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What *Drosophila* spermatocytes tell us about the mechanisms underlying cytokinesis

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Abstract

Cytokinesis separates the genomic material and organelles of a dividing cell equitably into two physically distinct daughter cells at the end of cell division. This highly choreographed process involves coordinated reorganization and regulated action of the actin and microtubule cytoskeletal systems, an assortment of motor proteins, and membrane trafficking components. Due to their large size, the ease with which exquisite cytological analysis may be performed on them, and the availability of numerous mutants and other genetic tools, *Drosophila* spermatocytes have provided an excellent system for exploring the mechanistic basis for the temporally programmed and precise spatially localized events of cytokinesis. Mutants defective in male meiotic cytokinesis can be easily identified in forward genetic screens by the production of multinucleate spermatids. In addition, the weak spindle assembly checkpoint in spermatocytes, which causes only a small delay of anaphase onset in the presence of unattached chromosomes, allows investigation of whether gene products required for spindle assembly and chromosome segregation are also involved in cytokinesis. Perhaps due to the large size of spermatocytes and the requirement for two rapid-fire rounds of division without intervening S or growth phases during meiosis, male meiotic mutants have also revealed much about molecular mechanisms underlying new membrane addition during cytokinesis. Finally, cell type-specific differences in the events that set up and complete cytokinesis are emerging from comparison of spermatocytes with cells undergoing mitosis either elsewhere in the organism or in tissue culture.

Introduction

Cytokinesis, which partitions the cytoplasmic and nuclear content of the mother cell into two daughters at the end of cell division (reviewed in Barr and Gruneberg, 2007; Green et al., 2012), is both a fundamental task in normal development and is crucial for maintaining ploidy in adult tissues produced throughout life in stem cell lineages. In animal cells, a contractile ring composed of F-actin filaments and active Myosin II assembled on the inner face of the plasma membrane around the cell equator (Schroeder, 1990; Miller, 2011) generates the constricting force that separates the cell into two. How the cell builds the

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contractile ring at just the right place to cleanly and reliably separate the genetic and cytoplasmic contents is both a key regulatory problem and a long-standing question in the field of cytokinesis. Constriction of the actomyosin ring draws the plasma membrane and the underlying cortex inward, generating a cleavage furrow. This furrow ingresses until the daughter cells remain connected by a thin cytoplasmic bridge, dubbed the midbody. During the last step of cytokinesis, abscission, the intercellular bridge is ultimately severed, leading to two fully-separated daughter cells.

Cytokinesis is an intricate process involving at least one hundred proteins and requiring tight coordination between different cytoskeletal components and the plasma membrane (Eggert et al., 2006; Barr and Gruneberg, 2007; Pollard, 2010; Green et al., 2012). Much of our knowledge regarding the molecular players and underlying mechanisms that regulate and drive cytokinesis derives from genetic dissection of this process in suitable model organisms, such as *Drosophila melanogaster*, *Caenorhabditis elegans* and *Schizosaccharomyces pombe* (reviewed in Giansanti et al., 2001; Glotzer, 2005; Eggert et al., 2006; Bathe and Chang, 2010; Pollard and Wu, 2010; Green et al., 2012). In this review we describe the advantages offered by *Drosophila* spermatocytes for combined genetic and cytological studies of the mechanisms that underlie cytokinesis and examine the novel contributions from such studies to the field.

***Drosophila* spermatocytes as a system for the study of cytokinesis**

Drosophila spermatocytes offer a well-suited cell system for immunocytological and *in vivo* analysis of the structural and morphological events that underly cytokinesis, in part due to the large size of male meiotic cells and to the fact that clusters of primary and secondary spermatocytes undergo meiotic division in synchrony. This, coupled with the ease of identification of mutants that affect male meiosis by a simple screen of unfixed tissue and the many other genetic tools available in *Drosophila*, has led to a detailed descriptive and mechanistic understanding of both common and cell type-specific events of cytokinesis in animal cells.

Male germ cells differentiate throughout reproductive life from adult stem cells. In *Drosophila*, when a male germ line stem cell located at the apical tip of the testis divides, one daughter normally self-renews and maintains stem cell identity, while the other daughter becomes enclosed in two somatic support cells and initiates differentiation as a gonialblast (Fuller and Spradling, 2007). The gonialblast initiates four rounds of synchronous spermatogonial mitotic divisions to produce a cyst of 16 germ cells, which undergo premeiotic S phase and become spermatocytes. During the extended G2 phase of the cell cycle that is meiotic prophase, each spermatocyte undergoes impressive growth, leading to a 25-fold increase in volume (Lindsley and Tokuyasu, 1980). As a result, by the onset of the first meiotic division, approximately 90 hours after premeiotic S phase, spermatocytes are considerably larger than most somatic cells. The 16 spermatocytes in the cyst then synchronously undergo the G2/M transition of meiosis I and embark in quick succession on the first then the second meiotic division, producing a cyst of 64 haploid round spermatids. *Drosophila* spermatogonia and spermatocytes, like male germ cells in mammals, undergo incomplete cytokinesis, both during the mitotic spermatogonial divisions and the two

meiotic divisions. In both cases, cleavage furrows constrict, but abscission does not take place, and daughter cells remain interconnected by intercellular bridges called ring canals. Other than this developmentally-programmed lack of abscission, the mechanism of cleavage furrow formation and ingression appears largely conserved in spermatocytes compared with other animal cells (Giansanti et al. 2001 and this review).

During both the first and second meiotic divisions, the nuclear envelope does not disassemble by metaphase, but becomes fenestrated near the spindle poles. A small number of microtubules penetrate these fenestrations to contact the kinetochores, while the bulk of the metaphase spindle remains outside the nuclear envelope (Tates, 1971; Church and Lin, 1982). From metaphase to telophase, nuclei become spindle-shaped and are surrounded by a system of 5 to 7 double membranes called parafusorial membranes. During each meiotic division, alignment of mitochondria along the pole-to-equator spindle microtubules outside of the parafusorial membranes ensures the equal partitioning of mitochondria between the two daughter cells at the end of cytokinesis. The nuclear envelopes eventually break down and new nuclear envelopes form around the separated daughter nuclei in telophase. After the second meiotic division, the mitochondria in each early round spermatid fuse to form a complex, interlaced structure called the nebenkern (Fuller, 1993; Fig. 1A).

When viewed by phase-contrast optics, wild type early round spermatids contain a phase-dark nebenkern and a single phase-light haploid nucleus (Fig. 1A–B), and all the 64 interconnected spermatids within a cyst have the same sized nebenkern and the same sized nucleus. Mutants defective in meiotic cytokinesis can be easily identified by examining the pattern of nuclei and nebenkern in onion-stage spermatid cysts. Failure of cytokinesis during one or both meiotic divisions impairs the partitioning of mitochondria into separate daughter cells, and if segregation of the chromosomes occurred normally, results in spermatids containing 2 or 4 haploid nuclei paired with a single abnormally large nebenkern (Fig. 1B–C). Defects in cytokinesis, coupled with abnormal chromosome segregation, give rise to spermatids containing a large nebenkern associated with multiple nuclei of variable size (Figure. 1D). In this context, one peculiarity of male meiotic cells that is particularly advantageous when studying cytokinesis, is the lack of a robust spindle assembly checkpoint (Rebollo and Gonzalez, 2000). The spindle assembly checkpoint (SAC) signaling pathway normally monitors kinetochore attachment to spindle microtubules (Musacchio and Salmon, 2007). In somatic cells, SAC blocks cells in metaphase in the presence of unattached chromosomes or malformed spindles. In spermatocytes, in contrast, such abnormalities only cause a small delay in anaphase onset. Thus, the spermatocyte system allows discovery of requirements for gene products in later stages of cell division that are masked in somatic cells by the strong SAC when those same gene products also have an earlier role in spindle assembly or bipolar attachment of kinetochores to spindle microtubules (Wakefield et al., 2000; Giansanti et al., 2001).

Cleavage furrow formation in *Drosophila* spermatocytes

The pioneering studies of Ray Rappaport (1961) indicated for the first time the key role of anaphase spindle microtubules in establishing the cleavage site in animal cells. Anaphase mitotic spindles are largely comprised of astral microtubules, which emanate radially from

the two separated centrosomes toward the poles of the cell, and the central spindle (CS), an array of antiparallel, interdigitating microtubule (MT) bundles that lies between the two sets of chromosomes (Glotzer, 2009). The cleavage furrow forms in a position that bisects the axis of chromosome segregation, thus ensuring that each daughter cell receives a single copy of the genome (Glotzer, 2009). While CS microtubules send stimulatory signals to the cell equator for contractile ring assembly, astral microtubules are thought to send inhibitory signal(s) that prevent the localization of contractile ring proteins at the poles of the cell (Dechant and Glotzer, 2003; Bringmann and Hyman, 2005; Lewellyn et al., 2010).

