TRAPPII is required for cleavage furrow ingression and localization of Rab11 in dividing male meiotic cells of *Drosophila*

Carmen C. Robinett[1,*,‡,§], Maria Grazia Giansanti[2,‡], Maurizio Gatti[2] and Margaret T. Fuller[1]

[1]Department of Developmental Biology and Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA
[2]Istituto Pasteur-Fondazione Cenci Bolognetti and Istituto di Biologia e Patologia Molecolari (IBPM) del CNR, Dipartimento di Genetica e Biologia Molecolare, Università di Roma "La Sapienza", Rome, Italy

*Author for correspondence (robinettc@janelia.hhmi.org)
‡These authors contributed equally to this work
§Present address: Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147, USA

Accepted 2 October 2009
Journal of Cell Science 122, 4256-4534 Published by The Company of Biologists 2009
doi:10.1242/jcs.054536

Summary

Although membrane addition is crucial for cytokinesis in many animal cell types, the specific mechanisms supporting cleavage furrow ingression are not yet understood. Mutations in the gene *brunelleschi* (*bru*), which encodes the *Drosophila* ortholog of the yeast Trs120p subunit of TRAPPII, cause failure of furrow ingression in male meiotic cells. In non-dividing cells, Brunelleschi protein fused to GFP is dispersed throughout the cytoplasm and enriched at Golgi organelles, similarly to another *Drosophila* TRAPPII subunit, dBet3. Localization of the membrane-trafficking GTPase Rab11 to the cleavage furrow requires wild-type function of *bru*, and genetic interactions between *bru* and *Rab11* increase the failure of meiotic cytokinesis and cause synthetic lethality. *bru* also genetically interacts with *four wheel drive* (*fwd*), which encodes a PI4Kβ, such that double mutants exhibit enhanced failure of male meiotic cytokinesis. These results suggest that Bru cooperates with Rab11 and PI4Kβ to regulate the efficiency of membrane addition to the cleavage furrow, thus promoting cytokinesis in *Drosophila* male meiotic cells.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/122/24/4526/DC1

Key words: Cytokinesis, Golgi, TRAPPII

Introduction

In animal cell cytokinesis, the dynamic processes of contractile ring constriction and concomitant cleavage furrow ingression serve to physically partition the nascent daughter cells during the late stage of cell division. To provide the surface area required for cleavage furrow ingression, many animal cell types insert new membrane into the furrow (Aimar, 1997; Bluemink and de Laat, 1973; Danilchik et al., 2003; Shuster and Burgess, 2002; Skop et al., 2001) or modify the organization of their pre-existing plasma membrane by rounding up or flattening microvilli (Blose, 1979; Boucrot and Kirchhausen, 2007; Erickson and Trinkaus, 1976; Knutton et al., 1975). These strategies for accommodating cleavage furrow ingression are probably governed by cell shape, surface membrane organization, and internal membrane stores.

Consistent with these modifications of the plasma membrane, cytokinesis depends on vesicle trafficking components that include the membrane fusion machinery, regulatory small GTPases and multi-protein complexes involved in both exocytosis and endocytosis (for reviews, see Albertson et al., 2005; Finger and White, 2002; Montagnac et al., 2008; Prekeris and Gould, 2008). For example, vesicle-targeting syntaxins have been shown to be required for cytokinesis in embryos of the sea urchin and *C. elegans* (Conner and Wessel, 1999; Jantsch-Plunger and Glotzer, 1999), as well as for the cognate process of cellularization in *Drosophila* embryos (Burgess et al., 1997). Additionally, in dividing cultured cells, the final separation of daughter cells following furrow ingression is blocked by reduced function of the proteins syntaxin-2, endobrevin, soluble *N*-ethylmaleimide sensitive factor attachment protein (α-SNAP), the exocyst complex, and the GTPases ARF6, Rab35 and Rab11 (reviewed in Montagnac et al., 2008). Many of these components highlight the increasingly prominent role of endosomal trafficking in cytokinesis and cellularization (Albertson et al., 2005; Montagnac et al., 2008; Prekeris and Gould, 2008).

Cytokinesis in male meiotic cells (spermatocytes) of *Drosophila melanogaster* also depends on a number of vesicle-trafficking components. These include the intra-Golgi trafficking conserved oligomeric Golgi (COG) complex subunit 5 encoded by *four way stop* (*fws*) (Farkas et al., 2003), the endoplasmic reticulum (ER)-to-Golgi vesicle-docking protein encoded by *Syntaxin 5* (Xu et al., 2002), the GTPases Rab11 (Giansanti et al., 2007) and Arf6 (Dyer et al., 2007), the phosphatidylinositol 4-kinase β (PI4Kβ) encoded by *four wheel drive* (*fwd*) (Brill et al., 2000), and the phosphatidylinositol transfer protein encoded by *giotto* (*gio*) (Gatt and Glover, 2006; Giansanti et al., 2006). Strikingly, mutations in *fws*, *Rab11*, *Arf6*, *fwd* and *gio* cause defects in actomyosin ring constriction and concomitant failure of cleavage furrow ingression in spermatocytes, suggesting an intimate relationship between membrane trafficking and contractile ring behavior during cytokinesis in this cell type.

Here, we show that function of the membrane trafficking transport protein particle (TRAPP) II complex is required for cleavage furrow ingression during cytokinesis in *Drosophila* spermatocytes. Mutations in the gene *brunelleschi* (*bru*), which encodes the *Drosophila* ortholog of the yeast TRAPPII TRS120p subunit, cause failure of both actomyosin ring constriction and cleavage furrow ingression. In addition, we found that *bru*

genetically interacts with *Rab11* and is required for the localization of Rab11 to the cleavage furrow of dividing spermatocytes. Furthermore, we found that *bru* genetically interacts with *fwd*, which has also been shown to be required for the localization of Rab11 to the cleavage furrow in spermatocytes (Giansanti et al., 2007). These genetic interactions suggest that *bru* also functions in membrane trafficking in *Drosophila*, possibly as a subunit of a TRAPPII complex. Thus, we propose that *bru* mediates the flow of membrane through the Golgi that is required to support cleavage furrow ingression in dividing spermatocytes.

Results

Wild-type function of *bru* is required for cleavage furrow ingression in spermatocytes

Two mutant alleles of *bru, bru*^{Z3358} and *bru*^{Z0704}, were previously isolated from a collection of male-sterile mutants that was screened for mutations causing failure of cytokinesis in spermatocytes (Giansanti et al., 2004). Our initial characterization of fixed spermatocytes from *bru* males raised at 25°C revealed defects in contractile ring constriction during telophase (Giansanti et al., 2004). We subsequently found that the defect in *bru* mutant males is temperature sensitive, because the failure of meiotic cytokinesis observed at 25°C was strongly exacerbated at 29°C and suppressed at 18°C. At 29°C, early spermatids from *bru*^{Z3358}/+ testes appeared normal and contained a single phase-light nucleus associated with a similarly sized, phase-dark mitochondrial derivative (MD) (Fig. 1A). By contrast, ~80% of early spermatids from *bru*^{Z3358}/*Df(2L)pr2b* mutant males raised at 29°C contained four nuclei associated with a single, enlarged MD (Fig. 1A and 1B), reflecting cytokinesis failures during both meiotic divisions (Fuller, 1993). The vast majority of spermatids from *bru*^{Z3358}/*Df(2L)pr2b* mutant males raised at 18°C appeared normal (Fig. 1B). The phenotype of *bru*^{Z0704} exhibited a similar temperature sensitivity in *bru*^{Z0704}/*Df(2L)pr2b* males (C.C.R., unpublished).

Our previous characterization of actin localization in fixed spermatocytes from *bru* mutants raised at the intermediate temperature of 25°C suggested that contractile rings formed normally at the onset of cytokinesis in early telophase, but were frequently unconstricted or broken in later stages of division (Giansanti et al., 2004). To analyze contractile rings in vivo, we examined the behavior of the myosin regulatory light chain fused to the green fluorescent protein (RLC-GFP) in live spermatocytes (Royou et al., 2002). RLC-GFP marks the contractile ring throughout telophase, allowing visualization of ring constriction (Dyer et al., 2007). In wild-type spermatocytes (n=10), RLC-GFP rings initiated constriction immediately after assembly and completed constriction within 25 minutes (Fig. 1C, supplementary material Movie 1). In all *bru*^{Z3358}/*Df(2L)pr2b* mutant spermatocytes raised at 29°C (n=9), seemingly normal RLC-GFP rings assembled in mid-anaphase. However, in eight of these cells, RLC-GFP rings did not constrict, or constricted minimally, throughout the observation period (30 minutes) (Fig. 1C, supplementary material Movie 2). In addition, the unconstricted RLC-GFP rings in these spermatocytes eventually fragmented. To quantify the defect in constriction, we scored a population of telophase spermatocytes from wild-type and *bru* mutant flies raised at 29°C and categorized the rings as either constricted or unconstricted based on their apparent size. In *bru*^{Z3358}/*Df(2L)pr2b*, unconstricted RLC-GFP rings were observed in 65% of telophase spermatocytes (n=83), whereas unconstricted rings were observed in only 10% of the wild-type telophase spermatocytes (n=117). Together, these results indicate

