Mars promotes dTACC dephosphorylation on mitotic spindles to ensure spindle stability

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Microtubule-associated proteins (MAPs) ensure the fidelity of chromosome segregation by controlling microtubule (MT) dynamics and mitotic spindle stability. However, many aspects of MAP function and regulation are poorly understood in a developmental context. We show that mars, which encodes a Drosophila melanogaster member of the hepatoma up-regulated protein family of MAPs, is essential for MT stabilization during early embryogenesis. As well as associating with spindle MTs in vivo, Mars binds directly to protein phosphatase 1 (PP1) and coimmunoprecipitates from embryo extracts with minispindles and Drosophila transforming acidic coiled-coil (dTACC), two MAPs that function as spindle assembly factors. Disruption of binding to PP1 or loss of mars function results in elevated levels of phosphorylated dTACC on spindles. A nonphosphorylatable form of dTACC is capable of rescuing the lethality of mars mutants. We propose that Mars mediates spatially controlled dephosphorylation of dTACC, which is critical for spindle stabilization.

Introduction

Microtubule-associated proteins (MAPs) ensure the fidelity of chromosome segregation during cell division by controlling the formation and stability of spindle microtubules (MTs). Because disruption of spindle formation can promote genomic instability, an understanding of MAP function and regulation is central to dissecting basic mechanisms of tumorigenesis and would be invaluable in designing new therapies for the treatment of cancer. Although much progress has been made in understanding the functions of spindle-associated MAPs in the last few years, many aspects of their role or regulation remain to be fully elucidated. Human hepatoma up-regulated protein (HURP) has been described as a highly charged MAP that can bind directly to MTs in vitro and enhance their polymerization (Santarella et al., 2007). In vivo, HURP is part of a Ran-dependent complex that stabilizes mitotic MTs and is required for the formation and function of bipolar mitotic spindles (Koffa et al., 2006; Sillje et al., 2006; Wong and Fang, 2006). However, it is not known how HURP-associated proteins functionally interact with one another in a developmental context to support normal cellular function.

Mars, a D. melanogaster sequence homologue of HURP, was previously identified as a protein phosphatase 1 (PP1) binding protein, implicating reversible phosphorylation in the control of Mars or Mars-associated proteins (Bennett and Alphey, 2004; Yang et al., 2005). In this paper, we report the essential role of mars during early embryogenesis, its interactions with other MAPs, and its key role in promoting protein dephosphorylation on mitotic spindles to ensure spindle stability.

Results and discussion

HURP is a component of the mitotic spindle apparatus. To determine the cell cycle distribution of Mars, we generated a Mars-specific antibody and used it to stain syncytial embryos undergoing nuclear division. In prophase, Mars antibody staining was predominantly around the centrosome. In metaphase and anaphase, Mars was localized on spindle MTs in a gradient along the pole-to-pole axis with more intense staining at the centrosome-proximal regions (Fig. 1A). This is distinct from the distribution of HURP, which has been shown to be predominantly at chromatin-proximal regions until telophase, when levels sharply decline (Koffa et al., 2006; Sillje et al., 2006; Wong and Fang, 2006). In telophase, we observed a low level of discrete Mars staining at the midbody (not depicted), but the majority of Mars protein appeared to be spread over the nuclear envelope where it persisted during interphase (Fig. 1A and Fig. S1, available at...
observed in *mars* mutants is caused by arrest in embryogenesis after fertilization. The viability of embryos laid by *mars* mutant mothers was restored by moderate overexpression of *mars* WT (wild-type *mars*) in the female germline (Fig. 3 A), indicating that the failure of *mars* mutant embryos to develop is caused by disruption of the *mars* transcription unit.