Cytological analysis of *Drosophila* mutants defective in early steps of spermatocyte cytokinesis has revealed the importance of central spindle microtubules in cleavage furrow formation (Giansanti et al, 1999; Giansanti et al., 2004). In spermatocytes, central spindle microtubules are the only spindle components that are strictly required for signaling cytokinesis. Dividing spermatocytes devoid of astral microtubules and anucleate secondary spermatocytes assemble regular central spindles and contractile rings and successfully complete cytokinesis (Bonaccorsi et al., 1998; Giansanti et al., 2008; Bucciarelli et al., 2003). In contrast, mutations that perturb central spindle assembly invariably result in defects in F-actin ring assembly and cause early cytokinesis failure (Giansanti et al, 1999; Giansanti et al., 2004). As in other animal cells, major central spindle components include MAPs, plus-end directed kinesins, and the chromosomal passenger complex in addition to the antiparallel microtubule array.

Among the MAPs, the *Drosophila* ortholog of the highly conserved Protein Regulating Cytokinesis I (PRC1), Fascetto (Feo), is one of the first markers that localizes to the overlap central region of the anaphase central spindle in spermatocytes (Verni et al, 2004; Szafer-Glusman et al., 2011). Although not studied in spermatocytes, Feo is necessary for central spindle assembly in both larval neuroblasts and S2 cells (Verni et al, 2004). Two other MAPs were found to contribute to central spindle stability in *Drosophila* spermatocytes, namely Orbit and Abnormal Spindle Poles (Asp). Orbit localizes to the central spindle midzone but, in contrast to Feo, it associates with a subset of microtubule bundles that reside inside the nuclear envelope, dubbed “interior central spindle” microtubules (Inoue et al., 2004). *orbit* mutant spermatocytes display defective interior central spindle bundles and fail to complete contractile ring constriction. The evolutionarily conserved MAP, Asp (known as ASPM in humans), is concentrated at the polar regions of spindles in both neuroblasts and spermatocytes and contributes to spindle pole formation (Saunders et al., 1997; Wakefield et al., 2001). During anaphase, this protein localizes at the ends of the central spindle microtubule array nearest to the separated chromosomes, where it might crosslink and stabilize central spindle microtubule minus ends (Wakefield et al., 2001; Riparbelli et al., 2002). Indeed, loss of *asp* function perturbs central spindle structure and impairs contractile ring assembly in spermatocytes (Wakefield et al., 2001)

Several plus-end directed microtubule motor proteins of the kinesin family are also structural components of the spermatocyte central spindle. Both KLP3A (the *Drosophila* ortholog of KIF4) and the kinesin 6 family member MKLP1/Pavarotti (Pav) localize at the center of the central spindle, where the microtubule plus-ends overlap in anaphase spermatocytes (Williams et al., 1995; for a review see D’Avino et al., 2005). KLP3A, like its

homolog KIF4, forms a conserved complex with the MAP Feo/PRC1 and is required for formation of both the central spindle and the contractile ring (D'Avino et al., 2007; Giansanti et al., 1998; Giansanti et al., 1999).

A key regulatory component for specifying the site of cleavage furrow formation is centralspindlin, composed of two molecules of the kinesin family member Pav and two molecules of the Rho family GAP RacGAP50C. Centralspindlin activates RhoA (named Rho1 in *Drosophila*) at the equatorial cortex to trigger actomyosin contractile ring assembly. During cytokinesis, the balance between the active state (GTP-bound) and inactive state (GDP-bound) of RhoA/Rho1 depends on the RhoGEF ECT2/Pebble, which is targeted to the equatorial cortex by binding to centralspindlin (Somers and Saint, 2003; Kamijo et al., 2006; Nishimura and Yonemura, 2006; Yuce et al., 2005; Zhao and Fang, 2005; Piekny et al., 2005).

Regulation of centralspindlin activity and recruitment of ECT2/Pebble involve the activities of Aurora B kinase and Polo-like kinase 1 (Plk1). During anaphase, Aurora B-mediated phosphorylation of MKLP1/Pavarotti is critical for centralspindlin formation (Guse et al., 2005; Neef et al., 2006; Douglas et al., 2010), while targeting of the RhoGEF ECT2/Pebble to the cortex depends, at least in part, on phosphorylation of MgRacGAP/RacGAP50 by Polo kinase (Brennan et al., 2007; Santamaria et al., 2007; Burkard et al., 2009; Wolfe et al., 2009).

Plk1 and Aurora B also control early events of cell division such as chromosome condensation, spindle formation and chromosome segregation (for reviews see Barr et al., 2004; van der Waal et al., 2012). Because of these earlier requirements, the analysis of the effects of mutants lacking either Polo or Aurora B on cytokinesis in most cell types has been hindered by the early mitotic arrest caused by activation of the spindle checkpoint (Llamazares et al., 1980; Giet and Glover, 2001). In *Drosophila* spermatocytes, however, the lack of stringent checkpoint control has enabled investigation of the functional roles of the Polo and Aurora B kinases during cytokinesis. *Drosophila* mutants carrying a hypomorphic *polo* allele are viable and progress through male meiosis, thus allowing investigation of the function of Polo in cytokinesis (Carmena et al., 1998). During anaphase, Polo accumulates at the spindle midzone and is required for proper localization of Pav and for formation of both the central spindle and F-actin ring (Carmena et al., 1998; Hermann et al., 1998). Although there are no extant viable *pav* alleles that could be examined in male meiosis, the Pav and Polo proteins interact structurally, and each depends on the other for localization to the central spindle midzone in *Drosophila* embryonic cells (Adams et al., 1998).

Aurora B is the enzymatic subunit of the Chromosomal passenger Complex (CPC). Three additional CPC subunits, Inner Centromere Protein, Survivin, and Borealin, localize and activate the kinase (Ruchaud et al., 2007a; van der Waal et al., 2012). Early in mitosis and meiosis, in the events leading to metaphase, the CPC regulates processes such as chromosome condensation, spindle formation, correct attachment of kinetochore-to-spindle microtubules, and the mitotic checkpoint (Ruchaud et al., 2007a). During anaphase, function of the CPC is required for central spindle assembly and cytokinesis (Carmena, 2008).

Consistent with its multiple roles during cell division, the CPC exhibits very dynamic, cell cycle-dependent localization patterns. The CPC associates with chromatin during G2, then moves to the inner centromeres by prometaphase-metaphase. In anaphase the CPC translocates to the central spindle microtubules and the equatorial cortex (Szafer-Glusman et al., 2011; Adams et al., 2000; Vagnarelli and Earnshaw, 2004; Vader et al., 2006; van der Waal et al., 2012). The *D. melanogaster* genome encodes two Borealin paralogs, Borealin-related (Borr) and Australin (Aust; Gao et al., 2008); the latter replaces Borr in male meiosis. Dividing spermatocytes from males carrying either a hypomorphic allele of *dmINCENP* or a null allele of *australin* (*aust*) display a similar phenotype, early cytokinesis arrest with defective central spindles and failure to recruit Pav to the cell equator (Resnick et al., 2006; Gao et al., 2008), thereby suggesting a role for the CPC in activation and/or localization of the centralspindlin complex.

The strict interdependence of the four CPC subunits (Ruchaud et al., 2007a; 2007b) has complicated the analysis of the function of each single component; indeed, in human cells, knockdown of each CPC protein impaired the localization and function of the whole complex (Adams et al., 2001; Carvalho et al., 2003; Honda et al., 2003; Lens et al., 2003; Gassmann et al., 2004). A “separation-of-function” allele of *Drosophila* Survivin/Deterin (*dSurvivin*), isolated after EMS mutagenesis, has allowed investigation of the role of Survivin, and by inference the CPC, specifically during anaphase and cytokinesis (Szafer-Glusman et al., 2011). The *scapolo* (*scpo*) mutation contains a missense mutation in the BIR domain of the Survivin polypeptide that allows recruitment and function of the CPC in metaphase, but disrupts its localization and function in anaphase and cytokinesis (Szafer-Glusman et al., 2011).