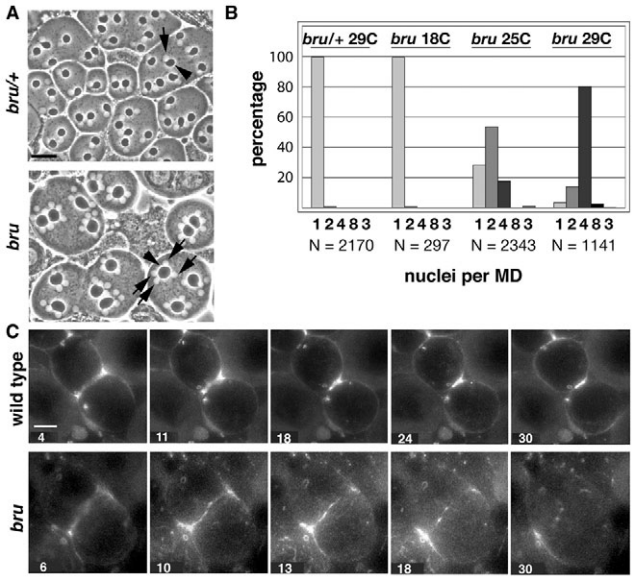

Fig. 1. Contractile ring constriction is inhibited in *bru* mutant spermatocytes. (A) Phase-contrast images of early spermatids in *bru*^{Z3358}/*CyO* (*bru*/+) and *bru*^{Z3358}/*Df(2L)pr2b* (*bru*) testes from males raised at 29°C. *CyO* is a balancer chromosome that provides wild-type *bru* function, and *Df(2L)pr2b* is a deficiency that deletes *bru*. Note that testis squash preparations typically disrupt the plasma membrane separation between early spermatids. Arrowheads indicate MDs and arrows indicate nuclei. Scale bar: 20 µm. (B) Percentages of early spermatids with 1, 2, 4, 8 or 3 nuclei per MD from *bru*^{Z3358}/+ and *bru*^{Z3358}/*Df(2L)pr2b* males raised at the indicated temperatures. N, total number of spermatids counted. (C) Behavior of contractile rings labeled with RLC-GFP in wild-type and *bru* mutant spermatocytes. Selected frames from supplementary material Movies 1 and 2 showing wild-type (+/+) and *bru*^{Z3358}/*Df(2L)pr2b* (*bru*) spermatocytes raised at 25°C. Spermatocytes were imaged for 30 minutes starting from late anaphase. The numbers at the bottom of each frame indicate time elapsed from the beginning of imaging. In the wild type, the RLC-GFP ring constricts fully. By contrast, the RLC-GFP ring in the *bru* mutant spermatocyte undergoes only slight constriction and eventually breaks down. Scale bar:10 µm.

that *bru* is required for ring constriction and concomitant furrow ingression.

The requirement for *bru* in cytokinesis appeared to be specific to spermatocytes. The *bru* mutants were fully viable through adulthood and female fertility was not grossly affected by loss of *bru* function. The quantity, viability and hatching frequency of eggs laid by *bru*^{Z3358}/*Df(2L)pr2b* females raised at 25°C and mated to wild-type Oregon R males were indistinguishable from that observed for their heterozygous *bru* ^{Z3358}/+ and *Df(2L)pr2b*/+ siblings (C.C.R., unpublished). Additionally, we did not detect polyploid cells in larval brains from *bru* mutant larvae, indicating that neuroblast cytokinesis is not affected (M.G.G., unpublished).

bru encodes the ortholog of the yeast TRAPPII subunit Trs120p

The *bru* locus was mapped to an ~24-kbp interval (see the Materials and Methods), and several annotated candidate genes in this interval were sequenced. Each of the two mutant alleles of *bru* was found to have a single base-pair change that introduced a premature stop codon in the annotated gene CG2478, which is predicted to encode a 1320 amino acid polypeptide (http://flybase.org) (Wilson et al., 2008). *bru*^{Z3358} and *bru*^{Z0704} would truncate this polypeptide after residues 514 and 754, respectively (Fig. 2A). Identification of *bru*

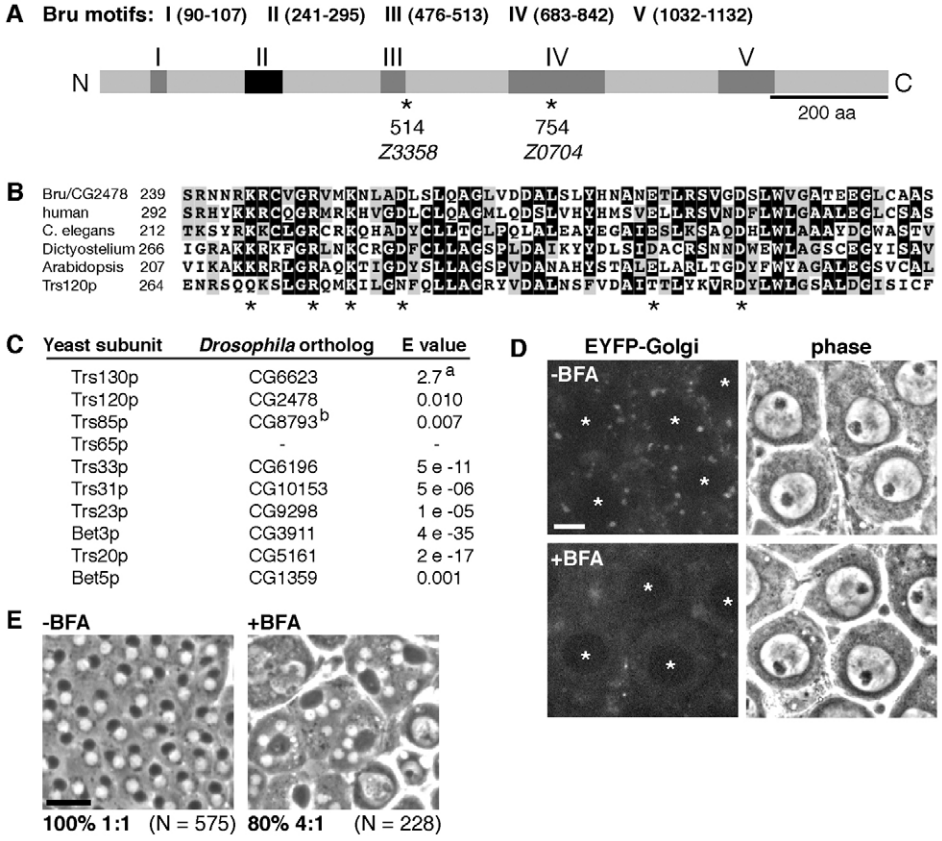

Fig. 2. *bru* encodes the *Drosophila* ortholog of Trs120p. (A) Predicted Bru protein with highly conserved motif II (black box) and motifs I and III-V (dark gray boxes) corresponding to indicated amino acid positions. Asterisks indicate positions of stop codons in *bru* mutant alleles. Motifs correspond to evolutionarily conserved domains (Cox et al., 2007). (B) Alignment of motif I amino acid sequences from Bru orthologs was generated by ClustalW 1.8 and rendered by BoxShade. Conserved residues that were mutated to alanine to test functional importance are indicated by asterisks. (C) Genes encoding *Drosophila* orthologs of yeast TRAPP proteins were identified by tBlastn analysis of the *Drosophila* genome using the *S. cerevisiae* protein sequence as a query. [a]Blastp analysis of the *Drosophila* proteome gave an E value of 6×10^{-5}. [b]The *S. pombe* ortholog of TRS85p was used to query the *Drosophila* genome. (D-E) Spermatocytes and early spermatids from larval testes cultured for 14 hours without BFA or with 30 μg/ml BFA. (D) Phase-contrast and fluorescent images of spermatocytes expressing *EYFP-Golgi*. In spermatocytes treated with BFA, the multiple Golgi organelles are disrupted. Asterisks indicate nuclei. Scale bar: 10 μm. (E) Phase-contrast images of early spermatids. Percentages of mononucleate (1:1) and tetranucleate (4:1) spermatids are indicated with the total number counted (N). Scale bar: 20 μm.

as CG2478 was confirmed by transgenic rescue of the male meiotic cytokinesis defect using a 5.9-kbp genomic fragment that contains only the complete wild-type CG2478 gene. Northern blot analysis revealed expression of the expected ~4.9-kbp message in adults of both sexes and in embryos (data not shown), indicating that *bru* expression was not restricted to the male germline.

Sequences predicted to encode homologs of the Bru protein were found in human, *Dictyostelium discoideum*, nematode, plant and yeast by tBlastn queries (Fig. 2B). The Bru homolog in *Saccharomyces cerevisiae* is Trs120p, an essential subunit of the TRAPPII complex that is required for membrane trafficking at the late Golgi (Cai et al., 2005; Sacher et al., 2000; Sacher et al., 2001). Bru was the only homolog of Trs120p identified in the *D. melanogaster* genome by tBlastn query (Fig. 2C), indicating that *bru* encodes the ortholog of Trs120p. In yeast, TRAPPII is a decameric complex composed of the seven TRAPPI subunits, plus three additional proteins, Trs120p, Trs130p and Trs65p (Sacher et al., 2001). *Drosophila* appears to have an orthologous TRAPPII complex because its genome encodes single, significant-scoring polypeptide orthologs for all of the *S. cerevisiae* TRAPPII subunits except Trs65p and Trs85p (Fig. 2C), which is consistent with a recent phylogenetic analysis (Cox et al., 2007). Although an ortholog of *S. cerevisiae* Trs85p was not identified, a tBlastn query with the *Schizosaccharomyces pombe* Trs85p ortholog revealed a *Drosophila* homolog (Fig. 2C). As expected, we did not find a *Drosophila* homolog of the TRAPPII-specific Trs65p, which is conserved only among fungi and some unicellular eukaryotes (Cox et al., 2007). To our knowledge, the *bru* mutations we describe are the first reported for a TRAPPI/II subunit in *Drosophila* and the first for a TRAPPII-specific subunit in animals.

