Role of Survivin in cytokinesis revealed by a separation-of-function allele

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ABSTRACT The chromosomal passenger complex (CPC), containing Aurora B kinase, Inner Centromere Protein, Survivin, and Borealin, regulates chromosome condensation and interaction between kinetochores and microtubules at metaphase, then relocalizes to midzone microtubules at anaphase and regulates central spindle organization and cytokinesis. However, the precise role(s) played by the CPC in anaphase have been obscured by its prior functions in metaphase. Here we identify a missense allele of Drosophila Survivin that allows CPC localization and function during metaphase but not cytokinesis. Analysis of mutant cells showed that Survivin is essential to target the CPC and the mitotic kinesin-like protein 1 orthologue Pavarotti (Pav) to the central spindle and equatorial cell cortex during anaphase in both larval neuroblasts and spermatocytes. Survivin also enabled localization of Polo kinase and Rho at the equatorial cortex in spermatocytes, critical for contractile ring assembly. In neuroblasts, in contrast, Survivin function was not required for localization of Rho, Polo, or Myosin II to a broad equatorial cortical band but was required for Myosin II to transition to a compact, fully constricted ring. Analysis of this “separation-of-function” allele demonstrates the direct role of Survivin and the CPC in cytokinesis and highlights striking differences in regulation of cytokinesis in different cell systems.

INTRODUCTION

The chromosomal passenger complex (CPC), composed of the Ser-Thr kinase Aurora B and three partner proteins, plays several key roles in mitosis and meiosis, including regulation of attachment of kinetochores to microtubules, the spindle checkpoint that delays anaphase onset until all chromosomes are under tension on the spindle, regulation of sister chromatid cohesion, and cytokinesis (Ruchaud et al., 2007a). To accomplish these different tasks, the Aurora B kinase associates with the microtubule-binding protein Inner Centromere Protein (INCENP; Cooke et al., 1987; Mackay et al., 1993), Borealin/DASRA/CSC-1 (Romano et al., 2003; Gassmann et al., 2004; Sampath et al., 2004; Hanson et al., 2005; Klein et al., 2006), and the small, multifunctional BIR-motif protein Survivin to form the CPC (Vagnarelli and Earnshaw, 2004).

Dissecting the role of individual CPC components has been hampered by the extraordinary interdependence of the four subunits (Ruchaud et al., 2007a, 2007b); depletion of any single CPC protein by RNA interference knockdown in human cells affected the structural unit, localization, and function of the CPC (Adams et al., 2001; Carvalho et al., 2003; Honda et al., 2003; Lens et al., 2003; Gassmann et al., 2004). The structural basis of this interdependence is evident in the crystal structure of the Survivin-Borealin INCENP core of the CPC complex, in which Borealin and INCENP associate with the C-terminal helical domain of Survivin to form a tight three-helix bundle (Jeyaprakash et al., 2007).

Strict localization of Aurora B by the CPC ensures that this kinase, which has multiple substrates, phosphorylates the correct targets at the proper points in cell cycle progression. Concentrated on chromosomes from G2, then at inner centromeres from prometaphase until the metaphase-to-anaphase transition, the CPC is required to regulate chromosome condensation, spindle formation and dynamics, kinetochore maturation, kinetochore-microtubule interaction, correct chromosome alignment, and control of the spindle
checkpoint (reviewed in Ruchaud et al., 2007a; Carmena, 2008). At anaphase onset the CPC then translocates to the central spindle (CS) midzone and equatorial cortex (Adams et al., 2001; Vagnarelli and Earnshaw, 2004; Vagnerelli et al., 2006) and is involved in CS formation (Kaitna et al. 2000; Severson et al. 2000; Giet and Glover, 2001; Carmena, 2008). At the CS Aurora B phosphorylates the centralspin- dlin components, Pav/mitotic kinesin-like protein 1 (MKLP1)/Zen-4 (Guse et al., 2005; Neef et al., 2006; Douglas et al., 2010) and the RacGAP50/MgcRacGAP/Cyc-4 (Minoshima et al., 2003). However, the mechanisms that target the CPC to the spindle midzone and equatorial cortex after onset of anaphase and the mechanisms by which the CPC regulates central spindle formation and cytokinesis are not understood. In addition, the requirements for CPC function for critical events in metaphase and at the metaphase–anaphase transition have complicated analysis of how the CPC is localized and functions at later stages for cytokinesis.

Here we characterize the role of Drosophila Survivin (dSurvivin), previously termed deterin and analyzed in its antiapoptotic activity (Jones et al., 2000), as a regulator of cell division, identifying a missense mutation (scapo1) in the dSurvivin BIR domain that allows recruitment and function of the CPC in metaphase but disrupts CPC localization and function in anaphase and telophase. Our findings reveal that Survivin plays a role in targeting the CPC and centralspin- dlin to the central spindle and the equatorial cell cortex during anaphase. In spermatocytes, Survivin function is also required to localize Polo and localize the small GTPase RhoA to set up the contractile ring machinery at the onset of cytokinesis (Giansanti et al., 2004; this study). In larval neuroblasts undergoing mitotic division, however, the scapo1 mutant did not block initial accumulation of Rho to a band at the equatorial cortex, although it did cause failure of cytokinesis. The different requirements for Survivin function for equatorial accumulation of Rho in spermatocytes versus neuroblasts may reflect a fundamental difference in the series of steps that lead to formation of the contractile ring in these two cell types.

RESULTS

The Drosophila homologue of mammalian survivin (dSurvivin) is localized in a pattern typical of chromosomal passenger proteins during cell division

Immunofluorescence analysis of fixed preparations of both larval neuroblasts undergoing mitosis (Figure 1A) and spermatocytes undergoing meiosis (Figure 1B) stained with an anti-dSurvivin antibody showed that Survivin accumulated at kinetochores during metaphase, concentrated at the interdigitating central spindle microtubules upon anaphase entry, and was enriched in the central spindle midzone by late anaphase and telophase (Figure 1, A and B). Likewise, live imaging of primary spermatocytes expressing green fluorescent protein (GFP)-tagged Survivin encoded on a genomic transgene revealed dynamic localization of GFP-Survivin to kinetochores at prometaphase, metaphase, and early anaphase and to the equatorial cortex and the central spindle midzone during late anaphase and telophase (Figure 1C and Supplemental Movies S1 and S2). Imaging of spermatocytes expressing GFP-tagged Survivin also revealed GFP-Survivin weakly associated with separating chromosomes during anaphase and telophase during the male meiotic divisions (Figure 1C and Supplemental Figure S1), as previously shown for INCENP (Resnick et al., 2006).

Separation of function of Drosophila Survivin in metaphase versus cytokinesis

A missense mutation in dSurvivin, identified in a large screen for ethyl methyl sulfonate–induced male sterile mutations that cause cytokinesis (Giansanti et al., 2004), differentially affected Survivin localization at metaphase versus ana/telophase, allowing investigation of Survivin function at the onset of cytokinesis. Genetic mapping and molecular cloning (Materials and Methods) revealed the scapo1 (scpo) mutant scpo2775 as a missense allele of the Drosophila orthologue of Survivin. Both a transgene carrying the wild-type survivin locus on a 2.5-kb genomic fragment and a transgene encoding GFP-survivin (used in Figure 1C and Supplemental Figure S1) fully rescued the scpo male sterile phenotype(s).

