Cenp-meta is required for sustained spindle checkpoint

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ABSTRACT

Cenp-E is a kinesin-like motor protein required for efficient end-on attachment of kinetochores to the spindle microtubules. Cenp-E immunodepletion in Xenopus mitotic extracts results in the loss of mitotic arrest and massive chromosome missegregation, whereas its depletion in mammalian cells leads to chromosome segregation defects despite the presence of a functional spindle assembly checkpoint (SAC). Cenp-meta has previously been reported to be the Drosophila homolog of vertebrate Cenp-E. In this study, we show that cenp-meta*D mutant neuroblasts arrest in mitosis when treated with colchicine. cenp-meta*D mutant cells display a mitotic delay. Yet, despite the persistence of the two checkpoint proteins Mad2 and BubR1 on unattached kinetochores, these cells eventually enter anaphase and give rise to highly aneuploid cells. Eventually triggers the mitotic exit observed. Thus, our data provide evidence for a role of Cenp-meta in sustaining the SAC response.

KEY WORDS: Mitosis, Spindle assembly checkpoint, Metaphase, Cenp-E, BubR1, APC/C, Kinetochore

INTRODUCTION

During normal mitosis, faithful chromosome segregation is assured by the robustness of kinetochore attachment to the spindle microtubules (K–MT attachments) and the surveillance mechanism called the spindle assembly checkpoint (SAC), which delays mitotic progression if incorrect K–MT attachments are detected, giving the cell time to correct them. The SAC functions to regulate temporally the activity of the anaphase promoting complex/cyclosome (APC/C), a ubiquitin ligase (Harper et al., 1993; Harper et al., 2002; Peters, 2002; Yu, 2002). By targeting Cyclin B and Securin for degradation by the proteasome, APC/C drives the cell to mitotic exit. Early in mitosis, several checkpoint proteins (including Mad1, Mad2, Bub1, BubR1, Bub3 and Mps1) bind to unattached or inappropriately attached kinetochores. This recruitment to the kinetochore generates a ‘stop anaphase’ signal that diffuses into the cytosol. This signal is composed of the checkpoint proteins Mad2, BubR1 and Bub3 bound to Cdc20, a key co-factor of the APC/C necessary for its activation (Musacchio and Salmon, 2007; Santaguida and Musacchio, 2009). Following correct attachment of all chromosomes to the spindle, the checkpoint is inactivated and Cdc20 is freed to activate the APC/C (Yu, 2002). Several additional factors have been shown to participate in mitotic checkpoint signalling in metazoa. These factors include the RZZ complex (Karess, 2005) and the protein CENP-E (Mao et al., 2003; Mao et al., 2005; Weaver et al., 2003).

Cenp-E is a plus-end directed molecular motor kinesin localized specifically to kinetochores during mitosis (Yen et al., 1991; Yen et al., 1992). Several studies have demonstrated that Cenp-E is required for efficient capture and attachment of microtubules to kinetochores (Lombillo et al., 1995; Wood et al., 1997; Yen et al., 1997; Mao et al., 2001; Putkey et al., 2002; Kapoor et al., 2006). In mammals, Cenp-E has been shown to be required for chromosome congression to the spindle equator and for stable kinetochore–microtubule attachment. Depletion of Cenp-E by antisense oligonucleotides (Yao et al., 2000) or RNA interference (Tanudji et al., 2004) and inhibition of CENP-E recruitment to kinetochores by antibody microinjection (Schaar et al., 1997; McEwen et al., 2001) in human cells all lead to problems in chromosome congression. In primary mouse fibroblasts, CENP-E depletion results in chromosome segregation defects with a few chromosomes clustered around the spindle poles (Putkey et al., 2002). Similarly, depletion of Cenp-E by siRNA in HeLa cells causes chromosome missegregation with the presence of mono-oriented chromosomes localized close to the spindle pole (Tanudji et al., 2004). More recently, Cenp-E was shown to be phosphorylated in vitro and in vivo by both Aurora kinases A and B at a conserved site close to the CENP-E kinesin neck domain (Kim et al., 2010). This phosphorylation leads to reduced affinity for microtubules in vitro whereas preventing its phosphorylation leads to chromosome alignment defects, thereby demonstrating that Aurora kinases control Cenp-E mediated promotion of chromosome biorientation (Kim et al., 2010). Overall these combined observations indicate that CENP-E stabilizes K–MT attachment and promotes chromosome movement toward the metaphase plate.