Amino acid sequence conservation between the Bru homologs identified by ClustalW alignment indicated that the region of highest conservation between Bru and Trs120p is a novel 50-residue motif spanning residues 243-293 (Fig. 2B), which corresponds to Trs120p domain II (Cox et al., 2007). Consistent with the possibility that one or more of the highly conserved charged residues of this motif are important for Bru function, simultaneous replacement of K244, R249, K252, D256, E276 and D283 with alanines (Fig. 2B) in the *bru* genomic rescue construct abolished rescue activity: none of the eight transgenic lines carrying the multiply mutated construct rescued the *bru* male meiotic cytokinesis defect, whereas all five transgenic lines carrying either wild-type *bru* or *bru-GFP* rescued fully.

Because TRAPPII function is required for membrane trafficking at the Golgi in *S. cerevisiae*, we asked whether meiotic cytokinesis in cultured larval testes is sensitive to the Golgi membrane trafficking inhibitor brefeldin A (BFA) (Klausner et al., 1992). Treatment of cultured testes with BFA caused disruption of Golgi organelles in spermatocytes expressing EYFP-Golgi, a derivative of galactosyltransferase (LaJeunesse et al., 2004) (Fig. 2D). Furthermore, wild-type testes cultured with BFA exhibited a marked failure of cytokinesis in meiosis I and II. Whereas early spermatids from control testes contained a single nucleus paired with a normal-sized MD, early spermatids from BFA-treated testes frequently contained four nuclei associated with an enlarged MD (Fig. 2E). These data indicate that BFA-sensitive components essential for Golgi membrane trafficking are required for male meiotic cytokinesis. Similarly, cytokinesis in *C. elegans* embryos, cellularizing *Drosophila* embryos and HeLa cells is also sensitive to BFA, although the sensitivity of cytokinesis varies between cell types (Albertson et al., 2005; Montagnac et al., 2008).

Bru-GFP is dispersed throughout the cytoplasm and localizes to Golgi organelles

To determine the subcellular localization of Bru in male germline cells, we generated a *bru-GFP* transgene. This transgene fully rescued the *bru* mutant phenotype, indicating that the encoded Bru-GFP fusion protein is functional. Live prophase spermatocytes expressing *bru-GFP* exhibited a general cytoplasmic fluorescence plus a number of fluorescent bodies that colocalized with EYFP-Golgi (Fig. 3A). Immunofluorescent localization of Bru-GFP in fixed spermatocytes confirmed the presence of the fluorescent bodies and showed that they were closely associated with the Golgi marker p120 (Fig. 3B), which is consistent with the subcellular localization of epitope-tagged Trs120p to the late Golgi compartment in yeast (Cai et al., 2005). However, in yeast there has been no report of a cytoplasmic pool of Trs120p. The distribution of Bru-GFP was similar to that of the *Drosophila* TRAPPI/II subunit ortholog, dBet3, which we detected as a fusion of YFP to the Bet3p ortholog encoded by CG3911. In live prophase spermatocytes, dBet3-YFP was distributed as a haze throughout the cytoplasm and was also enriched at fluorescent bodies that colocalized with the COG5 ortholog Four-way stop fused to GFP (GFP-Fws) (Farkas et al., 2003) (Fig. 3C). During meiotic division, Bru-GFP was distributed throughout the cytoplasm, without consistent enrichment at any subcellular compartment (Fig. 3D).

bru interacts genetically with *Rab11* and the PI4Kβ gene *fwd*

Similarly to *bru,* wild-type *Rab11* function is required for contractile ring constriction during *Drosophila* male meiotic cytokinesis (Giansanti et al., 2007), and in yeast, *TRS120* genetically interacts with the *Rab11* orthologs, *YPT31/32* (Zhang et al., 2002). This prompted us to ask whether *bru* genetically interacts with *Rab11*. Flies that were wild type for *bru* and homozygous for $Rab11^{93Bi}$ or transheterozygous for $Rab11^{93Bi}/Rab11^{E(To)3}$ produced viable adults (Giansanti et al., 2007; Jankovics et al., 2001). Strikingly, however, $Rab11^{93Bi}$ was fully lethal in combination with *bru*. Flies homozygous for both bru^{Z3358} and $Rab11^{93Bi}$ died in early larval stages at 25°C, as did flies homozygous for bru^{Z3358} and transheterozygous for $Rab11^{93Bi}/Rab11^{E(To)3}$. By contrast, bru^{Z3358} homozygotes that were wild type for *Rab11* were fully viable through adulthood at 25°C.

Consistent with a strong genetic interaction between *bru* and *Rab11*, the defect in male meiotic cytokinesis caused by loss of *bru* function was exacerbated by introducing a single copy of the recessive lethal $Rab11^{E(To)3}$ allele. Testes from $bru^{Z3358}/Df(2L)pr2b;Rab11^{E(To)3}/+$ males raised at 25°C exhibited a 4.5-fold increase in the percentage of tetranucleate early spermatids relative to testes from $bru^{Z3358}/Df(2L)pr2b;+/+$ males (Fig. 4A). Similarly, $bru^{Z3358}/+$ males that were homozygous for the weak allele $Rab11^{93Bi}$ displayed a fourfold increase in the total number of multi-nucleate spermatids compared with homozygous $Rab11^{93Bi}$ males that lacked mutations in *bru* (Fig. 4B).

bru also genetically interacted with *fwd*, a non-essential gene that encodes a PI4Kβ and is required for contractile ring constriction in spermatocytes (Brill et al., 2000; Giansanti et al., 2004). Testes from *bru;fwd* double-mutant males produced higher percentages of early spermatids containing four, eight or more nuclei associated with enlarged MDs than did testes from their siblings that contained one mutant and one wild-type copy of either *bru* or *fwd* (Fig. 4C,D). This indicated that simultaneous loss of both *bru* and *fwd* wild-type functions causes more frequent failure of cytokinesis during meiosis I and II, as well as a dramatic increase in the failure of cytokinesis during the mitotic amplification divisions that precede meiosis. In addition, the elongation of later-stage spermatids was defective in *bru;fwd* double-mutant testes (Fig. 4C). Thus, the function of *bru* appears to cooperate with the functions of *fwd* in the execution of cleavage furrow ingression and spermatid cyst elongation.

By contrast, *bru* did not interact genetically with *pbl*, which encodes a Rho guanine nucleotide (GTP) exchange factor that is required for assembly of the contractile ring (Giansanti et al., 2004; Prokopenko et al., 1999). The percentage of tetranucleate early spermatids in testes from *bru;pbl* double-mutant males was similar to that of testes from *pbl* mutants that were heterozygous for *bru*. There was no indication of cytokinesis failure during the preceding mitotic amplification divisions, and elongation of later-stage spermatids was similar to that of the *bru* mutant (Fig. 4E,F).

To determine whether simultaneous loss of either *bru* and *fwd* or and *Rab11* affects mitotic division in somatic cells, we examined brains from third-instar larvae of *bru;fwd* double mutants, and the brains of the rare surviving second- or third-instar larvae from *bru;Rab11* double mutants. In all cases, we did not detect polyploid cells in ~200 metaphases scored, suggesting that mitotic cytokinesis in somatic cells is not defective in these double mutants.

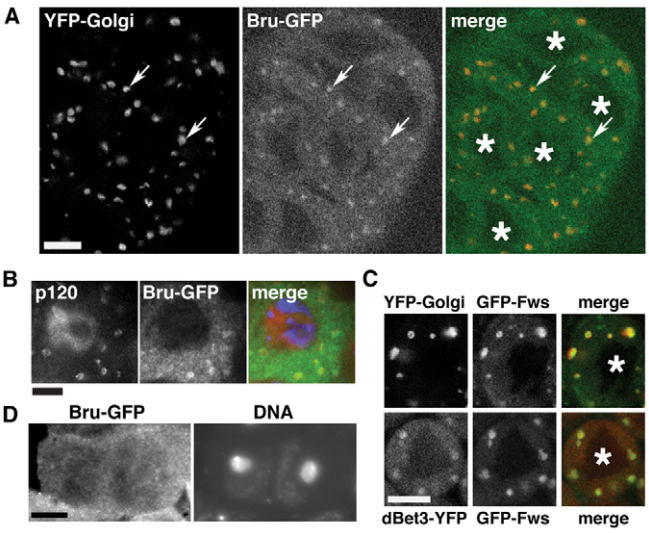

Fig. 3. Bru-GFP localizes to Golgi organelles and the cytoplasm. (A) Live, unsquashed spermatocyte cyst coexpressing *EFYP-Golgi* and *bru-GFP*, imaged by confocal microscopy. In the merged image, EYFP-Golgi (red) colocalizes (yellow) with fluorescent bodies of Bru-GFP (green). Two examples of signal colocalization are indicated by arrows. Asterisks indicate nuclei. (B) Fixed, squashed spermatocyte expressing *bru-GFP,* immunostained for p120 and GFP. In the merged image, p120 (red) colocalizes (yellow) with fluorescent bodies of Bru-GFP immunostained with anti-GFP (green). DAPI-stained DNA (blue). Nuclei are non-specifically stained with the p120 antiserum. (C) Live unsquashed spermatocytes coexpressing *EYFP-Golgi* or *dBet3-YFP* with *GFP-Fws* imaged by confocal microscopy. In the merged image, both EYFP-Golgi and dBet3-YFP (red) colocalize (yellow) with GFP-Fws (green). Asterisks indicate nuclei. (D) Fixed, squashed telophase spermatocyte expressing *bru-GFP*, immunostained with anti-GFP. DNA is stained with DAPI. Scale bars: 10 µm.