Interestingly, phenotypic analysis of *scapolo* (*scpo*) hemizygotes revealed striking differences in how determination of the site of cleavage furrow formation is regulated in different *Drosophila* cell types (Fig. 2). In both neuroblasts and spermatocytes at anaphase, Survivin is required to target the CPC and Pav to the central spindle and the equatorial cortex. In anaphase spermatocytes, the *scpo* mutation also impaired localization of Polo and Rho1 to the cell equatorial cortex and contractile ring assembly. These results suggest that in male meiotic cells Survivin is involved in the mechanism that leads to Rho1 activation. This may be through effects on Polo, as failure to recruit Polo would prevent localization of the RhoGEF ECT2/Pebble, which normally activates Rho1 locally, thereby resulting in the inability to form a contractile ring. In contrast, in anaphase neuroblasts from *scpo* hemizygotes, Polo, Rho1, and Myosin II accumulate in a broad equatorial cortical band, even in the absence of CPC and Pav. Such an initial wide equatorial belt of cytokinetic machinery components is also formed in wild type neuroblasts undergoing mitosis (Fig. 2). This initial wide band normally narrows gradually into a thin ring at the equatorial cell cortex, then the ring constricts for cleavage furrow formation during cytokinesis. In *scpo* neuroblasts, however, the broad equatorial band, visualized by Myosin II, failed to convert into the thin equatorial contractile ring and the mutant cells did not complete cytokinesis. These observations indicate a striking, cell type-specific difference in the events that set up the contractile ring. In neuroblasts, assembly of the contractile ring occurs in two steps, as proposed by Lewellyn et al. (2011) for *C. elegans* embryos. The first step, which involves

activation and localization of Rho1 and the recruitment of contractile ring proteins to a broad swath of the cortex about the midriff of the cell, appears independent of the central spindle and Pav (Fig. 2). Spermatocytes, in contrast, appear to skip the first step: Rho1 and contractile ring proteins first appear at the equatorial cortex in a tightly focused ring, without forming a preceding wide band (Fig. 2A). It may be that global inhibition of Rho activation is more stringent at the cortex of spermatocytes than in neuroblasts, where a more permissive cortex could allow early formation of a broad belt of Rho1 (Fig. 2B). Alternatively, a recent study has shown the existence in neuroblasts of a spindle-independent backup system that permits localization of Myosin II to the cell cortex under the control of the neuroblast cell polarity system (Cabernard et al., 2010). This redundant signaling system, not present in spermatocytes, may account for the broad band of Myosin II observed in *scpo* mutant neuroblasts during anaphase.

Structural and regulatory components of the contractile ring in *Drosophila* spermatocytes

In the GTP bound state, Rho activates downstream effectors that lead to actin polymerization and Myosin II activation at the equatorial cortex (Piekny et al., 2005; Goode and Eck, 2007; Matsumara et al., 2011). Non-muscle Myosin II in the contractile ring consists of hexamers that assemble into filaments. Each hexamer contains a dimer of heavy chains, two essential light chains and two regulatory light chains. Phosphorylation of the regulatory light chains (rMLC) is a key step required for activation of Myosin II motor activity and bipolar filament assembly. The importance of this process is demonstrated by the opposite effects of phosphomimetic and nonphosphorylatable alleles of the *Drosophila* rMLC Spaghetti Squash (*Sqh*) on cytokinesis (Jordan and Karess, 1997). Dividing spermatocytes from mutant males in which function of *sqh* has been lost are devoid of both central spindles and F-actin rings at telophase, indicating an early requirement for *Sqh* in cytokinesis (Giansanti et al., 2001).

The traditional “purse-string” model for how the contractile ring generates the force for cleavage furrow ingression proposes that bipolar filaments of Myosin II use their motor activity to walk along antiparallel actin filaments, drawing the F-actin strands together in a purse-string-like fashion (Schroeder et al., 1990; Satterwhite and Pollard, 1992). Because the cortex/plasma membrane is attached to the F-actin strands, the membrane is drawn inward into a cleavage furrow as the actomyosin ring constricts just under the cell cortex. However, the exact geometry of F-actin and Myosin II filaments in the contractile ring is still a matter of debate and alternative models for contractile ring organization and constriction have been proposed (Reichl et al., 2008; Carvalho et al., 2009; Kee et al. 2012; Ma et al., 2012; reviewed in Green et al., 2012). Recent studies in *Dictyostelium* and vertebrate cells have led to the proposal that Myosin II does not translocate actin, but is required to cross-link actin filaments and to exert tension on actin during cytokinesis (Reichl et al., 2008; Kee et al., 2012; Ma et al., 2012).

As the ring constricts, the cross-sectional area of the ring is stable, suggesting the importance of a balance between F-actin polymerization and disassembly during cytokinesis. F-actin ring assembly and disassembly are regulated by the actin-binding

proteins Profilin and Cofilin. Profilin binds to actin monomers and promotes actin filament formation in a formin-dependent pathway at the cell cortex (reviewed in Glotzer; 2005). Cofilin is an actin-severing protein required for F-actin disassembly (Ono et al., 2003). Consistent with the functions of these proteins, mutations affecting profilin and cofilin have opposite effects on cytokinesis in spermatocytes. Mutations in the gene *chickadee* (*chic*), which encodes the *Drosophila* profilin, impair F-actin ring formation and central spindle assembly (Giansanti et al., 1998; Giansanti et al., 1999). Mutations in the gene *twinstar* (*tsr*), which encodes a cofilin homolog, do not affect contractile ring assembly, but perturb F-actin disassembly at the end of cytokinesis (Gunsalus et al., 1995; Giansanti et al., 1999). Telophase spermatocytes from *tsr* males display abnormally large F-actin aggregates that fail to disassemble at the end of cytokinesis (Gunsalus et al., 1995).

In addition to Myosin II and F-actin, other structural components of the contractile apparatus, including the GTP-binding Septins and Anillin, act as scaffolding proteins at the cleavage furrow (for a review see D'Avino, 2009). Five *D. melanogaster* septins have been identified so far: Sep1, Sep2, Peanut, Sep4 and Sep5 (Adam et al., 2000). In testes, Peanut, Sep1 and Sep2 colocalize with Myosin II, Anillin and F-actin in the contractile rings, and are also components of the ring canals that remain to connect sister cells after the incomplete cytokinesis typical of mitotic spermatogonia and the meiotic divisions of spermatocytes (Hime et al., 1996; Giansanti et al., 2001). However, the role played by Septins in *Drosophila* spermatocytes has not been fully elucidated (Adam et al., 2000; Giansanti et al., 2001).

Anillin was first isolated as an F-actin-binding protein from *Drosophila* embryo extracts (Miller et al., 1989). Later studies showed that this widely-conserved protein also binds activated Myosin II and Septins, which are also filament-forming proteins (Kinoshita et al., 2002; Straight et al., 2005; D'Avino et al., 2008; reviewed in D'Avino et al., 2009). Recent studies have provided evidence suggesting that *Drosophila* Anillin interacts with RacGAP50C (D'Avino et al., 2008; Gregory et al., 2008). Based on these data, it has been suggested that Anillin might mediate interactions between central spindle microtubules and the equatorial cortex required to stabilize the division plane. In *Drosophila* spermatocytes, Anillin starts to concentrate at the cell equator of dividing spermatocytes during anaphase, before the appearance of F-actin (Giansanti et al., 1999; Fig. 1). Consistent with this temporal order, analysis of spermatocytes from mutants defective in F-actin ring assembly showed that initial recruitment of Anillin to the cortex did not depend on F-actin (Giansanti et al., 1999). Analysis of dividing spermatocytes depleted of Anillin by Goldbach and coauthors (2010) showed that Anillin function is required for localization of Septins to the cleavage furrow and to maintain Rho, F-actin and Myosin II in the contractile ring. Strikingly, ectopic expression of the adherens junction protein DE-cadherin suppressed the cytokinesis defects caused by loss of anillin in spermatocytes (Goldbach et al., 2010). DE-cadherin-GFP localized to the cleavage furrow and recruited β -catenin and α -catenin during cytokinesis, raising the possibility that either anillin-septin or cadherin-catenin complexes act as alternative cassettes to anchor F-actin and Myosin II at the cleavage furrow during spermatocyte cytokinesis.

A novel component of *Drosophila* contractile ring and ring canals, dubbed Nessun Dorma (Nesd), may play a role in anchoring the actomyosin ring to the plasma membrane during cleavage furrow ingression (Montembault et al., 2010). Nesd, identified as an evolutionarily-conserved partner of the centralspindlin complex proteins (Montembault et al., 2010), directly binds both Pav and RacGAP50C through a highly-conserved domain in its N-terminus. Phenotypic analysis of dividing spermatocytes from *nesd* mutants revealed that *nesd* function is required during late telophase in male meiotic cells. Nesd starts to concentrate in the cleavage furrow during late telophase and is required for ring canal formation (Montembault et al., 2010). Nesd function in ring canals might be associated with the presence of a pectin lyase-like domain in its C-terminal half and its high binding affinity for β -galactosides. Based on these results, it has been suggested that glycosylated proteins are involved in late cytokinesis of *Drosophila* male germ cells (Montembault et al., 2010). Consistent with this hypothesis, the glycoprotein mucin D is a component of the ring canals in both testes and ovaries of *Drosophila* (Kramerova and Kramerov, 1999).

Membrane traffic in male meiotic cytokinesis

In addition to the force generated by constriction of the actomyosin contractile ring at the cell equator, successful cleavage furrow ingression requires an increase in surface area of the plasma membrane to encapsulate each daughter cell into a separate membrane compartment. Indeed, recent studies have demonstrated that targeted vesicle transport plays an important role during cleavage furrow ingression, as well as in the later events of abscission (McKay and Burgess, 2011; Neto et al., 2011). Vesicle-mediated traffic to the ingressing cleavage furrow involves components of two main pathways: the recycling endosome (RE) pathway and the secretory pathway (reviewed in Prekeris and Gould, 2008; McKay and Burgess, 2011). A large part of our knowledge regarding the role of vesicle trafficking during cytokinesis has derived from experiments performed on cell lines grown in culture. *Drosophila* male meiosis offers a well-suited model for studying the requirement of membrane trafficking during cytokinesis in a tissue of a multicellular organism.