Although it is now well established that CENP-E has a role in efficient K–MT capture and chromosome congression, its implication in the signaling cascade of the mitotic checkpoint is more controversial. Inhibition of CENP-E expression in mammalian cells, by antisense oligonucleotides or by RNAi, leads to prolonged mitotic arrest (Yao et al., 2000; Tanudji et al., 2004). On the other hand, depletion of CENP-E in primary mouse fibroblasts does not result in long-term mitotic arrest (Putkey et al., 2002). Similarly, in Xenopus, immunodepletion of Cenp-E from Xenopus extracts results in loss of mitotic arrest in the presence of microtubule-depolymerizing agents (Abrieu et al., 2000). Moreover, CENP-E and BubR1 can form a stoichiometric complex, and the presence of CENP-E can greatly enhance the kinase activity of BubR1 toward itself or an exogenous substrate such as histone H1 (Mao et al., 2003; Weaver et al., 2003; Guo...
et al., 2012). These observations suggest that the loss of mitotic arrest observed in *Xenopus* egg extracts depleted of CENP-E is caused by a reduction of BubR1 kinase activity.

In *Drosophila*, Cenp-meta and Cenp-ana were previously reported to be closely related to Cenp-E (Yucel et al., 2000). While a *cenp-ana* mutation leads to an increased frequency of anaphase, a *cenp-meta* mutation leads to an elevated prometaphase frequency (Yucel et al., 2000), reflecting a role in promoting chromosome congression, an observation confirmed by others (Williams et al., 2003; Maia et al., 2007). Moreover, co-depletion of *cenp-ana* and *cenp-meta* in Drosophila S2 cells by RNAi yield to a phenotype and a mitotic index identical to the single RNAi depletion of *cenp-meta* (Goshima and Vale, 2003) strongly suggesting that Cenp-meta is likely to be the *Drosophila* homolog of vertebrate Cenp-E. Zygotic deletion of Cenp-meta, is strongly suggesting that Cenp-meta is likely to be the *Drosophila* homolog of vertebrate Cenp-E. Zygotic deletion of Cenp-meta, is lethal at the pupal stage. Homozygous mutants of *cenp-meta* show an elevated mitotic index, with retarded congression of chromosomes to the metaphase plate (Yucel et al., 2000). Such a phenotype suggests that the checkpoint is functional and activated in these mutants, delaying anaphase onset since the chromosomes are not properly bioriented. Paradoxically, *cenp-meta* mutants display a very high level of aneuploid cells (Yucel et al., 2000; Williams et al., 2003). This apparent discrepancy has not been further addressed until the present study. Here, we show that Cenp-meta is required for prolonged mitotic checkpoint maintenance.

**MATERIALS AND METHODS**

**Genetic stocks**

The strain containing the null allele mutation *cenp-meta* has already been described previously. Briefly, imprecise excision of the P element deleted ∼5 kb of *Cenp-meta* genomic DNA just upstream of the 5′ end of the initial P element insertion site (Yucel et al., 2000). Flies expressing GFP-Rod, RFP-Rod, the mutations *mad2* and *asp*, RFP-BubR1, GFP-Mad2, Spc25-mRFP1 transgene were described previously (Buffin et al., 2005; Buffin et al., 2007; Schittenhelm et al., 2007; Rahmani et al., 2009). GFP-cyclin B flies were a gift from J. Raff (Cancer Research UK Gurdon Institute, Cambridge, England, UK).

