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, HURP was found to be localized at the chromosomes. In metaphase and anaphase, HURP was found to be localized at the centromeres. In telophase, HURP was found to be localized at the midbody. In interphase, HURP was found to be localized at the nuclear envelope. In this study, we observed a low level of discrete Mars staining at the midbody. In interphase, 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.

Supplemental Material can be found at:
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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<sup>WT</sup> (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<sup>1</sup> 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<sup>1</sup> 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<sup>1</sup> 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). Double staining for Mars and either Klp10A, which is primarily localized at focused minus ends where it promotes polymerization 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<sup>1</sup>, by imprecise excision of a P element transposon (referred to as mars<sup>2</sup> hereafter), which we found inserted in the mars<sup>S</sup> untranslated region (Fig. 2, A and C). mars<sup>S</sup> flies express full-length Mars protein at a much lower level than wild type (Fig. 2 B). mars<sup>1</sup> 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<sup>1</sup>, mars<sup>S</sup>, and mars<sup>WT</sup> 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

Figure 1. Mars localizes to spindle MTs. (A) Fixed wild-type embryos stained to reveal the distribution of Mars (green), α-tubulin (red), and DNA (blue) during mitosis. (B and C) Mars is concentrated at MT minus ends. Fixed wild-type embryos stained to reveal distribution of Mars (green) and either KLP10A (red; B) or γ-tubulin (red) and DNA (blue; C). (D) An embryo treated with 500 μg/ml colchicine before fixation to depolymerize MTs. Under these conditions, Mars staining (green) disappears from the spindle treated with 500 μg/ml colchicine before fixation to depolymerize MTs.

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 polymerization 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).

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importance of the putative PPI binding motif for interaction with PP1, we tested a mutant form of Mars in which phenylalanine 839 was replaced with alanine (Mars839A) in our pulldown assay. The ability of Mars839A to bind PP1 was greatly reduced compared with wild-type Mars (Fig. 4 A), indicating that Phe839 is crucial for interaction with PP1. Immunoprecipitation with an HURP antibody followed by immunoblotting with PP1 antibody showed that endogenous human PP1 and HURP 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
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unaffected in

Conversely, the ability of dTACC to associate with MTs was largely

fig. S3 A, available at http://www

data). Mars staining was not affected in embryos that produce
centrosomes during mitosis but is also found on spindle MTs
protein (fig. 5 A ). In syncytial embryos, dTACC is concentrated at
body staining, indicate that PP1 binding is not necessary for

rescue the embryonic lethality of embryos laid by

factors. [ID]FIG4[/ID]  In immunoprecipitation assays, we found that Mars assoc-

placement of the minus ends of centrosome-associated MTs dur-

ratios (fig. 4 D ). PP1 is a pleiotropic enzyme. To test the specific
role of Mars-bound PP1, we introduced FM-tagged Mars in flies to create Mars complexes lacking PP1. Ectopic expression of
marts at comparable levels to those of marts failed to rescue the embryonic lethality of embryos laid by marts or
marts mothers (fig. 4 D ). Collectively, these data suggest that
binding to PP1 is critical for function. Identical distributions of
marts and Mars , as determined by FLAG antibody staining, indicate that PP1 binding is not necessary for normal Mars localization on the mitotic spindle (fig. 4 E ).

HURP has been shown to interact with other MAPs, including

TPX2 and XMAP215 in Xenopus laevis (Kofia et al., 2006), suggesting that its role in the stabilization of spindle MTs may be partly mediated via interactions with other spindle assembly factors. In immunoprecipitation assays, we found that Mars associates with two proteins that are known to cooperate with each other to stabilize MTs during cell division (Lappin et al., 2002): the D. melanogaster XMAP215 homologue encoded by minisplines (msps) and Drosophila transforming acidic coiled-coil (dTACC) protein (fig. 5 A ). In syncytial embryos, dTACC is concentrated at centrosomes during mitosis but is also found on spindle MTs (Gergely et al., 2000) where it colocalizes with Mars (unpublished data). Mars staining was not affected in embryos that produce no detectable dTACC protein (fig. S3 A, available at http://www.jcb.org/cgi/content/full/jcb.200712080/DC1; Lee et al., 2001). Conversely, the ability of dTACC to associate with MTs was largely unaffected in marts 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 stabi-