Spermatocyte cytokinesis is especially sensitive to mutations affecting vesicle trafficking components, as reflected by the large number of male sterile mutants defective in male meiotic cytokinesis that have been identified in mutant screens in *Drosophila* (Brill et al., 2000; Xu et al., 2002; Farkas et al., 2003; Gatt and Glover, 2006; Giansanti et al., 2006, 2007; Dyer et al., 2007; Polevoy et al., 2009; Robinett et al., 2009). Secretory traffic, involving vesicle transport from the endoplasmic reticulum (ER) to the Golgi, and then to the plasma membrane, plays a crucial role in spermatocyte cytokinesis (Xu et al., 2002; Farkas et al., 2003; Giansanti et al., 2006, 2007; Belloni et al., 2012).

Based on the transmission electron microscopy studies of Tates (1971), the endoplasmic reticulum (ER) of almost mature spermatocytes, just prior to the meiotic divisions, is arranged in a system of parallel cisternae around the nucleus, with one of the cisternae continuous with the nuclear envelope. During meiotic division, ER structure can be visualized in spermatocytes from males that express the ER marker Protein disulfide isomerase (Pdi) tagged with GFP (Bobiniec et al., 2003; Giansanti et al., 2006, 2007). In metaphase and ana-telophase, GFP-Pdi stains the parafusorial membranes that surround the

nuclear envelope (see above) and a system of 13 umbrella-shaped layers at each cell pole dubbed the “astral membranes” (AM) (Giansanti et al., 2006, 2007).

Golgi complexes in premeiotic primary spermatocytes are associated with the ER cisternae (Tates, 1971). One peculiarity of *Drosophila* cells (including spermatocytes) is the organization of Golgi in multiple separate stacks (Tates, 1971; Fuller, 1993; Kondylis and Rabouille, 2009). These approximately 20 small Golgi stacks can be visualized as conical structures with antibodies against the Golgi scaffolding protein Lava lamp (Lva) or the Golgi mannosidase α -mannosidase II (GMII) (Sisson et al., 2002; Farkas et al., 2003; Giansanti et al., 2006, 2007; Robinett et al., 2009). This characteristic organization in multiple, separate stacks is advantageous in phenotypic analysis of mutants, as it allows the detection of subtle structural defects that might be missed in mammalian cells with Golgi stacks interconnected into a pericentriolar Golgi ribbon (Kondylis and Rabouille, 2009). By metaphase to telophase, the Golgi are dispersed and Golgi markers are associated with small vesicle-like structures that are excluded from the cell midzone (Farkas et al., 2003; Giansanti et al., 2006, 2007).

Several studies have implicated Golgi trafficking in cytokinesis in male meiotic cells. For example, treatment of cultured testes with Brefeldin A, a fungal metabolite that interferes with anterograde transport from the ER to the Golgi, causes disruption of Golgi organelles and disrupts cytokinesis during meiosis I and II, as revealed by the presence of multinucleate spermatids (Robinett et al., 2009; Kitazawa et al., 2012). In addition, cytokinesis of spermatocytes requires the wild type function of several proteins implicated in Golgi trafficking. These include the intra-Golgi trafficking Cog5 and Cog7 homologs (Farkas et al., 2003; Belloni et al., 2012), the ER-to-Golgi vesicle docking protein Syntaxin 5 (Xu et al., 2002), and Brunelleschi, which encodes the *Drosophila* ortholog of the yeast TRAPP II TRS120p subunit (Robinett et al., 2009). Recent studies have also implicated proteins that function in retrograde transport from Golgi to ER in spermatocyte cytokinesis, including subunits of the coatomer protein complex I (COPI) and the small GTPase Arf1 (Kitazawa et al., 2012). Using markers for cis-Golgi and ER compartments, Kitazawa and coauthors found that COPI proteins localize in the ER-Golgi intermediate compartments of premeiotic spermatocytes. During metaphase, COPI proteins are associated with vesicles that follow a dynein-mediated poleward movement along astral microtubules. Testis-specific knockdown of COPI disrupted the localization of other membrane trafficking proteins and lipid droplets at the furrow. A striking finding of this study is the intimate relationship between the ER-based intracellular structures and spindle microtubules in dividing spermatocytes. Time lapse analysis of spermatocytes that express GFP-tagged tubulin and ER markers revealed that knockdown of COPI affects not only the structure of ER-based astral and parafusorial membranes, but also that of astral and central spindle microtubules. Conversely, in a tubulin mutant, a primary defect in microtubule structures resulted in secondary defects in ER-based structures. Based on these results, Kitazawa and coauthors (2012) suggested that ER membranes and microtubule bundles are mutually dependent structures during male meiotic division.

Drosophila Zw10, a subunit of the conserved kinetochore-associated Rod-Zwilch-Zw10 (RZZ) complex required for function of the spindle assembly checkpoint in both *Drosophila*

and mammals (Starr et al., 1998; Scaerou et al., 2001; Williams et al., 2003; reviewed in Karess, 2005; Musacchio and Salmon, 2007), has also recently been shown to play a role in membrane trafficking during cytokinesis in spermatocytes (Wainman et al., 2012). In both mammalian and yeast cells, the Zw10 orthologs form a second complex with the conserved RINT1/Tip20p and NAG/Sec39p proteins that is involved in vesicle transport between Golgi and ER (Hirose et al., 2004; Arasaki et al., 2006; Sun et al., 2007; Varma et al., 2006; Aoki et al., 2009; Schmitt, 2010). In *Drosophila* spermatocytes, Zw10 is enriched at both the Golgi stacks and the ER, and function of Zw10 and the *Drosophila* homologue of *RINT1* are both required for Golgi integrity. Function of both Zw10 and *RINT1* is essential for furrow ingression during cytokinesis in spermatocytes (Wainman et al., 2012). In contrast, Rod is required for proper Golgi function, but is dispensable for cytokinesis. These data suggest that Zw10 and Rint1 might associate to form a subcomplex involved in membrane trafficking during spermatocyte cytokinesis that either does not contain or does not require active Rod.

The small GTPases Rab11 and Arf6 are also essential for cytokinesis in *Drosophila* male meiotic cells (Giansanti et al., 2007). In spermatocytes, Rab11 has been implicated in both Golgi and recycling endocytic traffic, and is enriched at vesicles that mediate membrane addition at the cleavage furrow (Giansanti et al., 2007; Dyer et al., 2007; Polevoy, 2009). In dividing spermatocytes, ARF6 colocalized with Rab11 and with the early endosome marker Rab4 (Dyer et al., 2007). Loss of ARF6 did not affect localization of Rab4 and Rab11 to the spindle midzone, but did disrupt completion of cleavage furrow ingression. Remarkably, ARF6 directly binds Pav, raising the possibility that centralspindlin might help recruit ARF6-associated endosomes to the cleavage furrow (Dyer et al., 2007).

Cytological analysis of mutants in *fws*, *bru*, *Rab11*, *Zw10* and *Arf6* revealed a common cytokinetic phenotype: mutant spermatocytes assemble regular central spindles and contractile rings during early telophase, but the rings fail to complete constriction, thereby resulting in cytokinesis failure (Farkas et al., 2003; Giansanti et al., 2007; Dyer et al., 2007; Robinett et al., 2009; Wainman et al., 2012). In addition, time-lapse analysis of *arf6*, *rab11* and *zw10* mutant spermatocytes expressing β -tubulin-EGFP indicated similar defects in central spindles over time (Dyer et al., 2007; Giansanti et al., 2007; Wainman et al., 2012). Central spindles formed transiently and furrows initially ingressed during the early stages of cytokinesis in the mutants. However, later during telophase, cleavage furrows regressed, and central spindle microtubule bundles disassembled.

This failure to complete cytokinesis and the subsequent furrow regression phenotype are thought to arise from defects in membrane addition to the surface at the furrow. In wild type spermatocytes, the increase in perimeter during cytokinesis correlates well with surface area increase (Dyer et al., 2007). The perimeter increase of *arf6* and *zw10* mutant spermatocytes during cytokinesis was drastically affected, consistent with defects in membrane expansion in the mutant cells (Dyer et al., 2007; Wainman et al., 2012).

The large size of spermatocytes and the requirement that these cells must undergo two successive meiotic divisions in a restricted time window with only a very short intervening interphase (Fuller, 1993; Giansanti et al., 2001) may have contributed to the emergence of

Drosophila spermatocytes as a very attractive system for investigating the roles of membrane remodeling and vesicle trafficking in cytokinesis.

