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Introduction

Kinetochore localization BUB-1/BUB-3 complex promotes anaphase onset in C. elegans

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The conserved Bub1/Bub3 complex is recruited to the kinetochore region of mitotic chromosomes, where it initiates spindle checkpoint signaling and promotes chromosome alignment. Here we show that, in contrast to the expectation for a checkpoint pathway component, the BUB-1/BUB-3 complex promotes timely anaphase onset in Caenorhabditis elegans embryos. This activity of BUB-1/BUB-3 was independent of spindle checkpoint signaling but required kinetochore localization. BUB-1/BUB-3 inhibition equivalently delayed separase activation and other events occurring during mitotic exit. The anaphase promotion function required BUB-1’s kinase domain, but not its kinase activity, and this function was independent of the role of BUB-1/BUB-3 in chromosome alignment. These results reveal an unexpected role for the BUB-1/BUB-3 complex in promoting anaphase onset that is distinct from its well-studied functions in checkpoint signaling and chromosome alignment, and suggest a new mechanism contributing to the coordination of the metaphase-to-anaphase transition.

Kinetochore are multiprotein structures assembled on centromeres during mitosis to segregate chromosomes (Cheesman and Desai, 2008; Santaguida and Musacchio, 2009). Microtubule-generated forces on kinetochores are counteracted by cohesins, which hold sister chromatids together (Onn et al., 2008; Nasmyth and Haering, 2009). Once all sister kinetochores are bi-oriented, cohesin is proteolytically cleaved by separase to separate sister chromatids. This transition from metaphase to anaphase is controlled by the anaphase-promoting complex/cyclosome (APC/C), a multisubunit E3 ubiquitin ligase (Pines, 2011; Primorac and Musacchio, 2013), and by phosphatases that reverse mitotic phosphorylation events (Sullivan and Morgan, 2007). Significant progress is being made in understanding APC/C mechanism and regulation (Primorac and Musacchio, 2013; Chang and Barford, 2014) and the contributions of phosphatases during mitotic exit (Sullivan and Morgan, 2007; Bollen et al., 2009; Hunt, 2013; Wieser and Pines, 2015), but how anaphase onset is precisely coordinated with chromosome alignment remains an important question.

Multiple mechanisms control APC/C activity. Increasing Cdk1 phosphorylation of APC/C subunits promotes interaction with its coactivator Cdc20 (Peters et al., 1996; Kramer et al., 2000; Kraft et al., 2003). APC/C activation is opposed by the spindle assembly checkpoint, which inhibits the ability of Cdc20 to fully activate APC/C when unattached kinetochores are present (Musacchio and Salmon, 2007; Lara-Gonzalez et al., 2012). After attachment, checkpoint silencing enables progression into anaphase (Sacristan and Kops, 2014). Phosphorylation of Cdc20 by Cdk1 inhibits its ability to bind and activate APC/C, which suggests that reversal of these phosphorylation events is important for anaphase onset (Kramer et al., 2000; Yudkovsky et al., 2000; Labit et al., 2012). Phosphatase activities are also important for reversing Cdk1 phosphorylation but their control is less well understood. A PP2A regulatory pathway involving GtTwall kinase and its substrates endosulphine A and Arpp19, both PP2A inhibitors, has been implicated in both entry and exit from mitosis (Gharbi-Ayachi et al., 2010; Mochida et al., 2010). A phosphatase relay mechanism involving PP1 and PP2a that is important for mitotic progression has also been recently described (Grallert et al., 2015).
Here, we uncover a new role for two conserved checkpoint components, Bub1 kinase and its binding partner the WD40-fold protein Bub3 (Hoyt et al., 1991; Roberts et al., 1994; Taylor et al., 1998), in promoting anaphase onset. The Bub1/Bub3 complex is recruited to kinetochores by binding to phosphorylated repetitive motifs in the N terminus of Knl1, a scaffold component of the Knl1/Mis12 complex/Ndc80 complex (KMN) network (London et al., 2012; Yamagishi et al., 2012; Shepperd et al., 2012; Primorac et al., 2013). Kinetochore-localized Bub1/Bub3 recruits other spindle checkpoint components including Mad1/Mad2 and BubR1 (Sharp-Baker and Chen, 2001; Gillett et al., 2004; Johnson et al., 2004; Vanoosthuyse et al., 2004; London and Biggins, 2014; Moyle et al., 2014). Bub1 has also been proposed to inhibit the APC/C by phosphorylation of its activator Cdc20 (Tang et al., 2004).