lation of the minus ends of centrosome-associated MTs dur-
ing 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 marts mutant embryos with an antibody that specifically recognizes dTACC phosphorylated at Ser863 (p-dTACC). marts 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 marts mutants (fig. 5 B), although we cannot rule out that global ratios of DTACC/p-DTACC are affected. We used marts to examine whether Mars promotes the dephosphorylation of dTACC by binding to PP1. Embryos with moderate levels of ectopic marts in embryos laid by either marts or marts mothers were essentially wild type in appearance and had little or no p-dTACC staining on mitotic spindles. In contrast, marts mutant embryos ectopically expressing marts at comparable levels to those of ectopic marts retained elevated p-dTACC staining on spindles (fig. 5 B and not depicted).
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staining confirmed that dTACC S863L 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 1/P mothers was rescued by dTACC S863L but not dTACC WT (Fig. 5 D). Collectively, these data indicate that dephosphorylation of dTACC on the spindle is an essential function of mars. Homozygous mars 1 mutants

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 S863L) expressed under control of the dTACC promoter (Barros et al., 2005). dTACC S863L, but not dTACC WT (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 S863L 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 1/P mothers was rescued by dTACC S863L but not dTACC WT (Fig. 5 D). Collectively, these data indicate that dephosphorylation of dTACC on the spindle is an essential function of mars. Homozygous mars 1 mutants

Figure 5. An essential role of mars is to promote dTACC dephosphorylation. (A) Mars coprecipitates dTACC and MspS from D. melanogaster embryonic nuclear extracts. Immunoprecipitation of arm-GAL4 (−) and arm-mars WT (+) embryo extracts with Myc antibody, followed by immunoblotting with dTACC and MspS antibodies, showed binding to FM-tagged Mars. Immunoprecipitation with dTACC antibody followed by immunoblotting with Mars antibody confirmed dTACC binding. (B) Distribution of dTACC and p-dTACC on mitotic spindles in embryos from mothers of different genotypes, as indicated. In wild type, p-dTACC is only found at the centrosome but in mars mutants, p-dTACC abnormally accumulates on mitotic spindles. Total dTACC staining is largely unaffacted in mars mutants. A nonphosphorylatable mutant form of dTACC (dTACC S863L), but not wild-type dTACC (dTACC WT), restores spindle structure and normal distribution of p-dTACC in a mars 1/P mutant background. Similarly, arm-mars WT, but not arm-mars FA, restores normal spindle structure and p-dTACC staining in a mars 1 background. Bar, 10 μm. (C) Linescans of fluorescence intensity (arbitrary units) across spindles from embryos of different genotypes, as indicated. The distribution of p-dTACC (red trace) is shown relative to α-tubulin (green trace). (D) Top graph shows quantification of ratio of spindle/centrosomal p-dTACC staining. dTACC S863L, but not dTACC WT, restores a normal p-dTACC ratio in embryos from mars 1/P mothers. Bottom graph shows that dTACC S863L, but not dTACC WT, rescues lethality of embryos laid by mars 1/P mothers. Hatch ratios are plotted as the mean ± standard error from n = 5 experiments.
were not rescued by dTACC<sup>S863L</sup> (unpublished data), suggesting that residual Mars protein in <i>mars<sup>10P</sup></i> embryos may play a dTACC-independent role, such as MT bundling, or that the level of ectopic dTACC<sup>S863L</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 <i>mars</i> 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 <i>mars</i> implicates PP1 in dephosphorylation of Mars or a Mars-associated protein. 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 the spindle? One possibility is that <i>mars</i> functions to ensure that MT stabilization mediated by p-dTACC only occurs at the centrosome, allowing a more dynamic spindle. This is hard to test however, because the mechanism by which p-dTACC stabilizes MTs is unclear. The effect of dTACC on MT assembly appears to be mediated by effector proteins, such as MspS, as TACC has not been described to possess MT stabilizing activity on its own (Kinoshita et al., 2005). Phosphorylation of dTACC might help activate effectors because dTACC mutated at Ser863 is able to recruit MspS to the centrosome but not promote MT assembly (Barros et al., 2005). However, it is not known which aspect of Mps activity is affected by 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.