The temperature-sensitive, hypomorphic scpo mutation caused severe disruption of cytokinesis but only mild defects in chromosome
segregation during male meiosis. Testes from *scpo* mutant males grown at 25°C displayed postmeiotic early (onion stage) spermatids with multiple equal-sized nuclei associated with an enlarged mitochondrial derivative (arrow) associated with two or four nuclei of similar size. (D) Mitotic parameters in larval brain preparations from *Drosophila* Oregon-R and *scpo*/*Df(3R)Exel5780* mutants stained for tubulin and DNA. OF, optic field: the circular area defined by a phase-contrast Neofluar 100X Zeiss objective, using 10× oculars and the Optovar set at 1.25. MF, Number of mitotic cells scored. MI, mitotic index: average number of mitotic figures per optic field. (E) Alignment of *Drosophila* Deterin/Survivin with human Survivin. Arrowheads indicate amino acids mutated in the *scapolo* male sterile allele: open arrowhead, permissive substitution of glutamine for glutamic acid in position 83, also present in the background chromosome; solid arrowhead, substitution of Ser for wild-type proline at position 86 that underlies the *scpo* phenotype. Asterisks, acidic patch near the Pro substitution; #, residues involved in Zn coordination. (F) Ribbon representation of dSurvivin (amino acids 20–106 of the 152 amino acid–long protein) modeled with Swiss Model and visualized with PyMOL. Blue sticks, Pro-86; magenta ribbon, acidic patch; yellow ribbon, amino acids in the Zn coordination loop: Cys-70 and -73 and His-90 and -93 (Jeyaprakash et al., 2007). (G) Immunofluorescence images of *scpo*/*Df(3R)Exel5780* larval neuroblasts stained for Survivin (red), tubulin (green), and DNA (blue). Scale bar, 10 μm.

**FIGURE 2:** The *scpo* mutation separates action of survivin in cytokinesis from earlier functions. (A–C) Onion-stage spermatids from (A) *scpo* mutant males raised at 18°C showing normal spermatids, each containing a mitochondrial derivative (arrow) associated with a nucleus of similar size (arrowhead); (B) *scpo* males raised at 25°C; and (C) *scpo*/*Df(3L)Exel5780* males raised at 25°C. In B and C most spermatids contained a large mitochondrial derivative (arrow) associated with two or four nuclei of similar size. (D) Mitotic parameters in larval brain preparations from *Drosophila* Oregon-R and *scpo*/*Df(3R)Exel5780* mutants stained for tubulin and DNA. OF, optic field: the circular area defined by a phase-contrast Neofluar 100X Zeiss objective, using 10× oculars and the Optovar set at 1.25. MF, Number of mitotic cells scored. MI, mitotic index: average number of mitotic figures per optic field. (E) Alignment of *Drosophila* Deterin/Survivin with human Survivin. Arrowheads indicate amino acids mutated in the *scapolo* male sterile allele: open arrowhead, permissive substitution of glutamine for glutamic acid in position 83, also present in the background chromosome; solid arrowhead, substitution of Ser for wild-type proline at position 86 that underlies the *scpo* phenotype. Asterisks, acidic patch near the Pro substitution; #, residues involved in Zn coordination. (F) Ribbon representation of dSurvivin (amino acids 20–106 of the 152 amino acid–long protein) modeled with Swiss Model and visualized with PyMOL. Blue sticks, Pro-86; magenta ribbon, acidic patch; yellow ribbon, amino acids in the Zn coordination loop: Cys-70 and -73 and His-90 and -93 (Jeyaprakash et al., 2007). (G) Immunofluorescence images of *scpo*/*Df(3R)Exel5780* larval neuroblasts stained for Survivin (red), tubulin (green), and DNA (blue). Scale bar, 10 μm.

segregation during male meiosis. Testes from *scpo* homozygous males grown at 25°C displayed postmeiotic early (onion stage) spermatids with multiple equal-sized nuclei associated with an enlarged mitochondrial derivative (Figure 2B), indicating failure of cytokinesis during both meiotic divisions. Testes from *scpo* homozygous males grown at 29°C also had multinucleate spermatids, with 5% of the nuclei varying in size, suggesting defects in both chromosome segregation and cytokinesis in a small number of meiotic divisions. In contrast, onion-stage spermatids from *scpo* homozygous males grown at 18°C appeared normal, indicating no obvious cytokinesis defects during meiosis (Figure 2A). Hemizygous individuals carrying the *scpo* allele in combination with *Df(3R)* *Exel5780*, a genomic deficiency that uncovers the survivin locus (*Drosophila* Genetic Resource Center stock number 150046), failed to hatch and survived only to larval stages. Spermatids from *scpo*/*Df(3R)Exel5780* larval males grown at 25°C were also multinucleate with an enlarged mitochondria derivative (Figure 2C). DNA staining in spermatocytes undergoing anaphase of the first meiotic division confirmed mild defects in chromosome segregation during meiosis: chromosomes segregated properly in 83% of *scpo*/*Df(3R)Exel5780* mutant cells scored (N = 103; Supplementary Figure S2).

Analysis of mitotic chromosomes in *scpo*/*Df(3R)Exel5780* larval brain squashes (see Materials and Methods) indicated defects in cytokinesis also occurred during CNS neuroblast divisions: 23% of metaphase figures were polyploid in the mutant (n = 238), compared with 0.3% in wild type (n = 298). However, the *scpo* mutation did not appear to delay the metaphase-to-anaphase transition, as the frequency of prometaphases/metaphases relative to all the mitotic figures was not significantly increased in *scpo*/*Df(3R)Exel5780* compared with wild type in larval brain preparations stained for tubulin and DNA (Figure 2D). Rather, the mitotic index was slightly lower than in controls (Figure 2D). Analysis of anaphase figures from *scpo*/*Df(3R) Exel5780* larval neuroblasts failed to detect lagging chromosomes or aberrant chromosome separation to the poles (Figure 2G), consistent with defects mainly in cytokinesis rather than in chromosome segregation.
Substitution of serine for the wild-type Pro-86 affects localization of Survivin protein during anaphase

Sequencing of the scpo2775 allele revealed two point mutations in the dSurvivin open reading frame compared with the original sequence for Drosophila melanogaster deterrent (FlyBase), causing substitution of glutamine for a conserved glutamic acid at position 83 and serine for a conserved proline at position 86 (Figure 2E). However, the glutamic acid–to–glutamine substitution is not likely to be the cause of the mutant phenotype, as the glutamic acid 83–to–glutamine substitution was also present in the original fly line prior to mutagenesis (Zuker line; Koundakjian et al., 2004), and flies homozygous for the Zuker screen–background chromosome did not show evidence of cytokinesis defects. In addition, transgenic flies carrying the survivin genomic region with the glutamic 83–to–glutamine mutation fully rescued the scpo phenotype. The replacement of glutamic acid 83 by glutamine disrupts only one of several amino acids in a conserved patch of acidic amino acids (residues E83, D84, and D85 in Drosohila Survivin) in a solvent-exposed loop of the BIR domain (Figure 2E, asterisks; Figure 2F, magenta ribbon; Jeyaprakash et al., 2007) important for Survivin function at centromeres (Cao et al., 2006; Lens et al., 2006; Yue et al., 2008; Kelly et al., 2010; Wang et al., 2010), and so is not likely to strongly affect function of Survivin in binding to Aurora B (Cao et al., 2006). In contrast, a survivin genomic transgene with the Pro-86–to-serine substitution failed to rescue the scapolo phenotype, suggesting that the defects in cytokinesis in scpo mutants are due to this amino acid change.