In addition to its role in the checkpoint, the Bub1/Bub3 complex contributes to chromosome alignment and segregation (Warren et al., 2002; Vanoosthuyse et al., 2004; Meraldi and Sorger, 2005; Fernius and Hardwick, 2007; Klebig et al., 2009). Bub1 phosphorylates histone H2A to create a binding site for Shugoshin, which recruits protein phosphatase 2A (PP2A) and Aurora B kinase to the inner centromere (Kawashima et al., 2010; Yamagishi et al., 2010). In vertebrates, Bub1 also recruits BubR1, CENP-E, CENP-F, and dynein, which contribute to proper chromosome alignment (Sharp-Baker and Chen, 2001; Johnson et al., 2004; Klebig et al., 2009).

Here we show that, in the early Caenorhabditis elegans embryo, kinetochore-localized BUB-1/BUB-3 promotes anaphase onset, and that this function is independent of its roles in spindle checkpoint signaling and chromosome alignment. These results identify a new function embedded in the Bub1/Bub3 complex and suggest a potential mechanism contributing to the coordination of chromosome alignment and anaphase onset.

Results and discussion

BUB-1/BUB-3 removal delays anaphase onset independently of checkpoint signaling

Analysis of one-cell C. elegans embryos expressing GFP fusions to label chromosomes and spindle poles revealed that depletion of BUB-1 increased the time from nuclear envelope breakdown (NEBD) to anaphase onset by ~50% (Fig. 1, A and C). This was surprising because BUB-1 is required for the spindle checkpoint, which restrains anaphase onset; thus, one would not expect removal of BUB-1 to delay anaphase onset. As BUB-1 also functions in chromosome alignment (Fig. S1 A), one possible explanation is that partial penetration of the BUB-1 depletion resulted in improperly attached chromosomes with insufficient residual BUB-1 to generate a spindle checkpoint signal and delay anaphase onset, a scenario reported in multiple mammalian Bub1 RNAi experiments (Johnson et al., 2004; Meraldi and Sorger, 2005; Klebig et al., 2009). As BUB-1 was >94% depleted (Fig. 1 B), this seemed unlikely. However, to test this further, we co-depleted BUB-1 and MAD-2 (also known as MDF-2). MAD-2 depletion abrogates checkpoint signaling in C. elegans embryos (Essex et al., 2009; Espeut et al., 2012) but did not suppress the anaphase onset delay resulting from BUB-1 depletion (Fig. 1, A and C). Thus, the observed delay in anaphase onset is not due to residual BUB-1 generating a checkpoint signal.

BUB-1 associates with BUB-3 via a conserved binding motif that follows its N-terminal TPR domain (Taylor et al., 1998; Wang et al., 2001; Larsen et al., 2007). The BUB-1/BUB-3 complex docks onto phosphorylated motifs in the KNL-1 N terminus (Primorac et al., 2013). A BUB-3 deletion mutant (bub-3(ok3437), referred to as bub-3Δ; Fig. S1 B), exhibited a comparable NEBD–anaphase onset delay to that resulting from BUB-1 depletion (Fig. 1 C and Fig. S1 C). BUB-1 kinetochore localization (Fig. 1 D), as well as its overall protein levels (Fig. S1 D), was significantly reduced in bub-3Δ. Conversely, BUB-3 kinetochore localization was significantly disrupted by BUB-1 depletion (Fig. 1 D), albeit without a reduction in BUB-3 protein levels (Fig. S1 E). Thus, depletion of BUB-1 should be considered as perturbing the BUB-1/BUB-3 complex.

BUB-1 depletion had no effect on interphase duration, measured as the interval between anaphase onset in the one-cell embryo and NEBD of the AB cell in the two-cell embryo (Fig. 1 E); in contrast, the NEBD–anaphase onset interval was delayed in the AB cell (Fig. 1 E), indicating that promotion of timely anaphase onset by the BUB-1/BUB-3 complex is not restricted to the first embryonic division. Thus, inhibition of the BUB-1/BUB-3 complex delays anaphase onset, and this delay is independent of spindle checkpoint signaling.