<i>Is mars a functional homologue of HURP? </i>We have confirmed that various aspects of <i>mars</i> and HURP function are conserved, including spindle stabilization and binding of Mars to Mps 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>EP(2)2477</i>, referred to here as <i>mars<sup>1</sup></i>, is a homozygous viable P element insertion in the 5′ untranslated region of <i>mars</i>. GFP-dTACC<sup>S863L</sup> and GFP-dTACC<sup>S863G</sup> (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 mars-null allele**

Isolation of a null allele of <i>mars</i> by P element excision from <i>mars</i> was performed as follows. Jumosparter y w/Y, isogenic <i>mars<sup>1</sup>/CyO, P(Delta2-3)</i> males were crossed with y w, <i>Tfr</i>/CyO females. From each cross, only one w revertant male, y w; <i>mars<sup>1</sup>/CyO</i>, in which the P element was excised, was individually crossed back to y w; <i>Tfr</i>/CyO females. To determine the molecular lesion in the <i>mars</i> mutant, genomic DNA surrounding the original <i>mars</i> insertion site was amplified from <i>mars</i> homozygotes by PCR using flanking primers and sequenced.

**Statistical analysis**

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

**Site-directed mutagenesis and ectopic expression**

<i>mars</i><sup>S863A</sup> was constructed by PCR-based site-directed mutagenesis. For ectopic expression in flies, full-length <i>mars<sup>WT</sup></i> and <i>mars</i><sup>S863A</sup> were subcloned into pPFMW (<i>Drosophila</i> Genomics Resource Center, Indiana University, USA), a modified version of pUASP (Roth, 1998) that contains an N-terminal 3xFLAG 6xMyc (FM) tag. UASP-FM-mars flies were made by P 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>S863A</sup> by ectopic expression of UASP-FM-mars transgenes in the germ-line using arm-GAL4 (Sanson et al., 1996) or nanos-GAL4<sup>17F</sup> (Van Doren et al., 1998).

**Immunoprecipitation and GST-pulldown experiments**

We subjected lysates from arm-GAL4 or arm-GAL4 UASP-FM-mars 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 monocalonal; Santa Cruz Biotechnology, Inc.), α-tubulin (DM1a; Sigma-Aldrich), MspS (gift from H. Ohkura, University of Edinburgh, Edinburgh, Scotland, UK; Cullen et al., 1999), PP1α/β78 (gift from P.T. Cohen, University of Dundee, Dundee, Scotland, UK; Helpes et al., 2001), or dTACC (Gergely et al., 2000) antibodies. For GST-pulldown experiments, full-length Mars ORF was subcloned into pDEST-15 (Invitrogen) for expression in E. coli in frame with an N-terminal GST tag. Mars<sup>S863A</sup> 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 between PP1, bacterial cell lysates expressing GST-tagged Mars<sup>WT</sup> or Mars<sup>S863A</sup> were incubated with arm-GAL4 UAS-HA-PP1α/β78 (Vereshchagina 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; Kothe et al., 2006). Precipitates were analyzed by immunoblotting with PP1 antibody (Helpes 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 TLNRNVRNLRPSSEFM (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), KLPI10A (gift from D. Sharp, Albert Einstein College of Medicine, Bronx, NY; Rogers et al., 2004), dTACC (Gergely et al., 2000), α-tubulin (DM1α; Sigma-Aldrich) and α-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% 40 × 1.3 NA Plan Fluor or a 60 × 1.4 NA Plan Apo objective and a scanning confocal system (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. Quantification 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 AQUA Advance 6 software (Kinetic Imaging Ltd.), and mean α-tubulin intensities were measured within a circle around each centrosome from spindles in metaphase. The mean pixel intensity of astral MTs from at least 25 embryos of each genotype was calculated. All measurements were corrected for variation in staining conditions by subtracting the background intensity.

A similar approach was taken to quantify dTACC staining, except that measurements were also taken at four points on the spindle, both proximal and distal to the centrosome. Linescans were generated using LSM510 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 mutant mothers and reduction of cold-resistant kinetochore MTs in embryos laid by mars mothers. Fig. S3 shows both the spindle localization of Mars in dTACC mutant embryos and elevated dTACC spindle 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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