In addition, because spermatocytes lack plasma membrane protrusions and microvilli (Tates, 1971) that might be used as a plasma membrane reservoir during cytokinesis, they may rely more on traffic from internal membrane stores to provide the additional surface area to produce four cells from one. Whatever the underlying reason, multiple mutations identified in forward genetic screens have revealed that the meiotic divisions of *Drosophila* spermatocytes appear to be particularly sensitive to mutations affecting vesicle trafficking pathways (Brill et al., 2000; Xu et al., 2002; Farkas et al., 2003; Gatt and Glover, 2006; Giansanti et al., 2006; Giansanti et al., 2007; Dyer et al., 2007; Robinett et al., 2009; Polevoy et al., 2009).

Specialized lipids and cytokinesis

Successful cytokinesis also depends on a special lipid composition at the cleavage furrow. Special membrane domains are emerging as key factors in regulating both the dynamics of cytokinetic structures and membrane addition (reviewed in Neto et al. 2011).

Very-long-chain-fatty acids (VLCFAs), consisting of 22 or more carbons, are primarily found in sphingolipids, and participate in the stabilization of highly curved membrane domains such as those at the ingressing tip of the cleavage furrow (Schneider et al., 2004). The *Drosophila* gene *bond* (named after James Bond because of the lack of a ring) encodes a member of the Elovl family involved in the biosynthesis of VLCFAs (Szafer-Glusman et al., 2008). In *bond* mutant spermatocytes, central spindles fail to assemble and the contractile ring, visualized by Sqh-GFP, detaches from the membrane and collapses to one side of the cell (Szafer-Glusman et al., 2008). These findings suggest that VLCFAs are required to allow the plasma membrane to undergo cleavage furrow invagination and indicate an intimate relationship between membrane lipids and the dynamics of cytoskeletal structures during cytokinesis. In contrast with *bond*, mutants in peroxin genes (*pex*) accumulate elevated levels of VLCFAs and *pex*⁺ heterozygotes suppress the cytokinesis defects in *bond* homozygotes (Chen et al., 2010). However, dividing spermatocytes from males carrying *pex* mutations fail cytokinesis just like *bond*, indicating that cytokinesis requires proper regulation of VLCFA levels.

Phosphatidylinositol phosphates are also important signaling molecules during cytokinesis (for a review see Brill et al., 2011). The *Drosophila* phosphatidylinositol (PI) transfer protein Giotto/Vibrator (Gio) concentrates in both the ER membranes and the cleavage furrow in dividing spermatocytes. Spermatocytes and neuroblasts from *gio* mutants are defective in contractile ring constriction and exhibit an accumulation of Golgi-derived vesicles at the cleavage site, suggesting a defect in membrane addition (Gatt and Glover, 2006; Giansanti et al., 2006; Giansanti et al., 2007).

The phosphoinositide Phosphatidylinositol 4,5-bisphosphate (PIP₂), enriched in the plasma membrane of the cleavage furrow of *Drosophila* spermatocytes, is crucial for cleavage furrow stability (Wong et al., 2005). Compelling data indicate that PIP₂ promotes actin polymerization (reviewed in Yin and Janmey, 2003). In addition, the polybasic domain of

Septins and the pleckstrin homology domain of Anillin can mediate interactions with PIP2 (Bertin et al., 2010; Liu et al., 2012). Thus, disruption of PIP2 at the furrow might affect the recruitment and/or the stability of contractile ring proteins. PIP2 turnover is also important during cytokinesis; drugs that interfere with PIP2 hydrolysis mediated by phospholipase C affect the completion of furrow ingression in spermatocytes (Wong et al., 2005).

Genetic analysis in *Drosophila* also indicates that phosphatidylinositol 4-phosphate (PI4P) may play a key role in cytokinesis of male meiotic cells. *fwd* encodes a *Drosophila* PI4KIII β (Brill et al., 2002). Dividing spermatocytes from *fwd* mutant males exhibit a cytokinetic phenotype similar to that of *gio* and *Rab11* mutants (Giansanti et al., 2006; Giansanti et al., 2007; Polevoy et al., 2009). Phenotypic analysis of single and double mutants in *fwd*, *rab11*, and *gio* indicate that Rab11, Gio and Fwd might function in the same pathway during cytokinesis, with Gio and Fwd required upstream of Rab11 (Giansanti et al., 2007). Indeed, Fwd proteins bind the GTPase Rab11 and colocalize with both Rab11 and PI4P markers on Golgi membranes, and loss of *fwd* disrupts the localization of Rab11 and PI4P at the cell equator (Polevoy et al., 2009).

Conclusions and perspectives

Drosophila spermatocytes have proven to be an optimal cell system for dissecting the molecular mechanisms underlying cytokinesis in the context of a multicellular organism. Studies over the last two decades have revealed the complexity of this process, which requires tight coordination of cell cycle regulatory proteins, cytoskeletal components and membrane trafficking.

Genetic dissection of male meiotic cytokinesis in *Drosophila* has allowed identification of the key molecular players. However, we are still far from identifying all the pieces of this complex puzzle. Male meiotic divisions are particularly dependent on membrane trafficking from internal membrane compartments, making this an excellent model for investigating membrane remodeling during cytokinesis, and pursuing the open questions concerning membrane addition to the cleavage site (reviewed in McKay and Burgess, 2012)

Remarkably, several cytokinesis genes/proteins have also been found to play roles in cancer pathogenesis (reviewed in Normand and King, 2010; Lacroix and Maddox, 2012). Cytokinesis proteins are frequently upregulated in cancer cells or are encoded by genes that are deleted or amplified in human cancers (Roversi et al., 2006; Lind et al., 2011; Sinha et al., 2011). Thus, clarification of the molecular pathways involved in cytokinesis may also aid cancer diagnosis and the design of therapeutic strategies.