The conserved Pro-86 (Figure 2E, solid arrowhead; Figure 2F, blue residue) is positioned right before a short alpha helix that helps coordinate a structural Zn atom (Jeyaprakash et al., 2007; Figure 2E, yellow hatches, and Figure 2F, yellow residues, indicate residues involved in Zn coordination), raising the possibility that the alpha helix might help stabilize the Zn coordination loop. It is possible that, with increasing temperature, a conformational change imparted due to the scpo mutation (perhaps a slight displacement of the helix) may affect coordination of the Zn atom and reduce structure stability. Alternatively, the mutant may affect Survivin function because it creates a consensus site for phosphorylation not present in the wild-type protein. Of interest, the mutant sequence generated by substitution of serine for the wild-type proline at position 86 of dSurvivin (Figure 2E, DD-WKE) conforms exactly to the consensus motif for phosphorylation by Polo kinase, D/E-X-S/T-Φ-X-D/E (with Φ indicating hydrophobic residue (Nakajima et al., 2003), raising the possibility that phosphorylation by Polo of the mutant Ser-86 might cause abnormal conformation or spatial hindrance that affects dSurvivin activity.

Immunostaining of larval neuroblasts from scpo2775/Df(3R)Exel5780 animals with antibodies against Survivin showed that the mutant protein localized correctly to kinetochores in dividing mutant cells during metaphase (Figure 2G), similar to wild type (Figure 1A). However, Survivin failed to localize to the equatorial midzone in scpo2775/Df(3R)Exel5780 larval neuroblasts (Figure 2G), suggesting that substitution of serine for the wild-type Pro-86 might impede localization of Survivin during anaphase without compromising its localization at earlier steps of division.

Substitution of serine for Pro-86 in Survivin caused defects in localization of Aurora B and INCENP to the equatorial midzone during anaphase

The chromosomal passenger proteins Aurora B and INCENP behaved similarly to the defective Survivin protein in scpo mutant spermatocytes and neuroblasts. Immunofluorescence analysis of spermatocytes from scpo2775/Df(3)Exel5780 males showed that INCENP and Aurora B localized correctly to kinetochores at metaphase (N = 37 for INCENP; N = 32 for Aurora B), as in wild type (N = 22 for INCENP; N = 24 for Aurora; Figure 3, A and B). However, both CPC proteins failed to accumulate to the central spindle midzone during anaphase in scpo spermatocytes (Figure 3, A and B; for INCENP, N = 46 mutant cells, N = 28 wild-type cells; for Aurora B, N = 55 mutant cells, N = 33 wild-type cells). Similarly, immunostaining of larval neuroblasts indicated that Aurora B accumulated at kinetochores in metaphase cells in both wild type (N = 100) and scpo2775/Df(3)Exel5780 (N = 147) (Figure 3C) but failed to relocalize to the spindle midzone at anaphase–telophase in scpo2775/Df(3R)Exel5780 (N = 39). In wild-type larval neuroblasts, in contrast, Aurora B consistently relocalized to the spindle midzone at anaphase–telophase (N = 24) (Figure 3C).

Consistent with the indication that the scpo2775 allele of survivin does not compromise the function of the CPC during metaphase but specifically affects CPC function during later stages, histone H3S10 phosphorylation on metaphase chromosomes, which depends on Aurora B activity, appeared similar in scpo mutant metaphase spermatocytes and in wild type (Figure 3D),

A role for Survivin in central spindle organization in dividing cells

Dynamic live-cell analysis of microtubules in wild-type versus scpo2775/Df(3R)Exel5780 spermatocytes expressing enhanced GFP (EGFP)–tagged β-tubulin revealed that the scpo mutant impairs assembly of the central spindle during anaphase and telophase. In wild type (N = 10), centrosome-nucleated microtubules extended from the poles to contact the cell cortex at the equatorial region in anaphase. On contact, the pole-to-equator microtubules bundled and became stabilized at the cortex (Figure 4A, 0–5 min, and Supplemental Movie S3). This initial bundling was rapidly followed by cell elongation and organization of cytoplasmic microtubules into interdigitated arrays at the cell midzone (Figure 4A, 5–11 min, and Supplemental Movie S3). With ingestion of the cleavage furrow the arrays of microtubules compacted into the characteristic telo-phase central spindle (Figure 4A, 11–25 min, and Supplemental Movie S3).

In scpo2775/Df(3R)Exel5780 mutant spermatocytes (N = 6), in contrast, although microtubules extended from the poles and contacted the cortex at the equator during anaphase, (Figure 4B, 0–11 min), upon initial contact the microtubules either failed to bundle and stabilize or did so only transiently (Figure 4B, cortical dots at 11 min, and Supplemental Movie S4, cortical dots at 22–24 min). In addition, internal pole-to-equator microtubules failed to organize robust interdigitating arrays at the cell midzone (Figure 4B, 11–25 min, and Supplemental Movie S4). Finally, there was no evidence of furrowing at the equator in scpo2775/Df(3R)Exel5780 mutant spermatocytes, and the cells failed to elongate (Figure 4B, 15–25 min, and Supplemental Movie S4).

The scpo mutant affected localization of the centralspinulin component Pavarotti but not the microtubule-bundling protein Feo to the anaphase central spindle

Immunofluorescence staining of spermatocytes undergoing meiotic division with antibodies against the microtubule bundling protein Fascetto/PRC1 (Feo; Verni et al., 2004) showed initial accumulation of Feo protein to microtubules at the midzone in scpo2775/Df(3R)Exel5780 anaphases (N = 16 control cells; N = 17 mutant...
In spermatocytes examined (N = 22 control cells; N = 24 mutant cells). In neuroblasts, similar to spermato- 
cytes, Feo protein was present marking microtubule plus ends at the 
cell equator during anaphase and early telophase (N = 20 control 
cells; N = 18 mutant cells; Figure 6A). In contrast to spermatocytes, 
however, some initial constriction of the cleavage furrow and some 
irregular bundling of microtubule plus ends marked by Feo occurred 
in telophase scpoz2775/Df(3R)Exel5780 neuroblasts (Figure 6A), al-
though the Feo signal did not concentrate into the normal tight ring 
by late telophase (N = 18 control cells; N = 16 mutant cells; Figure 6A). In contrast, Pav-KLP displayed only faint or no accumu-
lation to the spindle midzone of anaphases and telophases from 
scpoz2775/Df(3R)Exel5780 mutant testes stained for tubulin (green), 
phosphohistone H3 Ser-10 (red), and DNA (blue). Scale bar, 10 μm.