Rab11 localization to the cleavage furrow in spermatocytes is dependent on *bru*

Because *bru* genetically interacted with *Rab11*, we examined the localization of Rab11 in *bru* mutant spermatocytes during telophase. In the wild type, endogenous Rab11 localized to small puncta at the cell poles and to the cleavage furrow at the cell midzone (Fig.

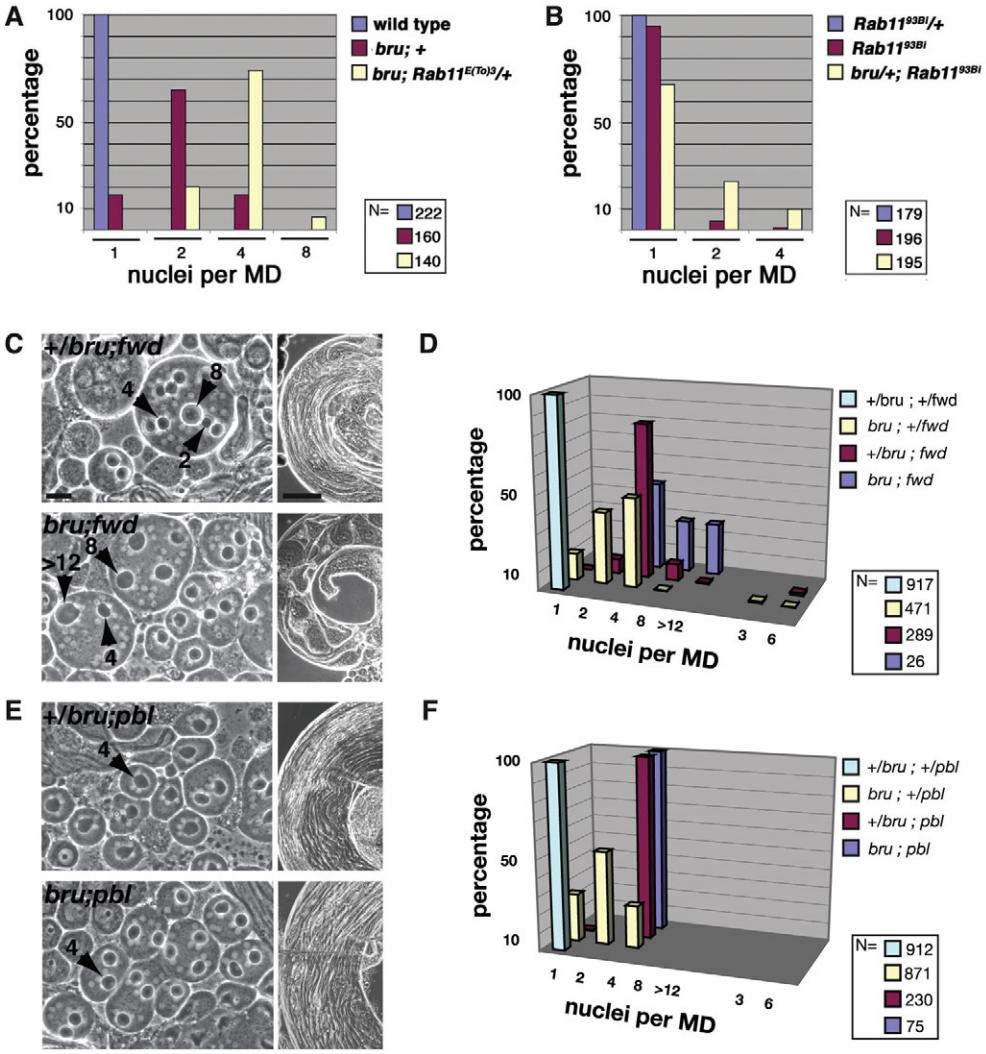

Fig. 4. *bru* genetically interacts with *Rab11* and *fwd*. (A,B,D,F) Percentage of early spermatids with the indicated number of nuclei per MD from males of the indicated genotypes raised at 25°C. N, total number of spermatids counted. + indicates the wild-type allele of the gene of interest. (A) Phenotypes for the progeny of *Df(2L)pr2b/CyO; +/+* crossed to bru^{Z3358}*/CyO;* $Rab11^{E(To)3}$*/MKRS*. (B) Phenotypes for the progeny of bru^{Z3358}*/CyO;* $Rab11^{93Bi}$*/MKRS* crossed to *+/+;* $Rab11^{93Bi}$*/TM6C*. (C,E) Early spermatids (left panels) and adult testes (right panels) from single- and double-mutant males of the indicated genotypes raised at 25°C and imaged by phase contrast. Numbered arrowheads indicate number of nuclei associated with the indicated MD. Scale bars: 20 μm (left panels) and 100 μm (right panels). (C,D) Phenotypes for the progeny of bru^{Z3358}*/CyO; Df(3L)7C/TM6B* crossed to bru^{Z0704}*/CyO;* fwd^{Z0453}*/TM6B*. (E,F) Phenotypes for the progeny of bru^{Z3358}*/CyO;* pbl^{5}*/TM6B* crossed to bru^{Z0704}*/CyO;* pbl^{Z4836}*/TM6B*.

5A) (Giansanti et al., 2007). By contrast, Rab11 did not show a specific pattern of localization in *bru* mutant spermatocytes undergoing telophase (Fig. 5A).

To confirm this result, we examined functional Rab11-GFP in dividing spermatocytes at different stages of the first meiotic division. Rab11-GFP exhibits dynamic localization behavior during the meiotic divisions, mirroring the localization of endogenous Rab11 (Giansanti et al., 2007). During the extended meiotic prophase that precedes cytokinesis, Rab11-GFP was dispersed throughout the cytoplasm and enriched at the multiple Golgi organelles in both wild-type and *bru* mutant spermatocytes (Fig. 5B). At anaphase of the first meiotic division in wild-type spermatocytes, Rab11-GFP fluorescence became fragmented and accumulated in puncta at opposite poles of the cell and at the parafusorial membranes (Fuller, 1993) that envelop the meiotic spindle (Fig. 5C, Fig. 6; supplementary material Movie 3). At telophase, fluorescent puncta persisted at the cell poles, and Rab11-GFP also accumulated in the cell midzone at the cleavage furrow (Fig. 5D and Fig. 6). In *bru* mutant spermatocytes, we did not observe distinct puncta during anaphase or telophase, and the overall distribution of Rab11-GFP appeared more diffuse than in the wild type (Fig. 5C,D, Fig. 6; supplementary material Movie 4). Additionally, Rab11-GFP did not accumulate at the cell midzone in *bru* mutant spermatocytes during telophase. Thus, *bru* function is required for proper Rab11 localization during spermatocyte cytokinesis.

Formation of the Golgi-derived acroblast fails in *bru* mutant spermatids

Following completion of the meiotic divisions, Bru-GFP was dispersed throughout the cytoplasm of early spermatids and also enriched in a perinuclear region corresponding to the acroblast (Fig. 7A). The acroblast is a developmentally regulated Golgi derivative that is formed by the coalescence of Golgi fragments following the completion of meiosis and is marked by GFP-Fws (Fig. 7A) and the Golgi protein Lava lamp (Lva) (Farkas et al., 2003; Sisson et al., 2000; Tates, 1971). The acroblast is situated adjacent to the acrosomal granule to which it secretes exocytic cargo that will support function of the acrosome in mature sperm (Wilson et al., 2006). Consistent with the perinuclear localization of Bru-GFP, acroblast formation failed in *bru* mutant spermatids. Analysis of Lva localization revealed that 35% (n=120) of early spermatids from bru^{Z3358}*/Df(2L)pr2b* mutant testes exhibited Golgi fragments that had not coalesced into a single acroblast but rather segregated into numerous small Golgi organelles (Fig. 7B). By contrast, 100% (n=96) of wild-type early spermatids formed a normal acroblast.