BUB-1/BUB-3 complex localization to kinetochores is required for promotion of anaphase onset

A significant pool of BUB-1/BUB-3 is retained on kinetochores after bipolar attachment of chromosomes (Fig. 2 A; Jablonski et al., 1998; Taylor et al., 2001; Gillett et al., 2004). To test if the ability of BUB-1/BUB-3 to promote anaphase required localization to kinetochores, we analyzed deletion mutants in the phosphorylation-dependent BUB-1/BUB-3 docking motifs in the KNL-1 N terminus (Fig. 2 B). The largest KNL-1 deletion tested (∆85–505) is expressed, localizes to kinetochores but prevents BUB-1 kinetochore localization without affecting BUB-1 protein levels (Fig. 2, C and D; Moyle et al., 2014), and is checkpoint signaling–defective (Fig. S2 A). KNL-1(∆85–505) delayed anaphase onset to a similar extent as BUB-1 depletion (Fig. 2, E and F). Two smaller nonoverlapping deletions within this region (∆85–290 and ∆291–505) both localized to kinetochores but reduced BUB-1 kinetochore localization by approximately half (Fig. 2 C; metaphase kinetochore BUB-1::GFP fluorescence intensity of 56 ± 17%; n = 11 for ∆85–290 and 40 ± 16%; n = 16 for ∆291–505, relative to wild-type [WT] KNL-1; data from Moyle et al., 2014) and caused an intermediate delay in anaphase onset (Fig. 2, E and F). Thus, kinetochore localization is required for the BUB-1/BUB-3 complex to promote anaphase onset, and the amount of kinetochore-localized BUB-1 influences the magnitude of the NEBD–anaphase onset interval.
Figure 1. BUB-1 depletion delays anaphase onset independently of checkpoint signaling. (A) Images from time-lapse movies of one-cell C. elegans embryos expressing GFP::H2b (arrow) and γ-tubulin::GFP (arrowheads) for the indicated conditions. Time is given in seconds after NEBD. The asterisks mark extra chromatin present due to defective meiotic segregation. Bar, 5 µm. (B) Immunoblot of bub-1(RNAi) worms next to a dilution of control N2 worms. Numbers indicate the amount loaded relative to the 100% lanes. α-Tubulin is a loading control. (C) Plot of mean NEBD–anaphase onset intervals for the indicated conditions in one-cell embryos. Error bars indicate SD; red broken lines are control values; n is the number of embryos. (D) BUB-1 and BUB-3 kinetochore localization in bub-3Δ (left) and bub-1(RNAi) (right), respectively; n is the number of embryos. The mean integrated fluorescence intensity at kinetochores is plotted; error bars indicate the 95% confidence interval (CI). Bars, 2 µm. (E) Schematic indicates the time intervals measured and plotted below. Error bars indicate SD; red broken lines are control values; n is the number of embryos.
signal for these fusions is not detected in early embryos (Liu et al., 2004; Wang et al., 2013; unpublished data), likely because the 20-min interval between meiosis II anaphase and NEBD of the first embryonic mitosis (Portier et al., 2007) is too short for GFP maturation at 20°C. This technical issue prevented us from directly monitoring APC/C activity to test if kinetochore-localized BUB-1/BUB-3 controls the timing of its activation. However, we engineered a sensor for activation of separase, the protease BUB-1 depletion delays separase activation and other mitotic exit events 

BUB-1/BUB-3 may promote anaphase onset by promoting APC/C activation or contributing to a pathway (e.g., activation of Cdk1-countering phosphatases) that functions in parallel to APC/C. While GFP fusions with Cyclin B (CYB-1; Liu et al., 2004) and Securin (IFY-1; Wang et al., 2013) enable monitoring of APC/C activation at meiosis I anaphase in C. elegans, GFP signal for these fusions is not detected in early embryos (Liu et al., 2004; Wang et al., 2013; unpublished data), likely because the ~20-min interval between meiosis II anaphase and NEBD of the first embryonic mitosis (Portier et al., 2007) is too short for GFP maturation at 20°C. This technical issue prevented us from directly monitoring APC/C activity to test if kinetochore-localized BUB-1/BUB-3 controls the timing of its activation. However, we engineered a sensor for activation of separase, the protease
that acts downstream of APC/C activation to cleave cohesin and separate sister chromatids (Uhlmann et al., 2000), based on our identification of a separase cleavage site in the N-tail of the CENP-A–related protein CPAR-1 (Monen et al., 2015). To create a mitotic separase sensor, we fused GFP to the N terminus of a chimeric protein in which the CPAR-1 N-tail was placed in front of the histone fold domain of the major C. elegans CENP-A–related protein HCP-3 (HCP-3 HFD; Fig. 3 A). GFP signal of this sensor was observed on metaphase chromosomes but was progressively lost starting 15 s before visible chromatid separation (Fig. 3, A–C). Mutation of two key residues in the predicted cleavage motif (Sullivan et al., 2004) abrogated the signal loss of the sensor (Fig. 3, B and C), confirming that separase cleavage liberated GFP from chromatin. Expression of the sensor did not affect NEBD–anaphase onset interval or embryo production/viability (Fig. S2, B and C).