**Western blot**

Protein extracts from 5 brains of wild-type and *cenp-meta* homozygous third instar larvae were loaded onto SDS 8%-acrylamide gels. Proteins were transferred to nitrocellulose membrane (Protran BA 85; Schleicher and Schuell, Dassel, Germany) using a BioRad electrophoretic blotting device. Membranes were blocked for 1 hour in TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) with 5% dry milk and incubated 1 hour 30 minutes at room temperature with rabbit anti-*Drosophila* Cenp-meta (a gift from M. L. Goldberg, Cornell University, Ithaca, NY) diluted 1:3000 in TBST plus 1% milk. After washing in TBST, the blot was incubated for 1 hour at room temperature with secondary antibody of goat anti-rabbit IgG conjugated with horseradish-peroxidase (Promega, Charbonnieres, France) diluted 1:6000. Immunodetection was carried out with the SuperSignal Kit (Perbio Science France, Brebières, France).

**Cytology**

Third instar larval brains were fixed and stained in aceto-orcein as previously described (Rahmani et al., 2009). The mitotic index (mean number of mitotic cells per microscopic field) in response to colchicine-induced depolymerization of microtubules was determined by preincubating brains in 10−4 M colchicine in 0.7% NaCl for 0, 30, or 60 minutes and then transferring them to 0.5% Na citrate hypotonic solution for 4 minutes before staining. Aneuploidy was determined by preincubating the brains in 10−4 M colchicine in 0.7% NaCl for 7 minutes to obtain a readable karyotype, then transferred to 0.5% Na citrate hypotonic solution for 4 minutes before being fixed and stained. Cells were observed with a microscope (Microphot; Nikon) and a 63x NA 1.4 phase contrast objective (Carl Zeiss, Inc.). A cell was reported as aneuploid if it clearly showed at least one extra chromosome.

**In vivo imaging**

*In vivo* imaging of living neuroblasts of third instar larval brains were carried out as described previously (Rahmani et al., 2009). Brains were imaged in a temperature-controlled room set at 21°C with a spinning disk confocal head (Ultraview; PerkinElmer) mounted on an inverted microscope (DMi6000; Leica) with a Zeiss 100× NA 1.4 lens and a camera (QuantEM 512SC; Photometrics), all piloted by MetaMorph 7 (MDS Analytical Technologies). At 20-seconds intervals, a z series of images consisting of seven 1-μm steps was acquired with 1× binning. Confocal video frames are maximum intensity projections. Time-lapse image series were converted into videos with ImageJ software (National Institutes of Health), and still images were processed using Photoshop (Adobe). NEB was defined as when the RFP-Spc25 or RFP- or GFP-Rod signal began to be visible on kinetochores. Anaphase onset was defined as the moment sister kinetochores (marked with either Spc25 or Rod) began to separate. GFP-cyclin B degradation dynamics was measured as described previously (Rahmani et al., 2009). Briefly, neuroblasts expressing one copy of GFP-cyclin B and RFP-Rod were filmed as above. GFP fluorescence in each z section was quantified for the whole cell and for a central region containing the kinetochores and most of the spindle. This gave a more robust measurement of the OCBD because spindle-associated cyclin B is the first to be degraded during metaphase (Buffin et al., 2007). The signal was adjusted for background and for bleaching relative to the signal of a neighboring non-mitotic cell (assumed to be constant). In the graphs for Fig. 4, the signal levels for the whole cell are displayed as normalized signal relative to the maximal intensity measured for the cell.

**Statistical analysis**

Data were expressed as mean ± s.d. The *P*-values were calculated using Student’s *t*-test. Values were considered statistically different whenever *P*<0.05.