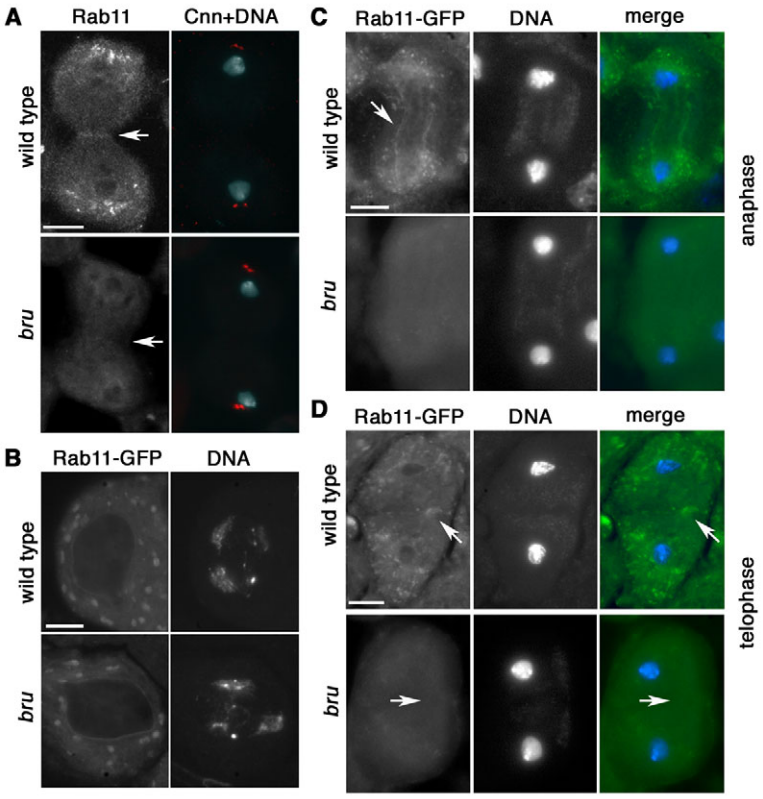

Fig. 5. Localization of Rab11 in dividing spermatocytes depends on *bru* function. (A-D) Localization of Rab11 in +/+ (wild type) and $bru^{Z3358}/Df(2L)pr2b$ (*bru*) spermatocytes. (A) Immunolocalization of endogenous Rab11 in telophase spermatocytes (left panels). The right panels show anti-Centrosomin (Cnn) staining (red) to label the cell poles and DAPI-stained DNA (blue). Arrows indicate the cell midzones. In the *bru* mutant spermatocyte, Rab11 does not form clear puncta at the cell poles or concentrate at the cell midzone. (B-D) Immunolocalization of Rab11-GFP in fixed wild-type and *bru* mutant spermatocytes stained with DAPI (DNA). (B) Prophase. (C) Anaphase. In the wild-type spermatocyte, an arrow indicates the parafusorial membranes that envelope the meiotic spindle. (D) Late telophase spermatocytes. Arrows indicate the cell midzones. Scale bars: 10 μm.

The *bru* defect in acroblast formation was not simply due to the multinucleate nature of the spermatids, because acroblasts were well formed in 100% (*n*=84) of tetranucleate early spermatids from the male meiotic cytokinesis mutant *pebble* (*pbl*) (Fig. 7B). *fws*, *gio* and *Rab11* mutants also exhibit failure of both acroblast formation and cleavage-furrow ingression, implying a mechanistic link between these processes (Farkas et al., 2003; Giansanti et al., 2007; Giansanti et al., 2006).

Discussion

To better understand the molecular mechanisms of cytokinesis, we characterized *brunelleschi* (*bru*), a gene that we had previously identified as being required for male meiotic cytokinesis in *Drosophila*. We have shown that *bru* encodes the *Drosophila* ortholog of the yeast protein TRS120p, a subunit of the TRAPPII vesicle-trafficking protein complex. Additionally, we found that *bru* genetically interacts with the membrane-trafficking genes *Rab11* and *fwd*.

In yeast, TRAPPII performs membrane-trafficking functions at the Golgi and is required for viability. The complex is composed of the seven TRAPPI complex subunits plus three TRAPPII-specific subunits encoded by the essential genes *TRS120* and *TRS130*, and the non-essential gene *TRS65* (Sacher et al., 2000; Sacher et al., 2001). The fact that all of the yeast TRAPPII subunits except Trs65p (Cox et al., 2007) have orthologs in *Drosophila* raises the possibility that flies have a TRAPPII complex that functions in membrane trafficking at the Golgi. Whereas yeast TRAPPI facilitates ER-to-Golgi transport (Barrowman et al., 2000; Sacher et al., 2001; Sacher et al., 1998), TRAPPII appears to regulate trafficking events at the late Golgi because mutations affecting Trs120p disrupt trafficking from the early endosome to the Golgi and mutations affecting Trs130p cause a block in general secretion from the Golgi (Cai et al., 2005; Sacher et al., 2001). Biochemically, both TRAPPI and TRAPPII in yeast act as GDP-GTP exchange factors (GEFs) that activate Rab homologs: TRAPPI acts as a GEF for the Rab1 ortholog Ypt1p, whereas there is debate as to whether the GEF activity of TRAPPII is directed toward Ypt1p or the Rab11 othologs Ypt31p/Ypt32p (Cai et al., 2008; Jones et al., 2000; Sacher et al., 2001; Sacher et al., 2008). While we were writing this manuscript, Yamasaki and co-workers (Yamasaki et al., 2009) reported that mammalian cells possess only a single, high molecular weight TRAPP complex that is analogous to the TRAPPII complex of yeast. This mTRAPPII complex was found to act as a GEF for Rab1 but not Rab11 (Yamasaki, 2009). In contrast to yeast, mTRAPPII is associated with markers of the early Golgi where it appears to function in early intra-Golgi trafficking. Thus, the functions of TRAPPII might differ between yeast and metazoans (Yamasaki et al., 2009).

Given the greater evolutionary conservation between the *Drosophila* and mTRAPPII subunits (Cox et al., 2007), we anticipate that the activity of *Drosophila* TRAPPII will be more similar to that of the mammalian complex. Several observations are consistent with this possibility. Bru and dBet3 are both enriched at the Golgi while also distributed throughout the cytoplasm, similarly to the pattern exhibited by mTrs130 and mTrs23, although the latter have a more punctate cytoplasmic distribution (Yamasaki et al., 2009). Also, TRAPPII subunits from both organisms contribute to the organization of Golgi membranes. *bru* wild-type function is required for formation of the acroblast, a Golgi-derivative in spermatids that has an extensive cisternal organization (Farkas et al., 2003; Tates, 1971), and mTrs130 is required for the maintenance of Golgi stacks (Yamasaki et al., 2009).

We have observed genetic interactions between *bru* and *Rab11*. *bru;Rab11* double mutants are synthetically lethal, and a single copy of *bru* exacerbates the failure of male meiotic cytokinesis in a *Rab11*

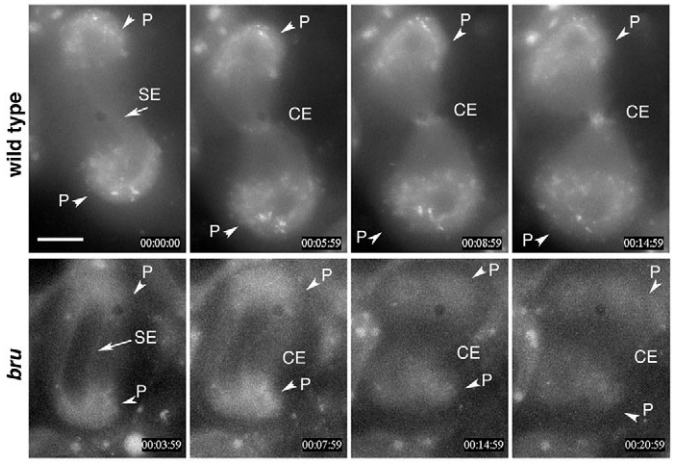

Fig. 6. Rab11-GFP fails to accumulate at the cleavage furrow of telophase spermatocytes from the *bru* mutant. Selected frames from supplementary material Movies 3 and 4 showing +/+ (wild type) and $bru^{Z3358}/Df(2L)pr2b$ (*bru*) spermatocytes expressing *Rab11-GFP* undergoing telophase of meiosis I at 25°C. The numbers at the bottom of each frame indicate time elapsed from the beginning of imaging. Both wild type and *bru* mutant spermatocytes display an enrichment of Rab11-GFP at the spindle envelope (SE) and at the cell poles (P) during anaphase, shown in the left panels. However, although Rab11-GFP appears to be associated with numerous puncta at the cell poles of the wild-type spermatocyte, only a few puncta are visible at the cell poles of the *bru* mutant spermatocyte. As meiosis proceeds, Rab11-GFP accumulates at the cell equator (CE) in the midzone of the wild-type spermatocyte but fails to accumulate in the midzone of the *bru* mutant spermatocyte. Scale bar: 10 μm.