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References

- Adam JC, Pringle JR, Peifer M. Evidence for functional differentiation among *Drosophila* septins in cytokinesis and cellularization. *Mol Biol Cell*. 2000; 11:3123–3135. [PubMed: 10982405]
- Adams RR, Tavares AA, Salzberg A, Bellen HJ, Glover DM. pavarotti encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis. *Genes Dev*. 1998; 12:1483–1494. [PubMed: 9585508]
- Adams RR, Wheatley SP, Gouldsworthy AM, Kandels-Lewis SE, Carmena M, Smythe C, Gerloff DL, Earnshaw WC. INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr Biol*. 2000; 10:1075–1078. [PubMed: 10996078]
- Adams RR, Maiato H, Earnshaw WC, Carmena M. Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J Cell Biol*. 2001; 153:865–880. [PubMed: 11352945]
- Aoki T, Ichimura S, Itoh A, Kuramoto M, Shinkawa T, Isobe T, Tagaya M. Identification of the neuroblastoma-amplified gene product as a component of the syntaxin 18 complex implicated in Golgi-to-endoplasmic reticulum retrograde transport. *Mol Biol Cell*. 2009; 20:2639–2649. [PubMed: 19369418]
- Arasaki K, Taniguchi M, Tani K, Tagaya M. RINT-1 regulates the localization and entry of ZW10 to the syntaxin 18 complex. *Mol Biol Cell*. 2006; 17:2780–2788. [PubMed: 16571679]
- Barr FA, Sillje HH, Nigg EA. Polo-like kinases and the orchestration of cell division. *Nat Rev Mol Cell Biol*. 2004; 5:429–440. [PubMed: 15173822]
- Barr FA, Gruneberg E. Cytokinesis: placing the final cut. *Cell*. 2007; 131:847–860. [PubMed: 18045532]
- Bathe M, Chang F. Cytokinesis and the contractile ring in fission yeast: towards a systems-level understanding. *Trends Microbiol*. 2010; 18:38–45. [PubMed: 19959363]
- 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.
- Bertin A, McMurray MA, Thai L, Garcia G 3rd, Votin V, Grob P, Allyn T, Thorner J, Nogales E. Phosphatidylinositol-4,5-bisphosphate promotes budding yeast septin filament assembly and organization. *J Mol Biol*. 2010; 404(4):711–731. [PubMed: 20951708]
- Bobinnec Y, Marcaillou C, Morin X, Debec A. Dynamics of the endoplasmic reticulum during early development of *Drosophila melanogaster*. *Cell Motil Cytoskeleton*. 2003; 54(3):217–225. [PubMed: 12589680]
- Bonaccorsi S, Giansanti MG, Gatti M. Spindle self-organization and cytokinesis during male meiosis in asterless mutants of *Drosophila melanogaster*. *J Cell Biol*. 1998; 142:51–61. [PubMed: 9660862]
- Brennan IM, Peters U, Kapoor TM, Straight AF. Polo-Like Kinase Controls Vertebrate Spindle Elongation and Cytokinesis. *PLoS One*. 2007; 2:e409. [PubMed: 17476331]
- Brill JA, Hime GR, Scharer-Schuks M, Fuller MT. A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. *Development*. 2000; 127:3855–3864. [PubMed: 10934029]
- Brill JA, Wong R, Wilde A. Phosphoinositide function in cytokinesis. *Curr Biol*. 2011; 21:R930–R934. [PubMed: 22115464]
- Bringmann H, Hyman AA. Cytokinesis furrow is positioned by two consecutive signals. *Nature*. 2005; 436:731–734. [PubMed: 16079852]
- 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; 160:993–999. [PubMed: 12654903]
- Burkard ME, Maciejowski J, Rodriguez-Bravo V, Repka M, Lowery DM, Clauser KR, Zhang C, Shokat KM, Carr SA, Yaffe MB, Jallepalli PV. 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. [PubMed: 19468302]
- Cabernard C, Prehoda KE, Doe CQ. A spindle-independent cleavage furrow positioning pathway. *Nature.* 2010; 467:91–95. [PubMed: 20811457]
- Carmena M. Cytokinesis: the final stop for the chromosomal passengers. *Biochem Soc Trans.* 2008; 36:367–370. [PubMed: 18481960]
- Carmena M, Riparbelli MG, Minestrini G, Tavares AM, Adams R, Callaini G, Glover DM. *Drosophila* polo kinase is required for cytokinesis. *J Cell Biol.* 1998; 143:659–671. [PubMed: 9813088]
- Carvalho A, Carmena M, Sambade C, Earnshaw WC, Wheatley SP. Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. *J Cell Sci.* 2003; 116:2987–2998. [PubMed: 12783991]
- Carvalho A, Desai A, Oegema K. Structural memory in the contractile ring makes the duration of cytokinesis independent of cell size. *Cell.* 2009; 137:926–937. [PubMed: 19490897]
- 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. [PubMed: 19933170]
- Church K, Lin HP. Meiosis in *Drosophila melanogaster*. II. The prometaphase-I kinetochore microtubule bundle and kinetochore orientation in males. *J Cell Biol.* 1982; 93:365–373. [PubMed: 6807996]
- D'Avino PP, Savoian MS, Glover DM. Cleavage furrow formation and ingression during animal cytokinesis: a microtubule legacy. *J Cell Sci.* 2005; 118:1549–1558. [PubMed: 15811947]
- D'Avino PP, Archambault V, Przewloka MR, Zhang W, Lilley KS, Laue E, Glover DM. Recruitment of Polo kinase to the spindle midzone during cytokinesis requires the Feo/Klp3A complex. *PLoS One.* 2007; 2:e572. [PubMed: 17593971]
- D'Avino PP, Takeda T, Capalbo L, Zhang W, Lilley KS, Laue E, Glover DM. Interaction between Anillin and RacGAP50C connects the actomyosin contractile ring with spindle microtubules at the cell division site. *J Cell Sci.* 2008; 121:1151–1158. [PubMed: 18349071]
- D'Avino PP. How to scaffold the contractile ring for a safe cytokinesis - lessons from Anillin-related proteins. *J Cell Sci.* 2009; 122:1071–1079. [PubMed: 19339546]
- Dechant R, Glotzer M. Centrosome separation and central spindle assembly act in redundant pathways that regulate microtubule density and trigger cleavage furrow formation. *Dev Cell.* 2003; 4:333–344. [PubMed: 12636915]
- Douglas ME, Davies T, Joseph N, Mishima M. Aurora B and 14-3-3 Coordinately Regulate Clustering of Centralspindlin during Cytokinesis. *Curr Biol.* 2010; 20:927–933. [PubMed: 20451386]
- Dyer N, Rebollo E, Domínguez P, Elkhatib N, Chavrier P, Daviet L, González C, González-Gaitán M. Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. *Development.* 2007; 134:4437–4447. [PubMed: 18039970]
- Eggert US, Mitchison TJ, Field CM. Animal cytokinesis: from parts list to mechanisms. *Annu Rev Biochem.* 2006; 75:543–566. [PubMed: 16756502]
- Farkas RM, Giansanti MG, Gatti M, Fuller MT. *Drosophila* Cog5/COG4 is required for cytokinesis, polarized cell growth and normal Golgi architecture during spermatogenesis. *Mol Biol Cell.* 2003; 14:190–200. [PubMed: 12529436]
- 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; 133:605–616. [PubMed: 8636235]
- Fuller, MT. Spermatogenesis. In: Bate, M.; Martinez-Arias, A., editors. *The Development of Drosophila melanogaster*. Cold Spring Harbor, NY: Cold Spring Harbor Press; 1993. p. 71-147.
- Fuller MT, Spradling AC. Male and female *Drosophila* germline stem cells: two versions of immortality. *Science.* 2007; 316(5823):402–404. [PubMed: 17446390]
- Gao S, Giansanti MG, Buttrick GJ, Ramasubramanian S, Auton A, Gatti M, Wakefield JG. Australin: a chromosomal passenger protein required specifically for *Drosophila melanogaster* male meiosis. *J Cell Biol.* 2008; 180:521–535. [PubMed: 18268101]

- Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R, Nigg EA, Gerloff DL, Earnshaw WC. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J Cell Biol.* 2004; 166:179–191. [PubMed: 15249581]
- 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; 119:2225–2235. [PubMed: 16684816]
- Giansanti MG, Bonaccorsi S, Williams B, Williams EV, Santolamazza C, Goldberg ML, Gatti M. Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. *Genes and Dev.* 1998; 12:396–410. [PubMed: 9450933]
- Giansanti MG, Bonaccorsi S, Gatti M. The role of anillin in meiotic cytokinesis of *Drosophila* males. *J Cell Sci.* 1999; 112:2323–2334. [PubMed: 10381388]
- 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; 26:609–617. [PubMed: 11942616]
- Giansanti MG, Farkas RM, Bonaccorsi S, Lindsley DL, Wakimoto BT, Fuller MT, Gatti M. Genetic Dissection of Meiotic Cytokinesis in *Drosophila*. *Mol Biol Cell.* 2004; 15:2509–2522. [PubMed: 15004238]
- Giansanti MG, Bonaccorsi S, Kurek R, Farkas RM, Dimitri P, Fuller MT, Gatti M. The class I P1TP giotto is required for *Drosophila* cytokinesis. *Curr Biol.* 2006; 16:195–201. [PubMed: 16431372]
- 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; 18:5034–5047. [PubMed: 17914057]
- 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; 18:303–309. [PubMed: 18291647]
- Giet R, Glover DM. *Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J Cell Biol.* 2001; 152:669–682. [PubMed: 11266459]
- Glötzer M. The molecular requirements for cytokinesis. *Science.* 2005; 307:1735–1739. [PubMed: 15774750]
- Glötzer M. The 3Ms of central spindle assembly: microtubules, motors and MAPs. *Nat Rev Mol Cell Biol.* 2009; 10(1):9–20. [PubMed: 19197328]
- 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; 21:1482–1493. [PubMed: 20237160]
- Goode BL, Ect MJ. Mechanisms and function of formins in the control of actin assembly. *Annu Rev Biochem.* 2007; 76:593–627. [PubMed: 17373907]
- Gregory SL, Ebrahimi S, Milverton J, Jones WM, Bejsovec A, Saint R. Cell division requires a direct link between microtubule-bound RacGAP and Anillin in the contractile ring. *Curr Biol.* 2008; 18(1):25–29. [PubMed: 18158242]
- 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; 131:1243–1259. [PubMed: 8522587]
- Guse A, Mishima M, Glötzer M. Phosphorylation of ZEN-4/MKLP1 by aurora B regulates completion of cytokinesis. *Curr Biol.* 2005; 15:778–786. [PubMed: 15854913]
- Herrmann S, Amorim I, Sunkel CE. The POLO kinase is required at multiple stages during spermatogenesis in *Drosophila melanogaster*. *Chromosoma.* 1998; 107:440–451. [PubMed: 9914376]
- 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; 109:2779–2788. [PubMed: 9013326]
- Hirose H, Arasaki K, Dohmae N, Takio K, Hatsuzawa K, Nagahama M, Tani K, Yamamoto A, Tohyama M, Tagaya M. Implication of ZW10 in membrane trafficking between the endoplasmic reticulum and Golgi. *EMBO J.* 2004; 23:1267–1278. [PubMed: 15029241]