In contrast, immunofluorescence staining with antibodies against 
a centralspindlin component, the kinesin-like PAV-KLP (Adams et al., 
1998), revealed that anaphase spindles in scpoz2775/Df(3R)Exel5780 mutant spermatocytes (N = 
24 control cells; N = 26 mutant cells) (Figure 5A), consistent with 
the failure of contractile ring formation and constriction in these 
cells. However, Feo protein failed to concentrate to a tight equatorial midzone in 
telophases in scpoz2775/Df(3R)Exel5780 mutant spermatocytes (N = 
24 control cells; N = 26 mutant cells (Figure 5A), indicating that pole-to-equator microtubules 
were present and arranged with their plus ends overlapping at the 
cell midzone in mid anaphases in mutant spermatocytes. However, 

In contrast, immunofluorescence staining with antibodies against 
a centralspindlin component, the kinesin-like PAV-KLP (Adams et al., 
1998), revealed that anaphase spindles in scpoz2775/Df(3R)Exel5780 mutant spermatocytes were either devoid of Pav-KLP or displayed only very 
faint Pav-KLP concentration at the central spindle (N = 18 control 
cells; N = 18 mutant cells; Figure 5B). In telophase cells, Pav-KLP 
was completely absent from cortical microtubules and central spin-

cell in all scpoz2775/Df(3R)Exel5780 spermatocytes examined (N = 22 
control cells; N = 24 mutant cells). In scpoz2775/Df(3R)Exel5780 neuroblasts, similar to spermat-
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lation to the spindle midzone of anaphases and telophases from 
scpoz2775/Df(3R)Exel5780 neuroblasts (N = 18 control cells; N = 16 
mutant cells; Figure 6B).
Defective recruitment of Polo kinase to the spindle midzone in spermatocytes but not in larval neuroblasts from scpo<sup>2775/Df(3R)Exel5780</sup>

Localization of the mitotic kinase Polo to the central spindle in scpo<sup>2775/Df(3R)Exel5780</sup> larval neuroblasts was comparable with Feo (Figures 6A and 7B), consistent with the finding that recruitment of Polo to the central spindle in Drosophila S2 cells requires function of the microtubule plus end-bundling protein Feo but is independent of Pav/MKLP1 localization/activity (D’Avino et al., 2007). In the scpo mutant neuroblasts, Polo protein was present at the cell equator at late anaphase as in wild type (N = 18 control cells, N = 18 mutant cells), although Polo failed to concentrate into the normal tight band during telophase (N = 16 control cells, N = 16 mutant cells; Figure 7B). However, although localization of Feo to anaphase I scpo spermatocytes appeared normal (Figure 5A), no Polo localization was detected in any of the scpo<sup>2775/Df(3R)Exel5780</sup> mutant spermatocytes in late anaphase (N = 29 control cells, N = 25 mutant cells; Figure 7A) and telophase scored (N = 25 control cells, N = 22 mutant cells; Figure 7A). As discussed later, this may reflect fundamental differences between spermatocytes and neuroblasts in the sequence of steps leading to contractile ring assembly and organization prior to ring constriction.

Survivin activity is required for localization and stabilization of Rho 1 and myosin to the equatorial cortex in spermatocytes but not in neuroblasts

Action of Survivin may play a key regulatory role enabling initial localization and perhaps maintaining the dynamic activation state of the F-actin assembly regulator Rho 1 at the equatorial cortex of dividing spermatocytes. Immunofluorescence staining of wild-type spermatocytes revealed that Rho 1 became concentrated in a narrow ring at the cell equator during late anaphase (N = 18; Figure 8A), and then the ring constricted as the cells proceeded through late telophase, forming a tight accumulation at the midbody (N = 28; Figure 8A). In scpo<sup>2775/Df(3R)Exel5780</sup> spermatocytes, in contrast, accumulation of Rho 1 was never detected at the cell midzone in either late anaphase (N = 16) or telophase (N = 24; Figure 8A), correlating with the failure of scpo mutant spermatocytes to initiate assembly of the F-actin ring (Giansanti et al., 2004).

Loss of function of Survivin also resulted in failure to form the cortical ring of myosin II complex proteins in spermatocytes undergoing anaphase, visualized by the Drosophila myosin regulatory light chain Spaghetti squash tagged with GFP (Sqh-GFP). In wild-type spermatocytes undergoing anaphase, Sqh-GFP first appeared as a thin cortical band at the cell equator; then the band constricted to a tight ring as the cleavage furrow ingressed (Figure 8B). In contrast, no ring of Sqh-GFP was detected in anaphase or telophase scpo<sup>2775/Df(3R)Exel5780</sup> spermatocytes (Figure 8B).

scpo mutant neuroblasts undergoing mitosis differed from scpo mutant spermatocytes undergoing meiosis in the concentration of Rho 1 and myosin at the equatorial cortex, perhaps reflecting differences in the mechanisms that first assemble these proteins at the cortex during anaphase in the two cell types. Immunostaining of scpo<sup>2775/Df(3R)Exel5780</sup> late anaphase–early telophase neuroblasts for either Rho1 (N = 18 control cells; N = 12 mutant cells; Figure 9A) or myosin II (N = 15 control cells; N = 10 mutant cells; Figure 9B) revealed normal accumulation of the contractile ring components to a broad band at the cortex, in spite of the absence of strong central spindle microtubule arrays (Figure 9B). However, the initial bands of Rho 1 and myosin II failed to resolve into the typical tight rings in scpo<sup>2775/Df(3R)Exel5780</sup> late telophase neuroblasts, and, in spite of considerable constriction, completion of cytokinesis was impaired (Figure 9B).

DISCUSSION

A missense mutation leading to substitution of serine for the wild-type Pro-86 of Drosophila Survivin uncouples the function of Survivin in metaphase from function during anaphase and telophase, indicating a direct requirement for Survivin and the chromosomal passenger complex in orchestrating the profound reorganization of the cortical cytoskeleton at the cell equator at the onset of cytokinesis. This "separation-of-function" allele allowed analysis of Survivin and CPC function during cytokinesis, which is normally obscured by the better-known roles of the CPC at centromeres during metaphase, when it facilitates alignment of chromosomes to the spindle equator and mediates the spindle checkpoint (Carmena, 2008). Our finding that a point mutation in the BIR domain disrupts activity of Survivin during cytokinesis challenges the model that the C-terminal domain of Survivin is sufficient for cytokinesis function (Lens et al., 2006) and indicates that residues in the BIR domain are important for localization and activity of Survivin at the central spindle.