**RESULTS**

*cenp-meta* mutant cells are checkpoint competent

Previous studies have shown that Cenp-E depletion leads to either a mitotic arrest in mammalian cells or a loss of mitotic arrest in *Xenopus* egg extracts. In order to address these apparent conflicting observations, we looked at the mitotic index in *cenp-meta* mutant larval neuroblasts. The *cenp-meta* allele is homozygous lethal at the larval stage (for further information on the nature of the *cenp-meta* mutation, see Materials and Methods and Fig. 1A). No Cenp-meta protein was detected by western blot of *cenp-meta* mutant protein extracts (Fig. 1B). Karyotypic analysis after staining chromosomes with aceto-orcein revealed a high level of aneuploidy, around 7% (Table 1; supplementary material Fig. S1). The functionality of the checkpoint in *cenp-meta* mutant cells was tested by first looking if there was a mitotic arrest in the presence of colchicine, a microtubule depolymerizing agent. *cenp-meta* mutant neuroblasts accumulated in M phase after 1 hour colchicine treatment, as the mitotic index increased by 2.46-fold, comparable to the 3-fold increase observed in wild type. Therefore, the checkpoint appeared to be functional in *cenp-meta* mutant cells. To further confirm this, we generated a *cenp-meta* *mad2* double mutant and looked at the mitotic index in the presence of colchicine. *mad2* is a null mutation that affects the spindle checkpoint but the cells display very little aneuploidy (Buffin et al., 2007). We found that the *cenp-meta* *mad2* double mutant flies were larval/pupal lethals, that the mitotic index no longer increased after treating the cells with colchicine.
To further dissect the phenotype observed in the cenp-metaD mutant neuroblasts, we analysed living mutant cells. To test if the spindle checkpoint was functional in cenp-metaD mutant cells, we measured the mitotic timing in cenp-metaD mad2 double mutant cells and found that it was similarly accelerated (7.2 ± 1.9 minutes, P < 0.0001) (Fig. 2A,D; supplementary material Movie 3), thereby suggesting again that the spindle checkpoint is functioning in cenp-metaD mutant cells.

Paradoxically, despite the fact that the cells have an active checkpoint, the level of aneuploidy was very high in cenp-metaD single mutant cells (6.7% vs 0.2% in wt, Table 1). Moreover, the mutant cells treated with colchicine for 30 minutes or 60 minutes also displayed a high percentage of Premature Sister Chromatid Separation (PSCS) around 4.3% and 6.8%, respectively (Table 1; supplementary material Fig. S1). PSCS is generally considered a sign of mitotic exit and spindle checkpoint dysfunction.

To further explore this apparent weakness in the SAC function of cenp-metaD, we generated a double mutant of cenp-metaD and a null allele of abnormal spindle (asp). The asp mutation, which perturbs spindle assembly, normally causes cells to arrest for hours in mitosis (Ripoll et al., 1985) in a SAC-dependent manner (Basto et al., 2000; Buffin et al., 2007), and results in a very high mitotic index. We found that the mitotic index was significantly reduced in the cenp-metaD asp double mutant (3.5 vs 7.4 for asp alone, Table 1). This result confirms that the SAC is not as robust as in asp mutant cells. Therefore, the high level of aneuploid cells observed in cenp-metaD cells may be due to an overall reduction (but not elimination) of the SAC intensity or duration caused by the cenp-metaD mutation.

The signal for the checkpoint proteins Mad2 and BubR1 persists on unaligned kinetochores present in cenp-metaD mutant cells

The possibility that the mitotic checkpoint may not be maintained in cenp-metaD mutant cells may be due to a reduced recruitment level of Mad2 and/or BubR1. In order to examine this, we looked at the dynamic behavior of these two checkpoint proteins during mitosis. For this, GFP-tagged Mad2 was expressed in wt or cenp-metaD mutant cells. As reported previously in wild-type neuroblasts, GFP-Mad2 was recruited to kinetochores of cenp-metaD mutant cells right at NEB (Buffin et al., 2005) and started very quickly to stream continuously toward the spindle poles during prometaphase. By the time, the cell reached anaphase, Mad2 signal was not visible anymore (Fig. 3A; supplementary material Movie 4). However, in cenp-metaD mutant cells, while the streaming of Mad2 appeared to be normal, Mad2 signal could still be detected on kinetochores of polar chromosomes by the time mutant cells underwent anaphase, and this signal lasted until late anaphase (Fig. 3B,C; supplementary material Movies 3, 6). The persistence of Mad2 on the unattached polar kinetochores is consistent with an active SAC in cenp-metaD mutant cells.