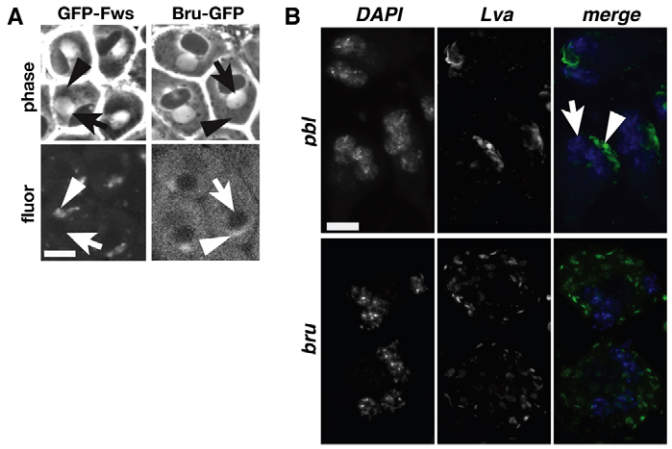

Fig. 7. *bru* is required for acroblast formation. (A) Live squashed early spermatids expressing the indicated fusion proteins, imaged by phase-contrast and conventional fluorescence. Arrows indicate nuclei and arrowheads indicate acroblasts. (B) Tetranucleate early spermatids from *bru* and *pbl* mutants fixed and stained with anti-Lva (green) and DAPI (blue). $pbl^{Z4836}/Df(3R)pbl\text{-}NR$ (*pbl*) and $bru^{Z3358}/Df(2L)pr2b$ (*bru*). In the *pbl* mutant spermatids, four nuclei (large arrow) are associated with a single acroblast (arrowhead). *bru* mutant spermatids do not exhibit an organized acroblast and their cytoplasm contains many Lva-positive bodies. Scale bars: 10 μm.

hypomorphic mutant background. Additionally, loss of *bru* wild-type function abrogates the localization of Rab11 to polar puncta and the cleavage furrow in dividing spermatocytes. These findings are consistent with observations in yeast, where overexpression of the *Rab11* ortholog *YPT31* suppresses lethality resulting from deletion of either *TRS120* or *TRS130* (Zhang et al., 2002), and loss of *TRS130* function via a temperature-sensitive mutation causes redistribution of the Rab1 and Rab11 orthologs, Ypt1p, Ypt31p/Ypt32p (Morozova et al., 2006). Rab11 has been ascribed roles in both exocytic and endocytic pathways in different cell types (Chen et al., 1998; de Graaf et al., 2004; Satoh et al., 2005; Ullrich et al., 1996; Urbe et al., 1993; Wilcke et al., 2000). For example, *Drosophila* photoreceptor cells lack a recycling endosome (RE), and Rab11 localizes to the *trans*-Golgi where it is required for proper trafficking of rhodopsin to the plasma membrane (Satoh et al., 2005). Similarly, in spermatocytes, which also lack a Rab11-enriched RE, Rab11 localizes to the Golgi and is likely to function in the Golgi-to-plasma membrane trafficking required for cytokinesis (Giansanti et al., 2007). Rab11 also performs an essential role in furrow formation during the cellularization of *Drosophila* embryos via the RE (Riggs et al., 2003), and in many cell types, Rab11 and/or endosomal components contribute to the late stages of cytokinesis (Montagnac et al., 2008; Prekeris and Gould, 2008). However, the functional requirement for the Golgi-specific *Syntaxin 5* and the COG5 ortholog *fws* (Farkas et al., 2003; Xu et al., 2002), as well as the sensitivity to BFA, imply that cytokinesis in spermatocytes depends on membrane trafficking through the Golgi. Thus, the genetic interaction between *bru* and *Rab11*, taken together with the localization of Bru to the Golgi, would be in agreement with the possibility that *bru* functions in early intra-Golgi traffic whereas *Rab11* promotes the trafficking of Golgi-derived membrane to the cell surface.

bru also genetically interacts with *fwd*, a PI4Kβ-encoding gene that is also required in spermatocytes for contractile ring constriction and the accumulation of Rab11 at polar puncta and the cleavage furrow (Brill et al., 2000; Giansanti et al., 2004; Giansanti et al., 2007). Here, we have shown that that *bru;fwd* double mutants display a cytokinesis phenotype that is much stronger than either single mutant. Interestingly, genetic and biochemical interactions have been reported between Rab11 and PI4Kβ, or their orthologs, in several systems (Meyers and Cantley, 1997; de Graaf et al., 2004; Sciorra et al., 2004; Giansanti et al., 2007). In mammalian cells, the efficiency of Golgi to plasma membrane transport correlates with the level of PI4Kβ-mediated recruitment of GTP-bound Rab11 to the Golgi (de Graaf et al., 2004). Previously, we have shown that *fwd* and *Rab11* are in the same epistasis group and therefore function in the same pathway in the context of spermatocyte cytokinesis (Giansanti, 2007). That *bru* genetically interacts with both *fwd* and *Rab11* is consistent with this finding. Thus, we propose that *bru* cooperates with a parallel *fwd-Rab11* pathway in supporting membrane addition to the cleavage furrow.

Although *TRS120* is an essential gene in yeast, we do not know whether *bru* is essential in *Drosophila*. Because the two alleles of *bru* were recovered from a collection of homozygous-viable, male-sterile mutants (Giansanti et al., 2004; Wakimoto et al., 2004), lethal alleles of *bru* could not have been found in our screen. The *bru* mutations we characterized each introduce a premature stop codon that would remove the C-terminal half of the encoded polypeptide. However, if the mutant mRNAs were translated, the regions of highest amino acid sequence conservation in the Bru N-terminal half would be retained. In this case, the temperature sensitivity of the *bru* allele might reflect the degree to which the truncated Bru protein physically associates with TRAPPII, with association being more stable at 18°C. In this regard, a number of yeast *TRS120* mutations that cause loss of the Trs120p C-terminus are viable and exhibit temperature-sensitive phenotypes in membrane trafficking (Cai et al., 2005). It is also possible that the process of membrane trafficking in *Drosophila* spermatocytes is itself sensitive to

temperature, such that higher temperatures generally exacerbate any defects in the membrane trafficking machinery.

We have shown that *bru* mutant spermatocytes form a regular contractile ring, but that the ring fails to constrict properly; this phenotype is shared with mutations in other membrane trafficking genes such as *fwd*, *fws*, *gio* and *Rab11* (Brill et al., 2000; Farkas et al., 2003; Gatt and Glover, 2006; Giansanti et al., 2004; Giansanti et al., 2006; Giansanti et al., 2007). In other cell types, the lack of membrane-trafficking components can interfere with the initial assembly of the contractile ring (Somma et al., 2002; Niswonger et al., 1997). Although the mechanisms underlying the interplay between ring behavior and membrane trafficking are currently unclear, there are two non-mutually exclusive possibilities. Lack of membrane addition to the advancing cleavage furrow might result in plasma membrane tension that counteracts the force of actomyosin ring constriction (Gatt and Glover, 2006; Giansanti et al., 2007). Alternatively, there might be vesicle-associated contractile ring components and actin-remodeling factors that fail to be transported to the cell midzone as observed for actin-vesicle transport in cellularizing *Drosophila* embryos (Giansanti et al., 2007; Albertson et al., 2008). Either of these possibilities might account for the defect in ring constriction and furrow ingression that we observe in dividing spermatocytes of the *bru* mutant.

We have not observed any phenotypes for *bru* mutants outside the male germline. However, the synthetic lethality resulting from simultaneous loss of both *bru* and *Rab11* wild-type functions, in conjunction with the expression of *bru* message in females and embryos, suggests that *bru* serves an important role in membrane trafficking homeostasis in other cell types. Thus, the specific requirement for a TRAPPII subunit in spermatocyte cleavage furrow ingression raises the possibility that dividing spermatocytes have an elevated requirement for fast and efficient vesicle transport during cytokinesis. This notion is consistent with the low surface-area-to-volume ratio of these cells and the challenge of producing four daughter cells via two meiotic divisions within a period of 194 minutes (Cenci et al., 1994; Dyer et al., 2007; Tates, 1971). The observation that spermatocytes lack membrane protrusions and microvilli (Tates, 1971) suggests that existing plasma membrane cannot be reorganized to provide the additional surface are required for cleavage furrow ingression. Thus, the success of male gamete formation in *Drosophila* might rely on a level of membrane-trafficking efficiency that is not essential in other cell types of this organism.

Materials and Methods

Fly strains and husbandry

Viable, male-sterile alleles bru^{Z3358}, bru^{Z0704} and pbl^{Z4836} are described (Giansanti et al., 2004). fwd^{Z0453}, *Rab11* alleles and *Rab11-GFP* have been described (Giansanti et al., 2007). The *Spaghetti squash (Sqh)-GFP* transgene was used to express RLC-GFP (Royou et al., 2002). Balancer chromosomes are described (Lindsley and Zimm, 1992). *EYFP-Golgi* (LaJeunesse et al., 2004), pbl^{5} (Lehner, 1992) and chromosomal deficiencies *Df(2L)pr2b*, *Df(3R)pbl-NR* and *Df(3L)7C* (Giansanti et al., 2004) were from the Bloomington *Drosophila* Stock Center at Indiana University.

Microscopy and immunofluorescence

Squashed adult or larval testes were imaged by phase-contrast on a Zeiss Axioskop or by fluorescence on a Zeiss Axiophot and processed as described (Farkas et al., 2003). For multinucleate spermatid counts, live testes were lightly squashed and imaged at multiple focal planes through the sample to prevent disruption of the large multinucleate cells while counting the number of nuclei. For RLC-GFP counts during meiosis I, live testes were squashed to flatten the cells so that the rings could be more easily viewed. Immunofluorescence staining of fixed testes used rabbit anti-Lava lamp (a gift from John Sisson, University of Texas, Austin, TX), anti-Rab11 and anti-Centrosomin (a gift from Tim Megraw, Florida State University, Tallahassee, FL) (Giansanti et al., 2007); rabbit anti-GFP (a gift from Gianluca Cestra, Università di Roma 'La Sapienza', Rome, Italy) and mouse anti-p120 (Calbiochem, anti-Golgi, *Drosophila*, 345867) were used at 1:100 to stain testes fixed as per (Giansanti et al., 2007). Live unsquashed spermatocyte cysts in Schneider's *Drosophila* Medium (Gibco) were imaged on a Leica SP2 AOBS confocal laser-scanning microscope. Serial excitation of fluorophores prevented signal bleed-through. EGFP was excited at 488 nm and 489-512 nm emissions collected. EFYP was excited at 514 nm and 520-576 nm emissions collected. All spermatocytes are primary spermatocytes in stages of meiosis I.