Survivin associates with kinetochores and the central spindle with different dynamics, being highly mobile in prometaphase and metaphase and strongly immobile at the anaphase central spindle (Beardmore et al., 2004; Delacour-Larose et al., 2004). This change in dynamics may underlie the largely normal localization and function of scpo mutant Survivin at metaphase but the fully penetrant effect on assembly of the F-actin contractile ring and cytokinesis observed in scpo mutants (Giansanti et al., 2004; this study).
Cytokinesis depends on the assembly of an equatorial actomyosin ring regulated by local activation of the small GTPase RhoA at the cortex, in turn catalyzed by the RhoGEF Ect2/Pebble (Yuce et al., 2005; Zhao and Fang, 2005; Kamijo et al., 2006; Nishimura and Yonemura, 2006). It has been proposed that association of RhoGEF/Pebble with centralspindlin promotes local RhoA activation at the cortex (Somers and Saint, 2003; Piekny et al., 2005; Chalamalasetty et al., 2006; Nishimura and Yonemura, 2006). In addition, the kinase polo (PLK1) has been implicated in RhoGEF localization and Rho activation, at least in part by phosphorylation of the centralspindlin component MgcRacGAP (Brennan et al., 2007; Santamaria et al., 2007; Burkard et al., 2009; Wolfe et al., 2009). Our observations that the Drosophila RhoA homologue, Rho1, failed to accumulate at the equatorial cortex in scpo mutant spermatocytes implicate Survivin and the CPC in the mechanism(s) that localize and activate RhoA at the equatorial cortex in these cells. This requirement may in part act through effects on Polo kinase. Failure to localize Polo to the central spindle in scpo mutant spermatocytes could prevent localization of RhoGEF by the centralspindlin complex and the consequent activation of Rho at the cortex. In this model, failure to localize Polo may contribute to the failure to form an equatorial ring of localized Rho1 and, in consequence, the inability to form a localized ring of myosin regulatory light chain and F-actin in scpo mutant male germ cells undergoing meiotic division. This mechanism may also explain the failure to maintain pole-to-equatorial microtubules observed in scpo mutant spermatocytes (Supplemental Movie S4 and Figure 4). It is likely that Rho-mediated activation of the Formin Dia helps stabilize microtubule arrays at the equatorial cortex of dividing cells, as active Rho and Formin (mDia) regulated stabilization of microtubule arrays at the cortex of migrating fibroblasts (Palazzo et al., 2001; Bartolini et al., 2008). Consistent with this model, we found that microtubules reached the plasma membrane at the equator of scpo dividing spermatocytes, but the bundles were transient and failed to form stable arrays at the cortex.

A striking finding of our work is the difference in requirement for Survivin function for localization of the Polo kinase and RhoA in anaphase neuroblasts versus spermatocytes. This difference raises two possibilities: either Survivin is not part of a universal signaling mechanism that directs cytokinesis, or different semiredundant mechanisms can drive cytokinesis, similar to redundancy between astral pulling and sliding of central spindle microtubules for anaphase B, and different cell types rely more strongly on one mechanism or the other. Indeed, consistent with the latter possibility, spermatocytes and neuroblasts display different cytoskeletal architectures during cytokinesis (Giansanti et al., 2006). In neuroblasts, actomyosin initially accumulates in a broad cortical band, presumably because this is the region of the cell cortex that escapes repression of Rho associated with the plus ends of astral microtubule (Werner et al., 2007; Chen et al., 2008; Foe and von Dassow, 2008; von Dassow et al., 2009). This initial wide band gradually narrows into a tight equatorial ring as the cell progresses into telophase (Giansanti et al., 2006). Thus assembly of the contractile apparatus in neuroblasts proceeds, as proposed by Lewellyn et al. (2011) for Caenorhabditis elegans embryos, in “two genetically separable steps” in which localization of contractile machinery is initially independent of the central spindle. In support of this model, we found that Rho1 accumulated in a broad cortical band in scpo mutant neuroblasts, suggesting that the first stage can occur independent of Survivin and CPC localization to the central spindle.

Spermatocytes, in contrast, do not form an initial wide equatorial band of contractile ring components. Instead, from their first appearance in early anaphase, the actomyosin rings in spermatocytes are tightly focused at the cell equator (Giansanti et al., 2006). We speculate that this restricted initial localization of contractile ring components and the apparent lack of a preceding wide equatorial band of contractile machinery may be the result of the difference in requirement for Survivin function in neuroblasts versus spermatocytes.
with and/or localized by central spindle microtubules (Burgess and Chang, 2005; Piekny et al., 2005; this study). Rho1 activation would then occur within a narrow peak exactly at the site where pole-to-equator microtubules interact to maximize RhoGEF deposition/concentration at the cortex (Somers and Saint, 2003; Piekny et al., 2005; Chalamalasetty et al., 2006; Nishimura and Yonemura, 2006).

Indeed, F-actin ring assembly occurs locally and cytokinesis initiates immediately after the pole-to-equator microtubules contact the cortex in Drosophila spermatocytes (Supplemental Movie S4 and Figure 4A). We propose that, according to this model, the defects in Survivin lead to lack of CPC activity and abnormal centralspindlin, resulting in absence of Rho1 and Polo kinase from the equator of scpo mutant spermatocytes.

In neuroblasts, where a more permissive cortex allows a broad belt of Rho1 activation at the cell equator, Survivin and CPC appear band may be a consequence of a more stringent global block to Rho1 activation at the cortex in spermatocytes than in neuroblasts (Canman et al., 2003; Foe and von Dassow, 2008; von Dassow et al., 2009; Canman, 2009). We propose that this global block is eventually overridden by positive regulation of Rho1 by local concentration of RhoGEF, in turn facilitated by CPC-dependent events associated with and/or localized by central spindle microtubules (Burgess and Chang, 2005; Piekny et al., 2005; this study). Rho1 activation would then occur within a narrow peak exactly at the site where pole-to-equator microtubules interact to maximize RhoGEF deposition/concentration at the cortex (Somers and Saint, 2003; Piekny et al., 2005; Chalamalasetty et al., 2006; Nishimura and Yonemura, 2006). Indeed, F-actin ring assembly occurs locally and cytokinesis initiates immediately after the pole-to-equator microtubules contact the cortex in Drosophila spermatocytes (Supplemental Movie S4 and Figure 4A). We propose that, according to this model, the defects in Survivin lead to lack of CPC activity and abnormal centralspindlin, resulting in absence of Rho1 and Polo kinase from the equator of scpo mutant spermatocytes.

In neuroblasts, where a more permissive cortex allows a broad belt of Rho1 activation at the cell equator, Survivin and CPC appear

FIGURE 6: Loss of Survivin function disrupts localization of the centralspindlin component Pav but not accumulation of the microtubule-bundling protein Feo in scpo^{2775/Df(3R)Exel5780} neuroblasts. Localization of Fascetto (Feo) and Pavarotti (Pav) in wild-type and scpo^{2775/Df(3R)Exel5780} mutant neuroblasts stained for tubulin (green), DNA (blue), and either (A) Feo (red) or (B) Pav (red). Arrows in A point to telophase nuclei. Scale bar, 10 μm.