We similarly examined RFP-BubR1 in wt and cenp-metaD mutant cells. BubR1 signal at metaphase kinetochores was totally undetectable in wt cells (Fig. 3D; supplementary material Movie 7). However, strong BubR1 signals were detected on unaligned kinetochores observed in cenp-metaD mutant cells and stayed visible until late anaphase (Fig. 3E,F; supplementary material Movies 8, 9). Based on these observations, it appears that anaphase onset can still occur in cenp-metaD mutant cells even if Mad2 and BubR1 are still retained on unattached or misaligned kinetochores.

Cyclin B is continuously degraded in cenp-metaD mutant cells

The level of inhibitory signal generated by Mad2 and BubR1 on unattached kinetochores observed in cenp-metaD mutant cells

(Table 1), and that the percentage of aneuploid cells was even higher than that observed in cenp-metaD single mutant (33.6% vs 6.7%). Therefore, these observations indicate that the spindle checkpoint appears to be functional in cenp-metaD mutant cells since removing the spindle checkpoint by removing Mad2 results in an even higher level of aneuploidy and lower mitotic index.

To further dissect the phenotype observed in the cenp-metaD single mutant, we analysed living cenp-metaD neuroblasts by time-lapse microscopy to determine the mitotic timing (i.e. the time that elapses between Nuclear Envelope Breakdown (NEB) and anaphase onset). For this, Spc25, a kinetochore protein that is part of the Ndc80 complex, tagged with the RFP fluorophore was expressed in cenp-metaD mutant cells to monitor the kinetochores. Whereas wild-type (wt) neuroblasts spent an average of 9.6 ± 1.6 minutes (Fig. 2A,B; supplementary material Movie 1) between the NEB and the anaphase onset, cenp-metaD mutant neuroblasts showed a prolonged prometaphase with an average of 15.1 ± 5.6 minutes (P < 0.0001) (Fig. 2A,C; supplementary material Movie 2). We have shown previously that in mad2 mutant cells, in which the spindle checkpoint is abolished, the average mitotic timing was accelerated (7.3 minutes) compared to wt cells (Buffin et al., 2007; Rahmani et al., 2009). To test if the spindle checkpoint was functional in cenp-metaD mutant cells, we measured the mitotic timing in cenp-metaD mad2 double mutant cells and found that it was similarly accelerated (7.2 ± 1.9 minutes, P < 0.0001) (Fig. 2A,D; supplementary material Movie 3), thereby suggesting again that the spindle checkpoint is functioning in cenp-metaD mutant cells.
Table 1. Analysis of the mitotic parameters in WT and cenp-meta mutant neuroblasts