To determine the cause of the lethality of *mars* mutant embryos, we fixed embryos from wild-type and *mars* mutant females and examined the distribution of nuclei and MTs. Embryos lacking maternal *mars* arrested during early embryogenesis after no more than five nuclear divisions. 81.9% (n = 144) of 15–45-min embryos laid by *mars* WT mothers exhibited at least two discrete DNA-containing regions (Fig. 3, B and C). The first of these was localized to the embryonic cortex and resembled a polar body, most likely containing the unused products of meiosis II (Wilson and Borisy, 1998). One or more additional DNA-containing regions, each surrounded by a bipolar spindle, were also observed more centrally, indicating that the vast majority of mutant embryos pass through meiosis to form one or more mitotic figure. Notably, the spindle structures in *mars* WT mutant embryos were very small and weak, albeit still bipolar (Fig. 3 C). Most spindles had at least one detached centrosome, possibly because of weakened spindle–centrosome interactions (Fig. 3, C–E). We also observed unaligned chromosomes in the *mars* WT mutant, which is indicative of insufficient MT attachment or tension (Fig. S2 A, available at http://www.jcb.org/cgi/content/full/jcb.200712080/DC1). Embryos laid by *mars* homozygous mothers resembled those from *mars* WT mothers, except the phenotype was somewhat less severe (Fig. 3, C–E). Quantitation of α-tubulin staining revealed no significant difference between astral MTs in embryos laid by wild-type and *mars* WT mothers (P = 0.416), whereas cold-resistant kinetochore MTs were destabilized in embryos laid by *mars* WT mothers (Fig. S2 B). Approximately 51% of embryos laid by *mars* WT homozygous mothers (n = 264) failed to develop beyond embryogenesis. Many showed terminal phenotypes at or shortly after gastrulation, presumably as a consequence of primary defects during the early cleavage divisions. Collectively, analysis of loss-of-function mutants suggest that *mars* has a role in spindle MT stabilization. HURP has bundling activity in vitro and in vivo (Koffa et al., 2006; Sillje et al., 2006; Wong and Fang, 2006). Correspondingly, strong overexpression of *mars* WT in syncytial embryos resulted in enlarged spindles with ectopic MT fibers (Fig. 3 F), suggesting that *mars* is limiting for MT bundling.

*mars* was originally isolated from a two-hybrid screen for putative PP1 binding proteins and contains a canonical PP1 binding motif (K/R,x,V,x,F; Bennett and Alphey, 2004; Bennett et al., 2006). To verify interactions with PP1, we performed pulldown experiments between Mars and PP1 (Koffa et al., 2006). To assess the

http://www.jcb.org/cgi/content/full/jcb.200712080/DC1). Double staining for Mars and either Klp10A, which is primarily localized at focused minus ends where it promotes depolymerization and poleward flux (Rogers et al., 2004), or γ-tubulin, which marks the face of the centrosome and nucleates MT polymerization (Jeng and Stearns, 1999), confirmed that Mars is localized at MT minus ends but not at the centrosome (Fig. 1, B and C). Mars’ localization during mitosis was completely disrupted upon treatment with colchicine to depolymerize MTs, indicating that Mars associates with spindle MTs (Fig. 1 D).

To determine the in vivo role of *mars*, we generated a null allele of *mars*, *mars* 1, by imprecise excision of a P element transposon (referred to as *mars* 2 hereafter), which we found inserted in the *mars* 5′ untranslated region (Fig. 2, A and C). *mars* 2 flies express full-length Mars protein at a much lower level than wild type (Fig. 2 B). *mars* 1 flies fail to produce Mars protein, which is consistent with molecular analysis revealing that ~0.84 kb of the coding region, including the translation start site, is deleted in this mutant (Fig. 2, B and C). *mars* mutant flies are viable but female sterile. Notably, eggs laid by *mars* 1, *mars* 2, and *mars* WT flies show a greatly reduced ability to hatch (Fig. 3 A). As we were able to visualize sperm tails in early-arrested embryos laid by *mars* mutant females (unpublished data), we conclude that the sterility
also interacted efficiently with each other in HeLa cell extracts (Fig. 4 C), suggesting that binding to PP1 is an evolutionarily conserved property of HURP proteins. Binding to PP1 prompted us to test functional interactions between Mars and PP1 in vivo. The ability of embryos laid by Mars or PP1a87B heterozygotes to hatch resembled that of the wild type (Fig. 4 D). Embryos laid by flies transheterozygous for Mars and PP1a87B showed a significantly reduced hatch also interacted efficiently with each other in HeLa cell extracts (Fig. 4 C), suggesting that binding to PP1 is an evolutionarily conserved property of HURP proteins.