FIGURE 7: Recruitment of Polo to the midzone is impaired in spermatocytes but not in neuroblasts from scpo^{2775/Df(3R)Exel5780} mutants. Wild-type and scpo^{2775/Df(3R)Exel5780} (A) spermatocytes or (B) neuroblasts stained for DNA (blue) and Polo (green). Polo is present at the midzone of anaphase wild-type and mutant neuroblasts but fails to form a tight band in telophase of scpo mutant neuroblast (arrows in B). Scale bar, 10 μm.
to promote gradual convergence of the initial broad band into a narrow ring centered at the maximum of RhoGEF activity at the cortex. In scpo mutants, which display irregular anaphase central spindles devoid of Pav, the broad Rho1 cortical band fails to narrow, the cells fail to form a focused, narrow ring of myosin, and cell division proceeds with inefficient and incomplete constriction.

A key difference between neuroblasts and spermatocytes that may account, at least in part, for the differences in behavior of Rho1 and myosin complex proteins we observed in scpo mutant mitotic versus male meiotic cells is in the relationship between Polo kinase and the CPC. In spermatocytes, Polo and the CPC are interdependent and Polo colocalizes with the CPC along its full journey from metaphase through anaphase and telophase (Goto et al., 2006; Vuoriluoto et al., 2011; this study). In neuroblasts, in contrast, Polo localization during cytokinesis appears to be independent of the CPC and centralspindlin, at least at early stages of cell division, but Polo appears to colocalize with Feo (this work). A second difference between neuroblasts and spermatocytes may be the recently described, spindle-independent backup system that can localize myosin to a broad band at the cell cortex near the future cleavage plane under control of the neuroblast cell polarity system (Cabernard et al., 2010). The broad localization of myosin to the cell cortex observed in ana/telophase neuroblasts in scpo mutants may be in part due to these redundant mechanisms.

**MATERIALS AND METHODS**

**Fly strains, husbandry, and transgenes**

Fly strains, husbandry, and transgenes

Flies were raised on cornmeal molasses or dextrose medium at 25°C. The scapolo allele of survivin was from the C. Zuker collection of ethylmethane sulfonate–mutated lines that were screened for male sterility (Giansanti et al., 2004). The Sqh-GFP stock was a gift from R. Karess (Royou et al., 2002). The β-tubulin–EGFP strain was kindly provided by M. Savoian and D. Glover (Inoue et al., 2004). The P-elements and chromosomal deficiencies used in the mapping of scpo were obtained from the Bloomington Drosophila Stock Center (Indiana University, Bloomington, IN) and the Szeged University Drosophila Stock Center (Szeged, Hungary). Transgenic flies were generated by P-element–mediated transformation of sequences cloned into pCaSpeR4. For the survivin rescue construct, a 2.5-kb fragment containing survivin was PCR amplified from the genomic BAC RP98-48C17 clone (BACPAC Resources, Oakland, CA) and cloned into pCaSpeR4. The survivin transgenes containing glutamine 83–to-glutamic substitution and Pro-86–to-serine substitutions were generated by PCR site-directed mutagenesis on the survivin rescue construct and cloned into pCaSpeR4. For the survivin-GFP fusion construct, survivin was fused in-frame to the 3′ end of EGFP using endogenous promoter and genomic sequences and cloned into pCaSpeR4.

**Mapping scapolo**

The scapolo locus was initially mapped by meiotic recombination to a region between the markers e and Pr on chromosome 3R.
Deficiency complementation tests showed that Exelixis Df(3R)Exel5780 and Df(3R)Exel5781 (from the Harvard Stock Center, Cambridge, MA) failed to complement the scpo mutant phenotype. Male recombination was used to narrow the scpo genomic region to the distal of P-element insertion P[w+;mC y(+;+Dmt2) = EPyg2]EY02138 and to the proximal of P(EPyg2) EY07690, leaving a region of 100 kb. scpo was identified by comparing sequences of candidate genes in the region in scpo homologous to sequences of the original Zuker-background chromosome.

Antibody generation
Antibodies were generated against a glutathione S-transferase (GST)—full-length dSurvivin fusion protein. survivin cDNA was PCR amplified from EST LP03704 (Berkeley Drosophila Genome Project, Berkeley, CA) and cloned in-frame into a pGEX-3X vector (Amersham Biosciences, Piscataway, NJ). The 50-kDa GST–Survivin fusion protein was expressed in Escherichia coli BL21 strain and purified on glutathione–Sepharose beads (Sigma-Aldrich, St. Louis, MO) according to the GST Gene Fusion System Handbook (Amersham Biosciences). Antibodies against the GST–Survivin fusion protein were produced in rabbits (GenScript, Piscataway, NJ).

Microscopy and immunofluorescence
Squashed adult or larval testes were imaged by phase contrast on a Zeiss Axioskop (Thornwood, NY) as described by Regan and Fuller (1988). Cytological preparations for immunostaining were made with testes or brains from third-instar larva or pupae. For all the preparations except for those shown in Figure 2, A and B, we used testes or brains from scpo2277/Df(3R)Exel5780 individuals raised at 25°C. To visualize tubulin and Aurora B, INCENP, or Survivin in spermatocytes, larval testes were fixed and stained according to Starr et al. (1998). To visualize α-tubulin along with either Scpo-GFP or α-tubulin with Ser-10 H3 histone, larval testes were fixed with 4% formaldehyde as described by Farkas et al. (2003). For all other immunostaining, preparations were fixed using 3.7% formaldehyde in phosphate-buffered saline and then squashed in 60% acetic acid according to Giansanti et al. (1999). To visualize mitotic chromosomes, larval brains preparations were dissected in NaCl, 0.7%, treated with hypotonic solution for 7 min, fixed in 45% acetic acid processed as per Giansanti et al. (1999), and stained with 4′,6-diamidino-2-phenylindole (DAPI). Monoclonal antibodies were used to stain α-tubulin (T6199; diluted 1:100; Sigma-Aldrich), Rho1 (diluted 1:100; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), and Polo mouse monoclonal anti-Polo M294 (diluted 1:20; a gift from D. Glover [Tavares et al., 1996]). Polyclonal antibodies were used to stain Pavlovotti (diluted 1:200; a gift from D. Glover [Adams et al., 1998]), Fascottio (diluted 1:200; a gift from F. Verni [Verni et al., 2004]), anti–Aurora B (diluted 1:200; a gift from P. P. D’Avino and D. Glover [Giet and Glover, 2001]), anti–Survivin (diluted 1:500; this study), anti–DmINCENP (diluted 1:400; a gift from J. Wakefield), anti–myosin II (diluted 1:400; a gift from R. Karess [Royou et al., 2002]), and anti–Ser-10 H3 histone (diluted 1:100; Cell Signaling Technology, Danvers, MA). Secondary antibodies Alexa 488/Alexa 555–conjugated anti–rabbit immunoglobulin G (Molecular Probes, Eugene OR) and rhodamine/fluorescein isothiocyanate–conjugated anti–mouse (Jackson ImmunoResearch, West Grove, PA) were used at dilution of 1:250 and 1:20, respectively. Slides were mounted in Vectashield medium with DAPI (Vector Laboratories, Burlingame, CA). Images were captured with a Photometrics (Tucson, AZ) cooled charge-coupled device camera connected to a Zeiss Axiohot epifluorescence microscope. Grayscale images were collected separately and were pseudocolored and merged in Photoshop 6 (Adobe, San Jose, CA).