| Strain (number of brains) | Time (min) in colchicine | Mitotic index (SD)a | Statisticsc | Relative mitotic index | % aneuploidy (SD) | Statisticsd | % PSCS (SD) | Statisticse | Notes |
|--------------------------|--------------------------|---------------------|-------------|------------------------|------------------|-------------|-------------|-------------|--------|-------|
| WT (5)                   | 0                        | 2.15 (0.13)         |             | 1                      | 0.17 (0.19)      |             | NA          | viable      |        |       |
| WT (5)                   | 30                       | 3.42 (0.16)         | P<0.00001   | 1.59                   | NA               |             | 0.15 (0.07) | NA          |        |       |
| WT (5)                   | 60                       | 6.44 (0.28)         | P<0.00001   | 2.99                   | NA               |             | 0.18 (0.05) | P>0.5       |        |       |
| cenp-meta.t (5)          | 0                        | 2.66 (0.42)         |             | 1                      | 6.67 (2.87)      | P<0.00001    | NA          | lethal      |        |       |
| cenp-meta.t (5)          | 30                       | 4.80 (0.98)         | P<0.0001    | 1.80                   | NA               |             | 4.31 (0.99) | NA          |        |       |
| cenp-meta.t (5)          | 60                       | 6.54 (0.67)         | P<0.0001    | 2.46                   | NA               |             | 6.82 (2.01) | P>0.5       | NA      |       |
| cenp-meta.t mad2 (4)     | 0                        | 0.44 (0.04)         |             | 1                      | 33.63 (6.18)     | P<0.0001     | NA          | lethal      |        |       |
| cenp-meta.t mad2 (4)     | 30                       | 0.39 (0.15)         | P>0.5       | 0.89                   | NA               |             | ND          | NA          |        |       |
| cenp-meta.t mad2 (4)     | 60                       | 0.31 (0.05)         | P>0.5       | 0.71                   | NA               |             | 0.19 (0.20) | P>0.5       | NA      |       |
| mad2 (5)                 | 0                        | 2.11 (0.26)b        |             | 1                      | 0.77 (0.37)b     | P<0.05       | NA          | viable      |        |       |
| mad2 (5)                 | 30                       | 1.58 (0.12)b        | P>0.05      | 0.75                   | NA               |             | 0.19 (0.18) | NA          |        |       |
| mad2 (5)                 | 60                       | 1.63 (0.19)b        | P>0.05      | 0.77                   | NA               |             | 0.19 (0.20) | P>0.5       | NA      |       |
| asp(4)                   | 0                        | 7.40 (0.21)b        |             | NA                     | 1.03 (0.26)b     | P<0.0001     | NA          | lethal      |        |       |
| cenp-meta.t asp (4)      | 0                        | 3.48 (0.57)         | P<0.00001   | NA                     | ND               |             | NA          | lethal      |        |       |

NA, not applicable.
ND, not determined.
SD, standard deviation.
aMitotic index is defined as the mean number of cells in mitosis per optic field.
bThese values were taken from Rahmani et al. (Rahmani et al., 2009).
cThe P-values is calculated for colchicine treated cells for 30 or 60 minutes versus not treated cells using Student’s t-test. Values are considered statistically different whenever P<0.05.
dThe P-values are calculated for the various mutants versus WT cells.
eThe P-values are calculated for the 60 minutes value versus the 30 minutes value.

Fig. 2. Mitotic timing in cenp-meta.t mutant and cenp-meta.t mad2 double mutant neuroblasts. (A) Comparative mitotic timing of cenp-meta.t and cenp-meta.t mad2 double mutant cells. NEB is defined as when RFP-Spc25 begins to be visible on kinetochores. cenp-meta.t (blue diamonds) are delayed, with an average 15.1 minutes vs 9.6 minutes in WT (yellow diamonds). cenp-meta.t mad2 (pink diamonds) enter anaphase earlier than WT cells (7.2 minutes vs 9.6 minutes, P<0.005). cenp-meta.t mad2 double mutant cells (pink diamonds) show the same timing as the one that was previously reported for mad2 alone (Buffin et al., 2007; Rahmani et al., 2009), thereby indicating that the prometaphase delay in cenp-meta.t is SAC-dependent. (B–D) Still frames extracted from typical movies used for the determination of mitotic timing (from NEB to anaphase). (B) WT, (C) cenp-meta.t, (D) cenp-meta.t mad2 double mutant. All cells are marked with RFP-Spc25. See also supplementary material Movies 1, 2, 3. Scale bars: 5 μm.
may not be high enough over time to efficiently suppress APC/C activation and consequently APC/C-mediated degradation of mitotic substrates such as cyclin B that leads to inactivation of Cdk1 activity and mitotic exit. Thus, measurement of cyclin B degradation during mitosis in wt and cenp-meta mutant cells was used as an in vivo readout of APC/C activation. In agreement with what we showed previously (Buffin et al., 2007; Rahmani et al., 2009), the onset on cyclin B degradation (OCBD) in wt Drosophila neuroblasts expressing GFP-tagged cyclin B started between 4 to 7 minutes after NEB and the timing between OCBD and anaphase onset was relatively constant with an average of 2.9 ± 0.6 minutes (Fig. 4A,C; supplementary material Movie 10). However, in cenp-meta mutant cells, the levels of cyclin B declined gradually but constantly until the cell entered anaphase (Fig. 4B,C; supplementary material Movie 11). This result suggests that in cenp-meta mutant cells, the functional SAC cannot effectively block APC/C mediated cyclin B degradation. Consequently, the continuous degradation of cyclin B mediated by the activated APC/C could eventually reach a threshold for which mitotic arrest could no longer be maintained.