Time-lapse imaging of RLC-GFP (Royou et al., 2002) or Rab11-GFP (Dollar et al., 2002) in live primary spermatocytes was carried out as described (Giansanti et al., 2007). Cells were examined with a Zeiss Axiovert 20 microscope equipped with a 63× (for RLC-GFP) or 100× objective (for Rab11-GFP) and a filter wheel combination (Chroma). Images were acquired with a CoolSnap HQ camera (Photometrics) controlled through a Metamorph software package (Universal Imaging). Images were collected at 1- or 1.5-minute intervals, and 10 fluorescent optical sections were captured at 1-μm *z* steps. Movies were created using the Metamorph software; each fluorescent image shown in Figs 1 and 6 is the maximum-intensity projection of all the sections. Wild-type spermatocytes contained no mutant alleles.

Cloning of *bru*

bru was mapped to 38B5-E1 (Giansanti et al., 2004), and additional deficiency complementation tests placed the locus between the proximal breakpoints of *Df(2L)pr-A16* and *Df(2L)pr2b* (Lindsley and Zimm, 1992). Single-embryo PCR analysis of homozygous deficiency embryos (protocol from C.C.R. available upon request) delimited the *bru* interval to 24 kbp. Annotated protein-coding genes in the interval were sequenced on the mutant chromosomes and compared with the wild-type Zuker background in which the mutations were made. Each *bru* mutant allele revealed a mutation in annotated gene *CG2478*. A 5.8-kbp *Hin*dIII genomic fragment containing the *CG2478* coding sequence, 5′ UTR and 700 bp of 5′ DNA that included the 500 bp of the upstream gene *CG2614*, but no other known genes, was subcloned from the *Drosophila* P1 clone DS02109 and fused to a PCR product containing an additional 100 bp of the 3′ untranslated region of CG2478. This 5.9-kbp fragment was transferred into pCaSpeR4, and the resulting pCaSpeR4-bru rescue construct was used to generate transgenic flies by standard methods as per (Farkas et al., 2003). The transgene was then crossed into the *bru* mutant background to test for phenotypic rescue of male meiotic cytokinesis.

Modified transgenes

For *bru-GFP*, the EGFP coding sequence (Clontech) was inserted immediately before the stop codon of the *bru* rescue construct. For *dBet3-YFP*, the EYFP coding sequence (Clontech) was inserted immediately before the stop codon of the cDNA RE68712, which corresponds to the message from *CG3911*, and flanked with 2 kbp of 5′ and 3′ *CG3911* genomic sequences from BAC RPC1-98 E20. For mutagenesis of the Bru motif I, a 475-bp *Nhe*I fragment was subcloned from the *bru* rescue fragment, and six selected codons were sequentially mutated (XL site-directed mutagenesis kit, Stratagene) to encode alanines. The mutated fragment was sequence-verified and subcloned back into pCaSpeR4-*bru* to generate transgenic flies.

BFA treatment

White pre-pupae larval testes were cultured in 25 μl Schneider's *Drosophila* medium with penicillin/streptomycin and 30 μg/ml of BFA (Sigma) from a 10 μg/ml stock in ethanol or an equal volume of ethanol on open slides in a humidified chamber at room temperature. Observation of multinucleate spermatids in wild-type *Oregon R* testes required incubation in BFA for 14 hours, consistent with the observation that one cyst of spermatocytes would be expected to execute meiotic cytokinesis every 10 hours (Lindsley and Tokuyasu, 1980).

We thank Robert Cohen, Gianluca Cestra, Tim Megraw, John Sisson and William Sullivan for reagents; Julie Brill and Bruce Baker for discussions; Matthew Fish for injection of transgenic constructs; Tom Clandinin, Roger Hoskins and Mark Hiller for assistance, and Susan Alexander for excellent administrative support. We are also grateful to Roberto Becattini and the Crisel Instruments staff for help with live imaging. We dedicate this paper to the memory of John Sisson. This work was funded by National Institutes of Health (NIH) NRSA fellowship 5 F32 GM20574-03 to C.C.R., NIH 1R01GM62276 to M.T.F., and a grant from Centro di Eccellenza di Biologia e Medicina Molecolare (BEMM) to M.G. Deposited in PMC for release after 12 months.

References

Aimar, C. (1997). Formation of new plasma membrane during the first cleavage cycle in the egg of Xenopus laevis: an immunocytological study. *Dev. Growth Differ.* **39**, 693-704.

Albertson, R., Riggs, B. and Sullivan, W. (2005). Membrane traffic: a driving force in cytokinesis. *Trends Cell Biol.* **15**, 92-101.

Albertson, R, Cao, J, Hsieh, T. S. and Sullivan, W. (2008). Vesicles and actin are targeted to the cleavage furrow via furrow microtubules and the central spindle. *J. Cell Biol.* **181**, 777-790.

Barrowman, J., Sacher, M. and Ferro-Novick, S. (2000). TRAPP stably associates with the Golgi and is required for vesicle docking. *EMBO J.* **19**, 862-869.

Blose, S. H. (1979). Ten-nanometer filaments and mitosis: maintenance of structural continuity in dividing endothelial cells. *Proc. Natl. Acad. Sci. USA* **76**, 3372-3376.

Bluemink, J. G. and de Laat, S. W. (1973). New membrane formation during cytokinesis in normal and cytochalasin B-treated eggs of Xenopus laevis. I. Electron microscope observations. *J. Cell Biol.* **59**, 89-108.

Boucrot, E. and Kirchhausen, T. (2007). Endosomal recycling controls plasma membrane area during mitosis. *Proc. Natl. Acad. Sci. USA* **104**, 7939-7944.

Brill, J. A., Hime, G. R., Scharer-Schuksz, M. and Fuller, M. T. (2000). A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. *Development* **127**, 3855-3864.

Burgess, R. W., Deitcher, D. L. and Schwarz, T. L. (1997). The synaptic protein syntaxin1 is required for cellularization of Drosophila embryos. *J. Cell Biol.* **138**, 861-875.

Cai, H., Zhang, Y., Pypaert, M., Walker, L. and Ferro-Novick, S. (2005). Mutants in trs120 disrupt traffic from the early endosome to the late Golgi. *J. Cell Biol.* **171**, 823-833.

Cai, Y., Chin, H. F., Lazarova, D., Menon, S., Fu, C., Cai, H., Sclafani, A., Rodgers, D. W., De La, Cruz, E. M., Ferro-Novick, S. et al. (2008). The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. *Cell* **133**, 1202-1213.

Cenci, G., Bonaccorsi, S., Pisano, C., Verni, F. and Gatti, M. (1994). Chromatin and microtubule organization during premeiotic, meiotic and early postmeiotic stages of Drosophila melanogaster spermatogenesis. *J. Cell Sci.* **107**, 3521-3534.

Chen, W., Feng, Y., Chen, D. and Wandinger-Ness, A. (1998). Rab11 is required for trans-golgi network-to-plasma membrane transport and a preferential target for GDP dissociation inhibitor. *Mol. Biol. Cell* **9**, 3241-3257.

Conner, S. D. and Wessel, G. M. (1999). Syntaxin is required for cell division. *Mol. Biol. Cell* **10**, 2735-2743.

Cox, R., Chen, S. H., Yoo, E. and Segev, N. (2007). Conservation of the TRAPPII-specific subunits of a Ypt/Rab exchanger complex. *BMC Evol. Biol.* **7**, 12.

Danilchik, M. V., Bedrick, S. D., Brown, E. E. and Ray, K. (2003). Furrow microtubules and localized exocytosis in cleaving Xenopus laevis embryos. *J. Cell Sci.* **116**, 273-283.

de Graaf, P., Zwart, W. T., van Dijken, R. A., Deneka, M., Schulz, T. K., Geijsen, N., Coffer, P. J., Gadella, B. M., Verkleij, A. J., van der Sluijs, P. et al. (2004). Phosphatidylinositol 4-kinasebeta is critical for functional association of rab11 with the Golgi complex. *Mol. Biol. Cell* **15**, 2038-2047.

Dollar, G., Struckhoff, E., Michaud, J. and Cohen, R. S. (2002). Rab11 polarization of the Drosophila oocyte: a novel link between membrane trafficking, microtubule organization, and oskar mRNA localization and translation. *Development* **129**, 517-526.

Dyer, N., Rebollo, E., Dominguez, P., Elkhatib, N., Chavrier, P., Daviet, L., Gonzalez, C. and Gonzalez-Gaitan, M. (2007). Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. *Development* **134**, 4437-4447.

Erickson, C. A. and Trinkaus, J. P. (1976). Microvilli and blebs as sources of reserve surface membrane during cell spreading. *Exp. Cell Res.* **99**, 375-384.

Farkas, R. M., Giansanti, M. G., Gatti, M. and Fuller, M. T. (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.

Finger, F. P. and White, J. G. (2002). Fusion and fission: membrane trafficking in animal cytokinesis. *Cell* **108**, 727-730.