Live imaging
Time-lapse imaging of living spermatocytes was carried out according to the protocol described in Inoue et al. (2004). Testes isolated from adult flies or third-instar larvae were dissected under 105 Voltaire gel (Elf Atochem, Philadelphia, PA) onto a clean coverslip attached to the underside of an aluminum slide. Cells were examined with a Zeiss Axiosview 20 microscope equipped with a 63×, 1.25 numerical aperture (NA) or a 63×, 1.4 NA objective and a filter wheel combination (Chroma Technology, Bellows Falls, VT). Images were acquired with a CoolSnap HQ camera (Photometrics), using a 2 × 2 bin controlled through Metamorph software (Molecular Devices, Sunnyvale, CA). Images were collected at 1-min intervals; 11 fluorescence optical sections were captured at 1-μm z-steps. Movies were created using the Metamorph software and show the maximum-intensity projection of all of the sections.

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REFERENCES
Adams RR, Maiato H, Earnshaw WC, Carmena M (2001). 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 153, 865–880.
Adams RR, Tavares AA, Salzberg A, Bellen HJ, Glover DM (1998). pavorti encodes a kinesis-like protein required to organize the central spindle and contractile ring for cytokinesis. Genes Dev 12, 1483–1494.
Bartolini F, Moseley JB, Schmaranzer J, Cassimeris L, Goode BL, Gundersen GG (2008). The formin mDia2 stabilizes microtubules independently of its actin nucleating activity. J Cell Biol 171, 523–536.
Beardmore VA, Ahonen LJ, Gorbsky GJ, Kallio MJ (2004). Survivin dynamics increases at centromeres during G2/M phase transition and is regulated by microtubule-attachment and Aurora B kinase activity. J Cell Sci 117, 4033–4042.
Brennan IM, Peters U, Kapoor TM, Straight AF (2007). Polo-like kinase controls vertebrate spindle elongation and cytokinesis. PLoS One 2, e409.
Burgess DR, Chang F (2005). Site selection for the cleavage furrow at cytokinesis. Trends Cell Biol 15, 156–62.
Burkard ME et al. (2009). Plk1 self-organization and priming phosphorylation of HsCYK-4 at the spindle midzone regulate the onset of division in human cells. PLoS Biol 7, e1000111.
Cabinard C, Prehoda KE, Doe CZ (2010). A spindle-independent cleavage furrow positioning pathway. Nature 467, 91–95.
Canman JC (2009). Cytokinetin astology. J Cell Biol 187, 757–759.
Canman JC, Cameron LA, Maddox PS, Staright A, Tirnauer JS, Mitchison TJ, Fang G, Kapoor TM, Salmon ED (2003). Determining the position of the cell division plane. Nature 424, 1074–1078.
Cao L, Yan X, Wu Y, Hu H, Li Q, Zhou T, Jiang S, Yu L (2006). Survivin mutant (Sun-DD70, 71AA) disrupts the interaction of Survivin with Aurora B and causes multinucleation in HeLa cells. Biochem Biophys Res Commun 346, 400–407.
Carmena M (2008). Cytokinesis: the final stop for the chromosomal passengers. Biochem Soc Trans 36, 367–370.
Carvalho A, Carmena M, Sambade C, Earnshaw WC, Wheatey SP (2003). Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. J Cell Sci 116, 2987–2998.
Koundakjian EJ, Cowan DM, Hardy RW, Becker AH (2004). The Zucker collection: a resource for the analysis of autosomal gene function in Drosophila melanogaster. Genetics 167, 203–206.

Lens SM, Rodriguez JA, Vader G, Span SW, Giaccone G, Medema RH (2006). Uncoupling the central spindle-associated function of the chromosomal passenger complex from its role at centromeres. Mol Biol Cell 17, 1897–1909.

Lens SM, Wolthuis RM, Klopmanmaker R, Kauw J, Agami R, Brummelkamp T, Kops G, Medema RH (2003). Survivin is required for a sustained spindle checkpoint arrest in response to lack of tension. EMBO J 22, 2943–2947.

Lewellyn L, Carvalho A, Desai A, Maddox AS, Oegema K (2011). The chromosomal passenger complex and centralspindlin independently contribute to contractile ring assembly. J Cell Biol 193, 155–169.

Mackay AM, Eckley DM, Chue C, Earnshaw WC (1993). Molecular analysis of the INCENPs (inner centromere proteins): separate domains are required for association with microtubules during interphase and with the central spindle during anaphase. J Cell Biol 123, 373–385.

Minoshima Y et al. (2003). Phosphorylation by aurora B converts MgcRacGAP to a RhoGAP during cytokinesis. Dev Cell 4, 549–560.

Nakajima H, Toyoshima-Morimoto F, Taniguchi E, Nishida E (2003). Identification of a consensu-specific motif for Plk (Polo-like kinase) phosphorylation reveals Myt1 as a Plk1 substrate. J Biol Chem 278, 25277–25280.

Neef R, Klein UR, Kopajtich R, Barr FA (2006). Cooperation between mitotic kinases controls the late stages of cytokinesis. Curr Biol 16, 301–307.

Nishimura Y, Yonenura S (2006). Centralspindlin regulates ECt2 and RhoA accumulation at the equatorial cortex during cytokinesis. J Cell Sci 119, 104–114.

Palazzo AF, Cook TA, Alberts BS, Gundersen GG (2001). MDia mediates Rho-regulated formation and orientation of stable microtubules. Nat Cell Biol 3, 723–729.

Pieńkowska K, Werner M, Glotzer M (2005). Cytokinesis: welcome to the Rho zone. Trends Cell Biol 15, 651–658.

Regan CL, Fuller MT (1998). Interacting genes that affect microtubule function: the nc2 allele of the haywire locus fails to complement mutations in the testis-specific beta-tubulin gene of Drosophila. Genes Dev 2, 82–92.

Resnick TD, Satinover DL, Maaslaag F, Stukenberg PT, Earnshaw WC, Orr-Weaver TL, Carmena M (2006). INCENP and Aurora B promote meiotic sister chromatid cohesion through localization of the Shugoshin MEI-5332 in Drosophila. Dev Cell 11, 57–68.

Romano A, Guse A, Krasnecsovova I, Schnabel H, Schnabel R, Gliick M (2003). CSC-1: a subunit of the Aurora B kinase complex that binds to the survivin-like protein BIR-1 and the INCENP-like protein ICP-1. J Cell Biol 161, 229–236.

Royou A, Sullivan W, Karess R (2002). Cortical recruitment of nonmuscle myosin II in early syncytial Drosophila embryos: its role in nuclear axial alignment and its regulation by Cdc2 activity. J Cell Biol 158, 127–137.

Ruchaud S, Carmena M, Earnshaw WC (2007a). Chromosomal passengers: conducting cell division. Nat Rev Mol Cell Biol 8, 798–812.