Binding to PP1 prompted us to test functional interactions between Mars and PP1 in vivo. The ability of embryos laid by Mars or PP1a87B heterozygotes to hatch resembled that of the wild type (Fig. 4 D). Embryos laid by flies transheterozygous for Mars and PP1a87B showed a significantly reduced hatch.
Conversely, the ability of dTACC to associate with MTs was largely moderate levels of ectopic Mars WT in embryos laid by either mars F839A mothers (Fig. 5 B), although we cannot rule out that global ratios of DTACC/p-DTACC was not simply a secondary consequence of aberrant spindle structure. Levels and distribution of p-DTACC were not affected in mutant embryos (Fig. 5 B), although we cannot rule out that global ratios of DTACC/p-DTACC were not affected in mutant embryos (Fig. 5 B). Therefore, although Mars and dTACC associate with one another, they do not appear to be dependent on each other for their localization.

Phosphorylation of dTACC on Ser863 is critical for stabilization of the minus ends of centrosome-associated MTs during mitosis (Barros et al., 2005). Although dTACC is found on both the centrosome and mitotic spindle, phosphorylated dTACC (p-dTACC) is tightly localized to the centrosomes (Barros et al., 2005), suggesting that once phosphorylated, p-dTACC is either unable to exchange with the soluble pool of dTACC or is rapidly dephosphorylated when it leaves the centrosome. The role of dephosphorylated TACC is not known, but it may function to stabilize MTs through lateral interactions with MTs or interactions with MT plus ends. The localization of Mars toward the minus ends of spindle MTs and association with both dTACC and PP1 prompted us to examine the involvement of Mars in maintaining low levels of p-dTACC on the spindle.

To examine the effect of Mars on dTACC phosphorylation, we stained mars mutant embryos with an antibody that specifically recognizes dTACC phosphorylated at Ser863 (p-dTACC). mars mutant embryos showed increased levels of p-dTACC on the mitotic spindles compared with the wild type (Fig. 5, B–D). On careful examination of these mutants, we noticed some spindles that looked normal but possessed elevated levels of p-dTACC (Fig. S3 B), indicating that increased p-dTACC was not simply a secondary consequence of aberrant spindle structure. Levels and distribution of total dTACC appeared normal in mars mutants (Fig. 5 B), although we cannot rule out that global ratios of DTACC/p-DTACC are affected. We used mars F839A to examine whether Mars promotes the dephosphorylation of dTACC by binding to PP1. Embryos with moderate levels of ectopic mars WT in embryos laid by either mars F839A mothers were essentially wild type in appearance and had little or no p-dTACC staining on mitotic spindles. In contrast, mars mutant embryos ectopically expressing mars F839A at comparable levels to those of ectopic mars WT retained elevated p-dTACC staining on spindles (Fig. 5 B and not depicted).
To test whether promoting the dephosphorylation of dTACC is a critical function of mars, we examined whether the sterility of mars mutants could be rescued by a nonphosphorylatable form of dTACC (dTACC<sub>SL</sub>) expressed under control of the dTACC promoter (Barros et al., 2005). dTACC<sub>SL</sub>, but not dTACC<sub>WT</sub> (wild-type dTACC), restored a normal distribution of p-dTACC staining on mitotic spindles (Fig. 5, B–C). Quantification of p-dTACC staining confirmed that dTACC<sub>SL</sub> restored a normal ratio of spindle/centrosomal p-dTACC staining in mars mutant embryos (Fig. 5 D). When we examined hatching of these embryos, we found that the lethality of embryos laid by mars<sup>1/P</sup> mothers was rescued by dTACC<sub>SL</sub> but not dTACC<sub>WT</sub> (Fig. 5 D). Collectively, these data indicate that dephosphorylation of dTACC on the spindle is an essential function of mars. Homozygous mars<sup>1</sup> mutants
were not rescued by dTACC<sup>205SL</sup> (unpublished data), suggesting that residual Mars protein in <i>mars<sup>3P</sup></i> embryos may play a dTACC-independent role, such as MT bundling, or that the level of ectopic dTACC<sup>205SL</sup> was insufficient to compensate for elevated p-dTACC in a <i>mars<sup>1</sup></i> background.