Fuller, M. T. (1993). Spermatogenesis. In *The development of Drosophila melanogaster* (ed M. Bate and A. Martinez-Arias), pp. 71-147. New York, Cold Spring Harbor Press.

Gatt, M. K. and Glover, D. M. (2006). The *Drosophila* phosphatidylinositol transfer protein encoded by vibrator is essential to maintain cleavage-furrow ingression in cytokinesis. *J. Cell Sci.* **119**, 2225-2235.

Giansanti, M. G., Farkas, R. M., Bonaccorsi, S., Lindsley, D. L., Wakimoto, B. T., Fuller, M. T. and Gatti, M. (2004). Genetic dissection of meiotic cytokinesis in Drosophila males. *Mol. Biol. Cell* **15**, 2509-2522.

Giansanti, M. G., Bonaccorsi, S., Kurek, R., Farkas, R. M., Dimitri, P., Fuller, M. T. and Gatti, M. (2006). The class I PITP giotto is required for Drosophila cytokinesis. *Curr. Biol.* **16**, 195-201.

Giansanti, M. G., Belloni, G. and Gatti, M. (2007). Rab11 is required for membrane trafficking and actomyosin ring constriction in meiotic cytokinesis of Drosophila males. *Mol. Biol. Cell* **18**, 5034-5047.

Jankovics, F., Sinka, R. and Erdelyi, M. (2001). An interaction type of genetic screen reveals a role of the Rab11 gene in oskar mRNA localization in the developing Drosophila melanogaster oocyte. *Genetics* **158**, 1177-1188.

Jantsch-Plunger, V. and Glotzer, M. (1999). Depletion of syntaxins in the early Caenorhabditis elegans embryo reveals a role for membrane fusion events in cytokinesis. *Curr. Biol.* **9**, 738-745.

Jones, S., Newman, C., Liu, F. and Segev, N. (2000). The TRAPP complex is a nucleotide exchanger for Ypt1 and Ypt31/32. *Mol. Biol. Cell* **11**, 4403-4411.

Klausner, R. D., Donaldson, J. G. and Lippincott-Schwartz, J. (1992). Brefeldin A: insights into the control of membrane traffic and organelle structure. *J. Cell Biol.* **116**, 1071-1080.

Knutton, S., Sumner, M. C. and Pasternak, C. A. (1975). Role of microvilli in surface changes of synchronized P815Y mastocytoma cells. *J. Cell Biol.* **66**, 568-576.

LaJeunesse, D. R., Buckner, S. M., Lake, J., Na, C., Pirt, A. and Fromson, K. (2004). Three new Drosophila markers of intracellular membranes. *Biotechniques* **36**, 784-788, 790.

Lehner, C. F. (1992). The pebble gene is required for cytokinesis in Drosophila. *J. Cell Sci.* **103**, 1021-1030.

Lindsley, D. L. and Tokuyasu, K. T. (1980). Genetics and Biology of *Drosophila*. New York: Academic Press.

Lindsley, D. L. and Zimm, G. G. (1992). The Genome of *Drosophila melanogaster*. San Diego, CA: Academic Press, Inc.

Meyers, R. and Cantley, L. C. (1997). Cloning and characterization of a wortmannin-sensitive human phosphatidylinositol 4-kinase. *J. Biol. Chem.* **272**, 4384-4390.

Montagnac, G., Echard, A. and Chavrier, P. (2008). Endocytic traffic in animal cell cytokinesis. *Curr. Opin. Cell Biol.* **20**, 454-461.

Morozova, N., Liang, Y., Tokarev, A. A., Chen, S. H., Cox, R., Andrejic, J., Lipatova, Z., Sciorra, V. A., Emr, S. D. and Segev, N. (2006). TRAPPII subunits are required for the specificity switch of a Ypt-Rab GEF. *Nat. Cell Biol.* **8**, 1263-1269.

Niswonger, M. L. and O'Halloran, T. J. (1997). A novel role for clathrin in cytokinesis. *Proc. Natl. Acad. Sci. USA* **94**, 8575-8578.

Prekeris, R. and Gould, G. W. (2008). Breaking up is hard to do-membrane traffic in cytokinesis. *J. Cell Sci.* **121**, 1569-1576.

Prokopenko, S. N., Brumby, A., O'Keefe, L., Prior, L., He, Y., Saint, R. and Bellen, H. J. (1999). A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in Drosophila. *Genes Dev.* **13**, 2301-2314.

Riggs, B., Rothwell, W., Mische, S., Hickson, G. R., Matheson, J., Hays, T. S., Gould, G. V. and Sullivan, W. (2003). Actin cytoskeleton remodeling during early *Drosophila* furrow formation requires recycling endosomal components Nuclear-fallout and Rab11. *J. Cell Biol.* **163**, 143-154.

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

Sacher, M., Jiang, Y., Barrowman, J., Scarpa, A., Burston, J., Zhang, L., Schieltz, D., Yates, J. R., 3rd, Abeliovich, H. and Ferro-Novick, S. (1998). TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. *EMBO J.* **17**, 2494-2503.

Sacher, M., Barrowman, J., Schieltz, D., Yates, J. R., 3rd and Ferro-Novick, S. (2000). Identification and characterization of five new subunits of TRAPP. *Eur. J. Cell Biol.* **79**, 71-80.

Sacher, M., Barrowman, J., Wang, W., Horecka, J., Zhang, Y., Pypaert, M. and Ferro-Novick, S. (2001). TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. *Mol. Cell* **7**, 433-442.

Sacher, M., Kim, Y. G., Lavie, A., Oh, B. H. and Segev, N. (2008). The TRAPP complex: insights into its architecture and function. *Traffic* **9**, 2032-2042.

Satoh, A. K., O'Tousa, J. E., Ozaki, K. and Ready, D. F. (2005). Rab11 mediates post-Golgi trafficking of rhodopsin to the photosensitive apical membrane of Drosophila photoreceptors. *Development* **132**, 1487-1497.

Sciorra, V. A., Audhya, A., Parsons, A. B., Segev, N., Boone, C. and Emr, S. D. (2004). Synthetic genetic array analysis of the PtdIns 4-kinase Pik1p identifies components in a Golgi-specific Ypt31/rab-GTPase signaling pathway. *Mol. Biol. Cell.* **2,** 776-793.

Shuster, C. B. and Burgess, D. R. (2002). Targeted new membrane addition in the cleavage furrow is a late, separate event in cytokinesis. *Proc. Natl. Acad. Sci. USA* **99**, 3633-3638.

Sisson, J. C., Field, C., Ventura, R., Royou, A. and Sullivan, W. (2000). Lava lamp, a novel peripheral golgi protein, is required for Drosophila melanogaster cellularization. *J. Cell Biol.* **151**, 905-918.

Skop, A. R., Bergmann, D., Mohler, W. A. and White, J. G. (2001). Completion of cytokinesis in C. elegans requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. *Curr. Biol.* **11**, 735-746.

Somma, M. P., Fasulo, B., Cenci, G., Cundari, E. and Gatti, M. (2002). Molecular dissection of cytokinesis by RNA interference in *Drosophila* cultured cells. *Mol. Biol. Cell* **13**, 2448-2460.

Tates, A. D. (1971). Cytodifferentiation during spermatogenesis in *Drosophila melanogaster*: An electron microscope study. In *Laboratorium voor Stralengenetica*. Leiden, ND: Rijksuniversiteit.

Ullrich, O., Reinsch, S., Urbe, S., Zerial, M. and Parton, R. G. (1996). Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* **135**, 913-924.

Urbe, S., Huber, L. A., Zerial, M., Tooze, S. A. and Parton, R. G. (1993). Rab11, a small GTPase associated with both constitutive and regulated secretory pathways in PC12 cells. *FEBS Lett.* **334**, 175-182.

Wakimoto, B. T., Lindsley, D. L. and Herrera, C. (2004). Toward a comprehensive genetic analysis of male fertility in Drosophila melanogaster. *Genetics* **167**, 207-216.

Wilcke, M., Johannes, L., Galli, T., Mayau, V., Goud, B. and Salamero, J. (2000). Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-golgi network. *J. Cell Biol.* **151**, 1207-1220.

Wilson, K. L., Fitch, K. R., Bafus, B. T. and Wakimoto, B. T. (2006). Sperm plasma membrane breakdown during Drosophila fertilization requires sneaky, an acrosomal membrane protein. *Development* **133**, 4871-4879.

Wilson, R. J., Goodman, J. L. and Strelets, V. B. (2008). FlyBase: integration and improvements to query tools. *Nucleic Acids Res.* **36**, D588-D593.

Xu, H., Brill, J. A., Hsien, J., McBride, R., Boulianne, G. L. and Trimble, W. S. (2002). Syntaxin 5 is required for cytokinesis and spermatid differentiation in Drosophila. *Dev. Biol.* **251**, 294-306.

Yamasaki, A., Menon, S., Yu, S., Barrowman, J., Meerloo, T., Oorschot, V., Klumperman, J., Satoh, A. and Ferro-Novick, S. (2009). mTrs130 is a component of a mammalian TRAPPII complex, a Rab1 GEF that binds to COPI-coated vesicles. *Mol. Biol. Cell* **20**, 4205-4215.

Zhang, C. J., Bowzard, J. B., Greene, M., Anido, A., Stearns, K. and Kahn, R. A. (2002). Genetic interactions link ARF1, YPT31/32 and TRS130. *Yeast* **19**, 1075-1086.