Ruchaud S, Carmena M, Earnshaw WC (2007b). The chromosomal passenger complex: one for all and all for one. Cell 131, 230–231.

Sambath SC, Ohi R, Leismann O, Salic A, Funabiki H (2004). The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. Cell 118, 187–202.

Santamaria A et al. (2007). Use of the novel Plk1 inhibitor ZK-thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. Mol Biol Cell 18, 4024–4036.

Severson AF, Hamill DR, Carter JC, Schumacher J, Bowman B (2000). The aurora-related kinase AiR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis. Curr Biol 10, 1162–1171.

Somers WG, Saint R (2003). A Rhogap and Rh family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. Dev Cell 4, 29–39.

Starr DA, Williams BC, Hays TS, Goldberg ML (1998). ZW10 helps recruit dynactin and dynein to the kinetochore. J Cell Biol 142, 763–774.

Tavares AA, Goldner DM, Sunkel CE (1996). The conserved mitotic kinase polo is regulated by phosphorylation and has preferred microtubule-associated substrates in Drosophila embryo extracts. EMBO J 15, 4873–4883.

Vader G, Medema RH, Lens SM (2006). The chromosomal passenger complex: guiding Aurora-B through mitosis. J Cell Biol 173, 833–837.

Vagnarelli P, Earnshaw WC (2004). Chromosomal passengers: the four-dimensional regulation of mitotic events. Chromosoma 113, 211–222.

Chalamalasetty RB, Hummer S, Nigg EA, Sillje HH (2006). Influence of human Ect2 depletion and overexpression on cleavage furrow formation and abscission. J Cell Sci 119, 3008–3019.

Chen W, Foss M, Tseng K-F, Zhang D (2008). Redundant mechanisms recruit actin into the contractile ring in silkworm spermatocytes. PLoS Biol 6, 1927–1941.

Cook CA, Heck MM, Earnshaw WC (1997). The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis. J Cell Biol 105, 2053–2067.

D’Avino PP, Archambault V, Przewlocka MR, Zhang W, Lilley KS, Laue E, Glover DM (2007). Recruitment of Polo kinase to the spindle midzone during cytokinesis requires the Fao/Klp3A complex. PLoS One 2, e572.

Delacour-Larose M, Molla A, Skoufias DA, Margolis RL, Dimitrov S (2004). Distinct dynamics of Aurora B and Survivin during mitosis. Cell Cycle 3, 1418–1426.

Douglas ME, Davies T, Joseph N, Mishima M (2010). Aurora B and 14-3-3 coordinate regulate clustering of centralspindlin during cytokinesis. Curr Biol 20, 927–933.

Farkas RM, Giansanti MG, Gatti M, Fuller MT (2003). The Drosophila Cog5 homologue is required for cytokinesis, cell elongation, and assembly of specialized Golgi architecture during spermatogenesis. Mol Biol Cell 14, 190–200.

Foe V, von Dassow G (2008). Stable and dynamic microtubules coordinately shape the myosin activation zone during cytokinetic furrow formation. J Cell Biol 183, 457–470.

Gasemann R, Carvalho A, Henzing AJ, Rudsaud S, Hudson DF, Honda R, Nigg EA, Gerloff DL, Earnshaw WC (2004). Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. J Cell Biol 166, 179–191.

Giansanti MG, Bonaccorsi S, Gatti M (1999). The role of anillin in meiotic cytokinesis of Drosophila males. J Cell Sci 112, 2323–2334.

Giansanti MG, Bonaccorsi S, Kurek R, Farkas RM, Dimitri P, Fuller MT, Gatti M (2006). The class I PTP giotto is required for Drosophila cytokinesis. Curr Biol 16, 195–201.

Giansanti MG, Farkas RM, Bonaccorsi S, Lindsey DL, Wakimoto BT, Fuller MT, Gatti M (2004). Genetic dissection of meiotic cytokinesis in Drosophila males. Mol Biol Cell 15, 2509–2522.

Gier T, Glover DM (2001). 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 152, 669–682.

Goto H, Kyono T, Tomono Y, Kawajiri A, Urano T, Furukawa K, Nigg EA, Inagaki M (2006). Complex formation of Plk1 and INCENP required for metaphase-anaphase transition. Nat Cell Biol 8, 180–187.

Guse A, Mishima M, Glotzer M (2005). Phosphorylation of ZEN-4/MKLP1 by aurora B regulates completion of cytokinesis. Cell Biol 15, 778–786.

Hales DK, Kellett AC, Earnshaw WC (2005). Loss of Drosophila borealin causes polyplody, delayed apoptosis and abnormal tissue development. Development 132, 4777–4787.

Honda R, Körner R, Nigg EA (2003). Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. Mol Biol Cell 14, 3325–3341.

Inoue YH, Savoian MS, Suzuki T, Mäthé E, Yamamoto MT, Glover DM (2004). Mutations in aristomat reveal that the central spindle is comprised of two microtubule populations, those that initiate cleavage and those that propagate furrow ingestion. J Cell Biol 166, 43–55.

Jeyaprakash AA, Klein UR, Lindner D, Ebert J, Nigg EA, Conti E (2007). Structure of a Survivin-Borealin-INCENP core complex reveals how the survivin-like protein BIR-1 and the INCENP-like protein ICP-1. J Cell Biol 171, 2547–2558.
Verni F, Somma MP, Gunsalus KC, Bonaccorsi S, Belloni G, Goldberg ML, Gatti M (2004). Feo, the Drosophila homolog of PRC1, is required for central-spindle formation and cytokinesis. Curr Biol 14, 1569–1575.

Von Dassow G, Verbrugghe KJ, Miller AL, Sider JR, Bement WM (2009). Action at a distance during cytokinesis. J Cell Biol 187, 831–845.

Vuoriluoto M, Laine LJ, Saviranta P, Pouwels J, Kallio MJ (2011). Spatio-temporal composition of the mitotic Chromosomal Passenger Complex detected using in situ proximity ligation assay. Mol Oncol 5, 105–111.

Wang F, Dai J, Daum JR, Niedzialkowska E, Banerjee B, Stukenberg PT, Gorbsky GJ, Higgins JM (2010). Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. Science 330, 231–235.

Werner M, Munro E, Glotzer M (2007). Astral signals spatially bias cortical myosin recruitment to break symmetry and promote cytokinesis. Curr Biol 17, 1286–1297.

Wolfe BA, Takaki T, Petronczki M, Glotzer M (2009). Polo-like kinase 1 directs assembly of the HsCyk-4 RhoGAP/Ect2 RhoGEF complex to initiate cleavage furrow formation. PLoS Biol 7, e1000110.

Yuce O, Piekny A, Glotzer M (2005). An ECT2-centralspindlin complex regulates the localization and function of RhoA. J Cell Biol 170, 571–582.

Yue Z et al. (2008). Deconstructing Survivin: comprehensive genetic analysis of Survivin function by conditional knockout in a vertebrate cell line. J Cell Biol 183, 279–296.

Zhao WM, Fang G (2005). MgcRacGAP controls the assembly of the contractile ring and the initiation of cytokinesis. Proc Natl Acad Sci USA 102, 13158–13163.