In summary, we have shown that <i>mars</i>, which encodes a <i>D. melanogaster</i> sequence homologue of HURP, is critical for mitotic spindle structure and chromosome segregation during early embryogenesis. The primary defect in <i>mars</i> mutants appears to be loss of spindle MT stability, whereas overexpression of <i>mars</i> leads to the production of enlarged spindles with ectopic MTs. These data are consistent with a role for <i>mars</i> in MT bundling/stabilization similar to that described for its human homologue HURP. However, our identification of Mars as an interacting subunit of PP1 suggests a novel mechanism by which this family of proteins can maintain normal spindle structure in vivo. Binding of PP1 to Mars implicates PP1 in dephosphorylation of Mars or a Mars-associated protein. [ID]FIG5[/ID] Although dTACC may be a substrate of PP1, it is also possible that Mars-bound PP1 may indirectly stimulate dephosphorylation of dTACC on the spindle by activating another protein phosphatase or inactivating a dTACC kinase such as Aurora-A, a known target of PP1 during mitosis (Katayama et al., 2001).

Our genetic experiments indicate that promoting dephosphorylation of dTACC on mitotic spindles is an essential role of Mars. Why is it important to maintain dephosphorylated TACC on MTs or interactions with plus ends and that these functions of dTACC seem to be lost in all the <i>mars</i> mutants and in the dTACC<sup>S863L</sup> mutant? For dTACC phosphorylation or to what extent the effect of dTACC phosphorylation is context dependent. It is conceivable that dTACC stabilizes spindle MTs by establishing lateral interactions with MTs or interactions with plus ends and that these functions of dTACC are impaired when phosphorylated at Ser863.

Is <i>mars</i> a functional homologue of HURP? We have confirmed that various aspects of <i>mars</i> and HURP function are conserved, including spindle stabilization and binding of Mars to MspS and PP1. However, Mars and HURP display apparently distinct spindle localizations, suggesting that there may be differences in how these proteins are used during cell division. This may reflect a wider difference in the organization of MAPs that control MT stability and the formation of bipolar spindles in flies and humans.

Spindle defects caused by lack of TACC phosphorylation or by alterations in TACC or HURP protein levels may lead to genetic instability and are implicated in cancer progression (Raff, 2002; Barros et al., 2005; Brittle and Ohkura, 2005). Our data indicate that spatially controlled dephosphorylation also plays a positive role in TACC function, suggesting that deregulation of either phosphorylation or dephosphorylation of TACC may also be involved in the molecular pathology of cancer by compromising the fidelity of chromosome segregation.

### Materials and methods

#### Fly strains

<i>E(p)2477</i>, referred to here as <i>mars<sup>3</sup></i>, is a homozygous viable P element insertion in the 5′ untranslated region of <i>mars</i>. GFP<sub>dTACC<sup>205SL</sup></sub> and GFP<sub>dTACC<sup>205SL</sup></sub> (gift from J. Raff, The Gurdon Institute, Cambridge, England, UK) have been previously described (Barros et al., 2005). Other fly stains are described in FlyBase (http://www.flybase.org).

#### Isolation and characterization of a <i>mars</i>-null allele

Isolation of a null allele of <i>mars</i> by P element excision from <i>mars<sup>3</sup></i> was performed as follows. Jumpstarter w<sup>y</sup>/Y; isogenic <i>mars<sup>3</sup>/CyO, P(Delta2-3) males were crossed with <i>y</i> w; <i>Trf</i>/CyO females. From each cross, only one w revertant male, <i>y</i> w; <i>mars<sup>3</sup>/CyO</i>, in which the <i>P</i> element was excised, was individually crossed back to <i>y</i> w; <i>Trf</i>/CyO females. To determine the molecular lesion in the <i>mars<sup>3</sup></i> mutant, genomic DNA surrounding the original <i>mars</i> insertion site was amplified from <i>mars<sup>3</sup></i> homozygotes by PCR using flanking primers and sequenced.