- Honda R, Körner R, Nigg EA. Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. *Mol Biol Cell*. 2003; 14:3325–3341. [PubMed: 12925766]
- 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. [PubMed: 15240569]
- Jordan P, Karess R. Myosin light chain-activating phosphorylation sites are required for oogenesis in *Drosophila*. *J Cell Biol*. 1997; 139:1805–1819. [PubMed: 9412474]
- Kamijo K, Ohara N, Abe M, Uchimura T, Hosoya H, Lee JS, Miki T. Dissecting the role of Rho-mediated signaling in contractile ring formation. *Mol Biol Cell*. 2006; 17:43–55. [PubMed: 16236794]
- Karess R. Rod-Zw10-Zwilch: a key player in the spindle checkpoint. *Trends Cell Biol*. 2005; 15:386–932. [PubMed: 15922598]
- Kee YS, Ren Y, Dorfman D, Iijima M, Firtel R, Iglesias PA, Robinson DN. A mechanosensory system governs myosin II accumulation in dividing cells. *Mol Biol Cell*. 2012; 23(8):1510–1523. [PubMed: 22379107]
- Kinoshita M, Field CM, Coughlin ML, Straight AF, Mitchison TJ. Self- and actin-templated assembly of Mammalian septins. *Dev Cell*. 2002; 3:791–802. [PubMed: 12479805]
- Kitazawa D, Yamaguchi M, Mori H, Inoue YH. COPI-mediated membrane trafficking is required for cytokinesis in *Drosophila* male meiotic divisions. *J Cell Sci*. 2012 May 2. jcs.103317; Advance Online Publication.
- Kondylis V, Rabouille C. The Golgi apparatus: lessons from *Drosophila*. *FEBS Lett*. 2009; 583:3827–3838. [PubMed: 19800333]
- Kramerova IA, Kramerov AA. Mucinoprotein is a universal constituent of stable intercellular bridges in *Drosophila melanogaster* germ line and somatic cells. *Dev Dyn*. 1999; 216:349–360. [PubMed: 10633855]
- Lacroix B, Maddox AS. Cytokinesis, ploidy and aneuploidy. *The Journal of Pathology*. 2012; 226:338–351. [PubMed: 21984283]
- Lens SM, Rodriguez JA, Vader G, Span SW, Giaccone G, Medema RH. Uncoupling the central spindle-associated function of the chromosomal passenger complex from its role at centromeres. *Mol Biol Cell*. 2006; 17:1897–1909. [PubMed: 16436504]
- Lewellyn L, Dumont J, Desai A, Oegema K. Analyzing the effects of delaying aster separation on furrow formation during cytokinesis in the *Caenorhabditis elegans* embryo. *Mol Biol Cell*. 2010; 21(1):50–62. [PubMed: 19889842]
- Lewellyn L, Carvalho A, Desai A, Maddox AS, Oegema K. The chromosomal passenger complex and centralspindlin independently contribute to contractile ring assembly. *J. Cell Biol*. 2011; 193:155–169. [PubMed: 21464231]
- Lind GE, Raiborg C, Danielsen SA, Rognum TO, Thiis-Evensen E, Hoff G, Nesbakken A, Stenmark H, Lothe RA. SPG20, a novel biomarker for early detection of colorectal cancer, encodes a regulator of cytokinesis. *Oncogene*. 2011; 30:3967–3978. [PubMed: 21499309]
- Lindsley, DL.; Tokuyasu, KT. Spermatogenesis. In: Ashburner, M.; Wright, TRF., editors. *Genetics and Biology of Drosophila*. New York: Academic Press; 1980. p. 225-294.
- Liu J, Fairn GD, Ceccarelli DF, Sicheri F, Wilde A. Cleavage furrow organization requires PIP(2)-mediated recruitment of anillin. *Curr Biol*. 2012; 22(1):64–69. [PubMed: 22197245]
- Llamazares S, Moreira A, Tavares A, Girdham C, Spruce BA, Gonzalez C, Karess RE, Glover DM, Sunkel CE. polo encodes a protein kinase homolog required for mitosis in *Drosophila*. *Genes and Dev*. 1991; 5:2153–2165. [PubMed: 1660828]
- Ma X, Kovács M, Conti MA, Wang A, Zhang Y, Sellers JR, Adelstein RS. Nonmuscle myosin II exerts tension but does not translocate actin in vertebrate cytokinesis. *Proc Natl Acad Sci U S A*. 2012; 109(12):4509–4514. [PubMed: 22393000]
- Matsumara F, Yamakita Y, Yamashiro S. Myosin light chain kinases and phosphatase in mitosis and cytokinesis. *Arch Biochem Biophys*. 2011; 510:76–82.
- McKay HF, Burgess DR. 'Life is a highway': membrane trafficking during cytokinesis. *Traffic*. 2011; 12:247–251. [PubMed: 21054718]

- Miller AL. The contractile ring. *Curr Biol.* 2011; 21(24):R976–R978. [PubMed: 22192825]
- 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; 109:2963–2975. [PubMed: 2512303]
- Montebault E, Zhang W, Przewloka MR, Archambault V, Sevin EW, Laue ED, Glover DM, D'Avino PP. Nessun Dorma, a novel centralspindlin partner, is required for cytokinesis in *Drosophila* spermatocytes. *J Cell Biol.* 2010; 191:1351–1365. [PubMed: 21187330]
- Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol.* 2007; 8:379–393. [PubMed: 17426725]
- Neef R, Klein UR, Kopajtich R, Barr FA. Cooperation between mitotic kinesins controls the late stages of cytokinesis. *Curr Biol.* 2006; 16:301–307. [PubMed: 16461284]
- Neto H, Collins LL, Gould GW. Vesicle trafficking and membrane remodelling in cytokinesis. *Biochem J.* 2011; 437:13–24. [PubMed: 21668412]
- Nishimura Y, Yonemura S. Centralspindlin regulates ECT2 and RhoA accumulation at the equatorial cortex during cytokinesis. *J Cell Sci.* 2006; 119:104–114. [PubMed: 16352658]
- Normand G, King RW. Understanding cytokinesis failure. *Adv Exp Med Biol.* 2010; 676:27–55. [PubMed: 20687468]
- Ono S. Mechanism of depolymerization and severing of actin filaments and its significance in cytoskeletal dynamics. *Int Rev Cytol.* 2007; 258:1–82. [PubMed: 17338919]
- Piekny A, Werner M, Glotzer M. Cytokinesis: welcome to the Rho zone. *Trends Cell Biol.* 2005; 15:651–658. [PubMed: 16243528]
- Polevoy G, Wei HC, Wong R, Szentpetery Z, Kim YJ, Goldbach P, Steinbach SK, Balla T, Brill JA. Dual roles for the *Drosophila* PI 4-kinase four wheel drive in localizing Rab11 during cytokinesis. *J. Cell Biol.* 2009; 187:847–858. [PubMed: 19995935]
- Pollard TD. Mechanics of cytokinesis in eukaryotes. *Curr Opin Cell Biol.* 2010; 22(1):50–56. [PubMed: 20031383]
- Pollard TD, Wu JQ. Understanding cytokinesis: lessons from fission yeast. *Nat Rev Mol Cell Biol.* 2010; 11:149–155. [PubMed: 20094054]
- Prekeris R, Gould GW. Breaking up is hard to do - membrane traffic in cytokinesis. *J Cell Sci.* 2008; 121:1569–1576. [PubMed: 18469013]
- Rappaport R. Experiments concerning the cleavage stimulus in sand dollar eggs. *J Exp Zool.* 1961; 148:81–89. [PubMed: 14490383]
- Rebollo E, Gonzalez C. Visualizing the spindle checkpoint in *Drosophila* spermatocytes. *EMBO Rep.* 2000; 1:65–70. [PubMed: 11256627]
- Reichl EM, Ren Y, Morphey MK, Delannoy M, Effler JC, Girard KD, Divi S, Iglesias PA, Kuo SC, Robinson DN. Interactions between myosin and actin crosslinkers control cytokinesis contractility dynamics and mechanics. *Curr Biol.* 2008; 18(7):471–480. [PubMed: 18372178]
- Resnick TD, Satinover DL, MacIsaac F, Stukenberg PT, Earnshaw WC, Orr-Weaver TL, Carmena M. INCENP and Aurora B promote meiotic sister chromatid cohesion through localization of the Shugoshin MEI-S332 in *Drosophila*. *Dev Cell.* 2006; 11:57–68. [PubMed: 16824953]
- Riparbelli MG, Callaini G, Glover DM, Avides Mdo C. A requirement for the Abnormal Spindle protein to organise microtubules of the central spindle for cytokinesis in *Drosophila*. *J Cell Sci.* 2002; 115:913–922. [PubMed: 11870210]
- 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; 122:4526–4534. [PubMed: 19934220]
- Robinson DN, Cooley L. Stable intercellular bridges in development: the cytoskeleton lining the tunnel. *Trends Cell Biol.* 1996; 6:474–679. [PubMed: 15157506]
- Roversi G, Pfundt R, Moroni RF, Magnani I, van Reijmersdal S, Pollo B, Straatman H, Larizza L, Schoenmakers EF. Identification of novel genomic markers related to progression to glioblastoma through genomic profiling of 25 primary glioma cell lines. *Oncogene.* 2006; 25:1571–1583. [PubMed: 16247447]

