and the spermatocyte meiotic divisions, cytokinesis is incomplete 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.

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interlaced structure resembling an onion, named the nebenkern\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 immunocytochemical and in vivo analysis of the cytokinetic structures (Fig. 2).\textsuperscript{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\textsuperscript{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,\textsuperscript{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 *fil* 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.\textsuperscript{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 microtubule bundling activity and is required for central spindle assembly in mammalian tissue culture cells.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{35} Thus, the activity of this kinesin is essential to mediate interactions between overlapping microtubule 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.\textsuperscript{96} The Drosophila ortholog of KIF4, KLP3A, is also concentrated at central spindle mid-zone and is required for central spindle assembly in spermatocytes.\textsuperscript{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.\textsuperscript{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.\textsuperscript{9,40,41} Indeed mutations that affect genes encoding central spindle components (Table I) such as the chromokinesin Klp3A,\textsuperscript{9,37} or the CLASP ortholog Orbit\textsuperscript{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.\textsuperscript{38} 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\textsuperscript{43} 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
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 ingress 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 ingress. The interior central spindle microtubules are essential for cytokinesis progress, 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. Nonetheless, the central spindles of klp67A mutant spermatocytes appear strikingly different from those of orbit mutants. 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 sufficiently 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                                        |               |                  |       |
| Pebble           | ECT2           | RhoGEF                                                           | ND            | Absence of both CS and CR | 41    |
|                  |                |                                                                  |               |                  |       |
|                  | Actin 5C       | Actin F-Actin                                                     | Contractile ring | ND          | 88    |
|                  | 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 | 5, 73, 74 |
| Peanut, Sep1, Sep2| Septin         | Septins, Scaffolding Protein                                     | Contractile ring | No defects in peanut mutants | 5, 18 |
|                  |                |                                                                  |               |                  |       |
| **Contractile ring components**                     |                |                                                                  |               |                  |       |
|                  |                |                                                                  |               |                  |       |
| **Actin filaments formation**                       |                |                                                                  |               |                  |       |
|                  |                |                                                                  |               |                  |       |
| **Associated with central spindle microtubules**     |                |                                                                  |               |                  |       |
|                  |                |                                                                  |               |                  |       |
| **Cytokinesis regulation**                          |                |                                                                  |               |                  |       |
| Aurora B         | Aurora B       | CPC subunit                                                       | CS midzone    | Defective CS     | 30, 33, 51 |
| INCENP           | Incenp         | CPC subunit                                                       | CS midzone    | Failure to localize Pav | 33, 51, 61 |
| Deterin /Scapolo| Survivin       | CPC subunit                                                       | CS midzone    | Absence of both CS and CR | 33    |
| Australin        | Borealin       | CPC subunit                                                       | CS midzone    | Defects in CPC localization | 51    |
| Polo             | Polo           | Polo kinase                                                       | CS midzone    | Absence of both CS and CR | 36, 67, 105 |
| Cdc37            | Cdc37          | Required for the activity 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.
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/

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|--------------------|----------------|----------------------------------------------------------------|--------------|------------------|-------|
| **Membrane remodeling and vesicle traffic** |
| Syntaxin 5         | Syntaxin 5     | Golgi traffic, Vesicle fusion                                  | Golgi        | Multinucleate spermatids | 79    |
| Four way stop      | COG complex    | Golgi integrity, vesicle trafficking and glycosylation        | Golgi        | Defects in CR constriction and CS stability | 77, 78 |
| Brunelleschi       | TRAPP1 subunit | Golgi traffic                                                  | Golgi        | Defects in CR constriction and CS stability | 76    |
| Giotto/vib         | PITP           | Phosphatidylinositol transfer vesicle traffic                 | ER, CF       |                  | 22, 23, 87 |
| Arf6               | Arf6           | Endocytic traffic                                             | Recycling endosomes | Defects in furrow ingestion | 82    |
| Four wheel drive   | PI4Kβ          | Phosphatidylinositol 4-kinase                                  | Golgi        | Defects in CR constriction | 23, 80, 81 |
| Ral1               | Rab11          | Rab11GTPase                                                   | Golgi, vesicles, CF | Defects in CR constriction | 23, 76, 81, 82 |
| Bond               | Elov1          | Biosynthesis of VLCAAs                                         | ND           | Defects in furrow ingression | 83    |
| Pex2, Pex10        | Peroxin proteins | Metabolism of VLCAAs                                      | ND           | Multinucleate spermatids | 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 | 91    |
| Nessun Dorma       | Nessun Dorma   | Centralspindlin Partner                                       | CF and ring canal | Defects in furrowing completion | 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 | 108   |
| Merlin             | Neurofibromatosis 2 (NF2) | Regulation of actin cytoskeleton Microtubule binding Regulation of microtubule cytoskeleton | CS microtubules | Multipolar Telophases II | 109   |

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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 central spindle 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 and it is involved in central spindle formation through phosphorylation of the central spindle 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 MglRacGAP/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)55,51,61 (Table 1). The protein Aust replaces Borr only in male meiotic cells. Strikingly, although Aust lacks a region corresponding to 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 of 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.6354 Interestingly, Aurora C knockout mice are viable but males are sterile and exhibit polyplody spermatids.53

Spermatocytes of CPC mutants share similar early cytokinesis defects. Analysis of male meiosis in INCENP (dmiINCENP) 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 dmiINCENP and aust mutants fail to recruit Pav at the cell equator, indicating a requirement for the CPC in centralspindlin localization.53,61

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 dmiINCENP 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.46 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.56,67

**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 sephin proteins form filaments at the cleavage site of several organisms including Drosophila.70 In Drosophila melanogaster five sephins have been identified so far named Sep1, Sep2, Sep3, Sep4 and Sep5.70 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 sephin proteins are required for cytokinesis in all Drosophila somatic cell types or in primary spermatocytes.16,71

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.77 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.78 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.83 The small GTPase Rab11, involved in both the secretory and the endocytic traffic, is also essential for cytokinesis in Drosophila male meiotic cells.23,25,82 Time-lapse analysis of arf6 and rab11 mutant spermatocytes undergoing cytokinesis indicated similar defects in furrow ingression.23,82 In spermatocytes expressing B-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 pexin 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-biphosphate (PIP2), Wong and coworkers found this phosphoinositide localized in the plasma membrane and the cleavage furrow of dividing spermatocytes.80 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.23,25,80,87 The Drosophila phosphatidylinositol 4-kinase β (P4Kβ) Fwd is also involved in spermatocyte cytokinesis.80,82 Recent data have shown that wild type function of Fwd is required for the synthesis of PIP4P on Golgi membranes and for the formation of Rab11 and P4P 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 Rab11.83

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 ingestion. 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-terminal 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 PLC8-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 examining mutations affecting membrane trafficking. Spermatocyte cytokinesis appears 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.22,23,77,80-82,87,94-96 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 how membrane remodeling is coordinated with cytoskeletal dynamics (for a review, see ref. 11).

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