#### Statistical analysis

We used unpaired two-tailed <i>t</i> tests to compare mean hatch ratios of eggs from different strains and unpaired one-tailed <i>t</i> tests to compare mean number of mitotic spindles in wild-type and <i>mars<sup>3</sup></i> mutant embryos.

#### Site-directed mutagenesis and ectopic expression

<i>mars<sup>2055</sup></i> was constructed by PCR-based site-directed mutagenesis. For ectopic expression in flies, full-length <i>mars<sup>WT</sup></i> and <i>mars<sup>2055</sup></i> were subcloned into pPFMW (Drosophila Genomics Resource Center, Indiana University, USA); a modified version of pUASP (Roth, 1998) that contains an N-terminal 3xFLAG 6xMyc (FM) tag. UASP-F<sub>Mars</sub>flies were made by <i>P</i> element-mediated germline transformation into a w<sup>1118</sup> strain by Genetic Services, Inc.

Embryos were provided with moderate levels of tagged Mars<sup>WT</sup> and Mars<sup>2055</sup> by ectopic expression of UASP-F<sub>Mars</sub>transgenes in the germ-line using arm-GAL4 (Sanson et al., 1996) or nanos-GAL4<sup>1716</sup> (Van Doren et al., 1998).

#### Immunoprecipitation and GST-pulldown experiments

We subjected lysates from arm-GAL4 or arm-GAL4 UASP-F<sub>Mars</sub>flies to immunoprecipitation with dTACC (gift from J. Raff; Gergely et al., 2000) or Myc antibody (A14 rabbit polyclonal; Santa Cruz Biotechnology, Inc.). After adsorption on protein G bound to GammaBind Plus Sepharose (GE Healthcare), we analyzed immunoprecipitates and total cell extracts by immunoblotting with Myc (PE10 mouse monoclonal; Santa Cruz Biotechnology, Inc.), α-tubulin (DM1α; Sigma-Aldrich), MspS (gift from H. Ohkura, University of Edinburgh, Edinburgh, Scotland, UK; Cullen et al., 1999), P<sub>1</sub>α<sub>878</sub> (gift from P.T. Cohen, University of Dundee, Dundee, Scotland, UK; Helps et al., 2001), or dTACC (Gergely et al., 2000) antibodies. For GST-pulldown experiments, full-length <i>mars ORF</i> was subcloned into pDEST-15 (Invitrogen) for expression in E. coli in frame with an N-terminal GST tag. <i>mars<sup>2055</sup></i> was made by PCR-based site-directed mutagenesis and subcloned into pDEST-15 in the same way. Constructs were sequenced to confirm that they contained no sequence errors. To test binding to PP1, bacterial cell lysates expressing GST-tagged Mars<sup>WT</sup> or Mars<sup>2055</sup> were incubated with arm-GAL4 UAS-HA-PP<sub>1</sub><sup>α<sub>878</sub></sup> (Vereschchagina et al., 2004) D. melanogaster embryo extracts, and GST-labeled protein was precipitated with GST Bind Resin (EMD). Precipitates were examined by immunoblotting with HA antibody (12CA5; Roche). To test binding between HURP and PP1, Hela cell nuclear extracts were subjected to immunoprecipitation with HURP antibody (gift from I.W. Mattaj, European Molecular Biology Laboratory, Heidelberg, Germany; Kofta et al., 2006). Precipitates were analyzed by immunoblotting with PP1 antibody (Helps et al., 2001).

#### Mars antibodies and immunofluorescence

Anti-peptide antibodies against Mars were raised by Eurogentec in rabbits by simultaneous immunization with two peptides: LVPEGTKTPPRRESN (residues 512–526) and 5TLNRVRNLPSSESFM (residues 906–921). Embryos were fixed with either methanol or formaldehyde and were processed for immunofluorescence as described previously (Huang and Raff, 1999). Colchicine treatment of embryos before fixation was as previously described (Gergely et al., 2000). Antibodies used for indirect immunofluorescence
were as follows: FLAG (rabbit polyclonal; Sigma-Aldrich), KLP10A (gift from D. Sharp, Albert Einstein College of Medicine, Bronx, NY; Rogers et al., 2004), pDACC (Greggely et al., 2000), pα-tubulin (gift from J. Raft, Barros et al., 2005), α-tubulin (DM1α; Sigma-Aldrich) and γ-tubulin (rabbit polyclonal or GTU88 monoclonal; Sigma-Aldrich). Secondary antibodies conjugated with Alexa Fluor 488 (Invitrogen), Cy3, or Cy5 (Jackson ImmunoResearch Laboratories) were used at 1:500–1,000 dilutions. DNA was counterstained with 1 μg/ml propidium iodide (Sigma-Aldrich).