- Ruchaud S, Carmena M, Earnshaw WC. Chromosomal passengers: conducting cell division. *Nat Rev Mol Cell Biol.* 2007a; 8:798–812. [PubMed: 17848966]
- Ruchaud S, Carmena M, Earnshaw WC. The chromosomal passenger complex: one for all and all for one. *Cell.* 2007b; 131:230–231. [PubMed: 17956723]
- Santamaria A, Neef R, Eberspächer U, Eis K, Husemann M, Mumberg D, Prectl S, Schulze V, Siemeister G, Wortmann L, Barr FA, Nigg EA. Use of the novel Plk1 inhibitor ZK-thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. *Mol Biol Cell.* 2007; 18:4024–4036. [PubMed: 17671160]
- Satterwhite LL, Pollard TD. Cytokinesis. *Curr Opin Cell Biol.* 1992; 4:43–52. [PubMed: 1313686]
- Saunders RD, Avides MC, Howard T, Gonzalez C, Glover DM. The *Drosophila* gene *abnormal spindle* encodes a novel microtubule-associated protein that associates with the polar regions of the mitotic spindle. *J Cell Biol.* 1997; 137(4):881–890. [PubMed: 9151690]
- Scaerou F, Starr DA, Piano F, Papoulas O, Karess RE, Goldberg ML. The ZW10 and Rough Deal checkpoint proteins function together in a large, evolutionarily conserved complex targeted to the kinetochore. *J Cell Sci.* 2001; 114:3103–3114. [PubMed: 11590237]
- Schmitt HD. Dsl1p/Zw10: common mechanisms behind tethering vesicles and microtubules. *Trends Cell Biol.* 2010; 20:257–268. [PubMed: 20226673]
- Schneider R, Brügger B, Amann CM, Prestwich GD, Epan RF, Zellnig G, Wieland FT, Epan RM. Identification and biophysical characterization of a very-long-chain-fatty-acid-substituted phosphatidylinositol in yeast subcellular membranes. *Biochem J.* 2004; 381(3):941–949. [PubMed: 15270698]
- Schroeder TE. The contractile ring and furrowing in dividing cells. *Ann NY Acad Sci.* 1990; 582:78–87. [PubMed: 2192604]
- 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. [PubMed: 15051485]
- Sinha S, Mondal G, Hwang EJ, Han da W, Dutta SK, Iyer S, Karumanchi SA, Kim KI, Couch FJ, Mukhopadhyay D. Von Hippel-Lindau gene product directs cytokinesis: a new tumor suppressor function. *J Cell Sci.* 2011; 124:2132–2142. [PubMed: 21652636]
- Sisson JC, Field C, Ventura R, Royou A, Sullivan W. Lava lamp, a novel peripheral golgi protein, is required for *Drosophila melanogaster* cellularization. *J Cell Biol.* 2000; 151:905–918. [PubMed: 11076973]
- 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. [PubMed: 12530961]
- Starr DA, Williams BC, Hays TS, Goldberg ML. ZW10 helps recruit dynactin and dynein to the kinetochore. *J Cell Biol.* 1998; 142:763–774. [PubMed: 9700164]
- Straight AF, Field CM, Mitchison TJ. Anillin binds nonmuscle myosin II and regulates the contractile ring. *Mol Biol Cell.* 2005; 16:193–201. [PubMed: 15496454]
- Sun Y, Shestakova A, Hunt L, Sehgal S, Lupashin V, Storrie B. Rab6 regulates both ZW10/RINT-1 and conserved oligomeric Golgi complex-dependent Golgi trafficking and homeostasis. *Mol Biol Cell.* 2007; 18:4129–4142. [PubMed: 17699596]
- Szafer-Glusman E, Giansanti MG, Nishihama R, Bolival B, Pringle J, Gatti M, Fuller MT. A role for very-long-chain fatty acids in furrow ingression during cytokinesis in *Drosophila* spermatocytes. *Curr Biol.* 2008; 18:1426–1431. [PubMed: 18804373]
- Szafer-Glusman E, Fuller MT, Giansanti MG. Role of Survivin in cytokinesis revealed by a separation-of-function allele. *Mol Biol Cell.* 2011; 22:3779–3790. [PubMed: 21865602]
- Tates, AD. Ph.D Thesis Leiden. Netherlands: Rijksuniversiteit de Leiden; 1971. Cyto differentiation during spermatogenesis in *Drosophila melanogaster*: an electron microscope study.
- Vader G, Medema RH, Lens SM. The chromosomal passenger complex: guiding Aurora-B through mitosis. *J Cell Biol.* 2006; 19:833–837. [PubMed: 16769825]
- Vagnarelli P, Earnshaw WC. Chromosomal passengers: the four-dimensional regulation of mitotic events. *Chromosoma.* 2004; 113:211–222. [PubMed: 15351889]

- van der Waal MS, Hengeveld RC, van der Horst A, Lens SM. Cell division control by the Chromosomal Passenger Complex. *Exp Cell Res.* 2012; 318(12):1407–2140. [PubMed: 22472345]
- Varma D, Dujardin DL, Stehman SA, Vallee RB. Role of the kinetochore/cell cycle checkpoint protein ZW10 in interphase cytoplasmic dynein function. *J Cell Biol.* 2006; 172:655–662. [PubMed: 16505164]
- Verni F, Somma MP, Gunsalus KC, Bonaccorsi S, Belloni G, Goldberg ML, Gatti M. Feo, the *Drosophila* homolog of PRC1, is required for central-spindle formation and cytokinesis. *Curr Biol.* 2004; 14:1569–1575. [PubMed: 15341744]
- Wainman A, Giansanti MG, Goldberg ML, Gatti M. The *Drosophila* RZZ complex: roles in membrane traffic and cytokinesis. *J Cell Sci.* 2012 Jun 8. jcs.099820; Advance Online Publication.
- Wakefield JG, Bonaccorsi S, Gatti M. The *Drosophila* Protein Asp Is Involved in Microtubule Organization during Spindle Formation and Cytokinesis. *J Cell Biol.* 2001; 153:637–648. [PubMed: 11352927]
- 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; 129:709–723. [PubMed: 7730406]
- Williams BC, Li Z, Liu S, Williams EV, Leung G, Yen TJ, Goldberg ML. Zwilch, a new component of the ZW10/ROD complex required for kinetochore functions. *Mol Biol Cell.* 2003; 14:1379–1391. [PubMed: 12686595]
- Wolfe BA, Takaki T, Petronczki M, Glotzer M. Polo-like kinase 1 directs assembly of the HsCdk-4 RhoGAP/Ect2 RhoGEF complex to initiate cleavage furrow formation. *PLoS Biol.* 2009; 7:e1000110. [PubMed: 19468300]
- Wong R, Hadjiyanni I, Wei HC, Polevoy G, McBride R, Sem KP, Brill JA. PIP2 hydrolysis and calcium release are required for cytokinesis in *Drosophila* spermatocytes. *Curr Biol.* 2005; 15:1401–1406. [PubMed: 16085493]
- Yin HL, Janmey PA. Phosphoinositide regulation of the actin cytoskeleton. *Annu Rev Physiol.* 2003; 65:761–789. [PubMed: 12471164]
- 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; 251:294–296. [PubMed: 12435359]

Figure 1. *Drosophila* spermatogenesis provides a highly suitable system for genetic and cytological analysis of cytokinesis

(A) Each primary spermatocyte undergoes two meiotic divisions. Light blue, nuclei. Grey, Cytoplasm. Each wild type spermatid at the so-called onion stage, contains a single nucleus (light blue) associated with a nebkern (black) of similar size. (B–D) Irregular spermatids at the onion-stage in males defective in cytokinesis. (B) One spermatid contains a single light nucleus associated with a dark nebkern (arrow, 1NK). The other spermatid contains two nuclei of identical size associated with a large nebkern (2NK). (C) Spermatids from a mutant failing both meiotic divisions exhibit a large nebkern (arrow, 4NK) associated

with 4 nuclei of similar size. (D) Spermatids from a mutant failing both cytokinesis and chromosome segregation have a large nebenkern (2NK) associated with two nuclei of irregular size.

(E–F) Localization of Anillin in dividing spermatocytes during late anaphase (E) and telophase (F). Tubulin is in green, Anillin is in red and DNA is in blue. Bar; 10 μ m.

Figure 2. Schematic representation of dividing spermatocytes (A, C) and neuroblasts (B, D) during late anaphase (A, B) and mid-telophase (C, D)

(A, C) In spermatocytes, a set of central spindle microtubules dubbed “peripheral astral microtubules” contacts the equatorial cortex (arrows) at the cell equator (green arrowheads) and leads to local activation of the small GTPase Rho (a process that depends on CPC and Polo). A distinct population of central spindle microtubule bundles merges with peripheral astral microtubules and is required for furrow ingression during cytokinesis. Note that Rho, Polo and contractile ring proteins accumulate at a tightly focused ring (red) since their first appearance at the cell cortex.

(B, C) In neuroblasts, assembly of the contractile ring occurs in two steps. During late anaphase, Rho, Polo and contractile ring proteins concentrate in a broad equatorial cortical band (red), even in the absence of CPC and Pav proteins. During telophase (D), the initial wide band typically narrows gradually into a thin ring that constricts during cytokinesis.

Blue, nuclei; light blue, centrosomes.