**DISCUSSION**

Here we have provided several lines of evidence that Cenp-meta is required for long-term maintenance of the spindle checkpoint. First, while cenp-meta mutant cells arrested in mitosis when treated with colchicine (as reflected by the increased number of mitotic cells seen in cenp-meta mutant treated cells), the relatively high level of cells with PSCS revealed that these cells were not able to maintain a prolonged arrest when treated with spindle poisons. Second, the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by the high mitotic index caused by 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We showed previously that the potential kinase activity of BubR1 is dispensable for initiating the spindle checkpoint response in Drosophila larval neuroblasts (Rahmani et al., 2009) but the presence of PSCS in BubR1-KD expressing cells treated with colchicine for 60 minutes suggests that the potential BubR1 kinase activity may be needed for long-term maintenance of metaphase arrest (Rahmani et al., 2009). Interestingly, the percentage of cells with PSCS in centripetaA mutant is much higher when compared to bubR1-KD mutant (6.8±2 vs 1.4±0.7, Table 1; Rahmani et al., 2009, respectively). This indicates that the percentage of cells that display signs of premature mitotic exit (reflected by the presence of PSCS) after colchicine treatment is greater in the absence of Centripeta than in the absence of BubR1 potential kinase activity and suggests that Centripeta may also affect the SAC independently of its presumed function in modulating BubR1 potential kinase activity. In conclusion, we have shown that Centripeta is important for prolonged SAC-dependent mitotic arrest. Whether Centripeta acts through BubR1 potential kinase activity and/or an unknown mechanism remains to be tested.

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Competing interests
The authors have no competing interests to declare.

Author contributions
R.E.K. and Z.R. designed the experiments. T.R. and Z.R. performed the majority of experiments and data analysis. Z.R. wrote the paper with suggestions from R.E.K.

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Fig. 4. Cyclin B degradation profiles in WT and centripetaA mutant neuroblasts. Typical onset of cyclin B degradation (OCBD) in a single neuroblast. (A) WT, (B) centripetaA mutant neuroblast. (A,B) The frames showing RFP-Rod (red) and GFP-cyclin B (green) at various time points (indicated as a,b,c, on both graphs) in WT (A) and centripetaA mutant (B) cells were taken from supplementary material Movies 10 and 11, respectively. (C) Mean time of OCBD versus anaphase (ANA) onset. OCBD begins 2.9 minutes before anaphase onset in WT cells (see also Rahmani et al., 2009) whereas it is a slow continuous process in centripetaA mutant cells. See also supplementary material Movies 10, 11. Scale bars: 5 μm.

Drosophila BubR1) was more likely to be a pseudokinase with no catalytic activity and that the pseudokinase domain was important for BubR1 protein stability (Suijkerbuijk et al., 2012). Thus, whether vertebrate BubR1 possesses real kinase activity still remains a controversial issue. However, the three catalytic residues essential for a conventional kinase are retained in Drosophila BubR1 domain (Suijkerbuijk et al., 2012), and suggest that Drosophila BubR1 may potentially be catalytically active. If it is the case, the absence of Centripeta may affect Drosophila BubR1 potential kinase activity in centripetaA mutant cells, thereby mimicking a phenotype that resembles the one observed with BubR1-KD (Kinase mutated) expressing cells.

References