Image acquisition and processing
Fixed embryos, mounted in 85% glycerol and 2.5% D-propylgalactoside in PBS, were examined using a microscope (Eclipse E800; Nikon) with either a 40x 1.3 NA Plan Fluor or a 60x 1.4 NA Plan Apo objective and a scanning confocal system (Andorra Plus; Bio-Rad Laboratories) equipped with LaserSharp 2000 software (Bio-Rad Laboratories). Images were imported to Photoshop (Adobe) and adjusted for brightness and contrast uniformly across entire fields. Quantitation of astral MTs was performed by making maximum intensity projections of 8–12 image stacks that were taken at 0.5-μm intervals from 15–90-min methanol-fixed embryos stained with α-tubulin, γ-tubulin, and DNA. The projections were imported into AQM (AQM software [Carl Zeiss, Inc.]). The mean intensity through the center of the unprocessed spindle images, parallel to the long axis of the structure, was calculated for each channel of fluorescence by the software.

Online supplemental material
Fig. S1 shows individual channel images of Mars distribution during mitosis. Fig. S2 shows both misaligned chromosomes on mitotic spindles in embryos laid by mars mothers and reduction of cold-resistant kinetochore MIs in embryos laid by mars mothers. Fig. S3 shows both the spindle localization of Mars in pDACC mutant embryos and elevated pα-tubulin staining on a normal looking spindle from an embryo laid by mars mothers. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200712080/DC1.

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References
Baksa, K., H. Morawietz, V. Dombrádi, M. Axton, H. Taubert, G. Szabo, I. Torok, A. Udvardy, H. Gyurkovics, and B. Szoó. 1993. Mutations in the protein phosphatase 1 gene at 97B can differentially affect suppression of position-effect variegation and mitosis in Drosophila melanogaster. Genetics. 135:117–125.
Barros, T.P., K. Kinoshita, A.A. Hymen, and J.W. Raft. 2005. Aurora A activates D-TACC–Mps complexes exclusively at centrosomes to stabilize centrosomal microtubules. J. Cell Biol. 170:1039–1046.
Bennett, D., and L. Alphey. 2004. Cloning and expression of mars, a novel member of the guanylate kinase associated protein family in Drosophila. Gene Expr. Patterns. 4:529–535.
Bennett, D., E. Lyulcheva, and L. Alphey. 2006. Towards a comprehensive analysis of the protein phosphatase 1 interactome in Drosophila. J. Mol. Biol. 364:196–212.

Brittle, A.L., and H. Ohkura. 2005. Centrosome maturation: Aurora lights the way to the poles. Curr. Biol. 15:R880–R882.
Cullen, C.F., P. Deak, D.M. Glover, and H. Ohkura. 1999. mini spindles: A gene encoding a conserved microtubule-associated protein required for the integrity of the mitotic spindle in Drosophila. J. Cell Biol. 146:1005–1018.
Dombrádi, V., J.M. Axton, H.M. Barker, and P.T.W. Cohen. 1990. Protein phosphatase activity in Drosophila mutants with abnormalities in mitosis and chromosome condensation. FEBS Lett. 275:39–43.
Greggely, F., D. Kidd, K. Jeffers, J.G. Wakefield, and J.W. Raft. 2000. D-TACC: a novel centrosomal protein required for normal spindle function in the early Drosophila embryo. EMBO J. 19:241–252.
Helps, N.R., P.T. Cohen, S.M. Bahri, W. Chia, and K. Babu. 2001. Interaction with protein phosphatase 1 is essential for bifenocal function during the morphogenesis of the Drosophila compound eye. Mol. Cell. Biol. 21:2154–2164.
Huang, J., and J.W. Raft. 1999. The disappearance of cyclin B at the end of mitosis is regulated spatially in Drosophila cells. EMBO J. 18:2184–2195.
Jeng, R., and T. Sterns. 1999. Gamma-tubulin complexes: size does matter. Trends Cell Biol. 9:339–342.
Katayama, H., H. Zhou, Q. Li, M. Tatsuka, and S. Sen. 2001. Interaction and feedback regulation between STK15/BTKAK/Aurora-A kinase and protein phosphatase 1 through mitotic cell division cycle. J. Biol. Chem. 276:46219–46224.
Kinoshita, K., T.L. Noetzel, L. Pelletier, K. Mechtil, D.N. Drechsel, A. Schwager, M. Lee, J.W. Raft, and A.A. Hyman. 2005. Aurora A phosphorylation of TACC3/maskin is required for centrosome-dependent microtubule assembly in mitosis. J. Cell Biol. 170:1047–1055.
Kirchner, J., S. Gross, D. Bennett, and L. Alphey. 2007. Essential, overlapping and redundant roles of the Drosophila protein phosphatase 1 alpha and beta genes. Genetics. 176:273–281.
Koffa, M.D., C.M. Casanova, R. Santarella, T. Kocher, M. Wilm, and I.W. Mattaj. 2006. HURP is part of a Ran-dependent complex involved in spindle formation. Curr. Biol. 16:743–754.
Lappin, T.R., R.N. Mullan, J.P. Stewart, N.A. Morgan, A. Thompson, and A.P. Maxwell. 2002. AINT/ERIC/TACC: an expanding family of proteins with C-terminal coiled coil domains. Leuk. Lymphoma. 43:1455–1459.
Lee, M.J., F. Greggely, K. Jeffers, S.Y. Peak-Chew, and J.W. Raft. 2001. Mps1/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behaviour. Nat. Cell Biol. 3:643–649.
Raft, J.W. 2002. Centrosomes and cancer: lessons from a TACC. Trends Cell Biol. 12:222–225.
Rogers, G.C., S.L. Rogers, T.A. Schwimmer, S.C. Emc-McClang, C.E. Walczak, R.D. Vale, J.M. Scholzy, and D.J. Sharp. 2004. Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. Nature. 427:364–370.
Rorth, P. 1998. Gal4 in the Drosophila female germline. Mech. Dev. 78:113–118.
Sanson, B., P. White, and J.P. Vincent. 1996. Uncoupling cadherin-based adhesion from wingless signalling in Drosophila. Nature. 383:627–630.
Santarella, R.A., M.D. Koffa, P. Tittmann, H. Gross, and A. Hoenger. 2007. HURP wraps microtubule ends with an additional tubulin sheet that has a beta-sheets conformation of tubulin. J. Mol. Biol. 365:1587–1595.
Sillje, H.H., S. Nagel, R. Koner, and E.A. Nigg. 2006. HURP is a Ran-importin beta-regulated protein that stabilizes kinetochore microtubules in the vicinity of chromosomes. Curr. Biol. 16:731–742.
Van Doren, M., A.L. Williamson, and R. Lehmann. 1998. Regulation of zygotic gene expression in Drosophila primordial germ cells. Curr. Biol. 8:243–246.
Vereschagina, N., D. Bennett, B. Szoór, J. Kirchner, S. Gross, E. Vissi, H. White-Cooper, and L. Alphey. 2004. The essential role of PP1beta in Drosophila is to regulate nonsynucle myosin. Mol. Biol. Cell. 15:4395–4405.
Wilson, P.G., and G.G. Borisy. 1998. Maternally expressed gamma tub37CD in Drosophila is differentially required for female meiosis and embryonic mitosis. Dev. Biol. 199:273–280.
Wong, J., and G. Fang. 2006. HURP controls spindle dynamics to promote proper interkinetochore tension and efficient kinetochore capture. J. Cell Biol. 173:879–891.
Yang, C.P., M.S. Chen, G.J. Liaw, S.F. Chen, G. Chou, and S.S. Fan. 2005. Using Drosophila eye as a model system to characterize the function of mars gene in cell-cycle regulation. Exp. Cell Res. 307:183–193.