Figure 3. BUB-1 depletion delays separase activation and other mitotic exit events. [A] Schematic of sensor used to monitor separase activation in one-cell embryos. [B] Images from time-lapse sequences of strains coexpressing mCh::H2b and either the separase sensor or an uncleavable mutant. GFP imaging at 5-s intervals was initiated ∼50–80 s before anaphase onset; mCherry imaging was initiated earlier to score NEBD. Bar, 2 µm. (C) Plot of mean chromosomal GFP fluorescence for the separase sensor and the uncleavable mutant, relative to sister separation onset. n is the number of embryos. Error bars indicate the 95% CI. (D) Plot of mean chromosomal GFP fluorescence of the separase sensor over time, for the indicated conditions. Error bars indicate the 95% CI. (E) Schematic (top) and mean intervals from NEBD (bottom) of other mitotic exit events scored in time-lapse sequences. n is the number of embryos analyzed. Error bars indicate SD; red broken lines are control values.
Figure 4. Mutations in the BUB-1 kinase domain delay anaphase onset to nearly the same extent as BUB-1 depletion. (A) Schematic of RNAi-resistant bub-1 transgene and three transgene-encoded variants. (B) BUB-1 immunoblots of the indicated conditions; α-tubulin is a loading control. (C) Embryo viability analysis for the indicated strains and conditions. Error bars indicate the SD of individual worm progeny viability. (D) Images from representative time-lapse sequences as in Fig. 1 A. Bar, 5 µm. (E) Plot of mean NEBD–anaphase onset intervals for the indicated conditions. Error bars indicate SD; the red broken line is the control value; n is the number of embryos. (F) Plot of the mean delay in anaphase onset for the indicated conditions, generated by subtracting the matched control values and propagating errors. Error bars indicate the 95% CI. bub-1(RNAi) and bub1&mad-2(RNAi) values are from Fig. 1 C; other data are from E. (G) Plot of mean chromosomal GFP fluorescence of the separase sensor, for the indicated conditions. Error bars indicate the 95% CI.

Analysis of sensor-expressing embryos revealed that separase activation was delayed by BUB-1 depletion to the same extent as anaphase onset (Fig. 3 D). Once initiated, the kinetics of sensor chromosomal signal loss was not significantly different between the control and the BUB-1 depletion (Fig. 3 D). Thus, kinetochore-localized BUB-1/BUB-3 controls the timing of separase activation relative to NEBD. To determine if the delay induced by BUB-1 depletion was limited to control of separase or represented a general delay in mitotic exit, we examined the timing of two other events that follow sister chromatid separation: cytokinesis onset and chromosome decondensation (Fig. 3 E). Both were delayed in the BUB-1 depletion relative to NEBD (Fig. 3 E), indicating that kinetochore-localized BUB-1/ BUB-3 does not act solely to control separase activation but instead functions in a pathway that is broadly relevant for triggering mitotic exit.

Anaphase onset promotion requires the BUB-1 kinase domain but is independent of its kinase activity

To identify features of BUB-1/BUB-3 required to promote anaphase onset, we focused on BUB-1, using single-copy RNAi-resistant transgenes (Fig. 4 A; Moyle et al., 2014). Both untagged
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The anaphase onset promotion function of the BUB-1/BUB-3 complex is independent of its role in chromosome alignment

Bub1 kinase activity is important for the recruitment of Shugoshin family proteins via histone H2a phosphorylation (Kawashima et al., 2010; Ricke et al., 2012). However, neither deletion nor mutation of SGO-1, the only C. elegans member of the Shugoshin protein family, resulted in a NEBD–anaphase onset delay (Figs. 4 A and C; compare to Fig. 1, A and C). The D814N mutation led to a prolonged NEBD–anaphase onset interval (Fig. 4, D–F), and the K718R;D847N mutant exhibited delayed anaphase onset, separate activation, cytokinesis onset, and chromosome decondensation comparable to those resulting from BUB-1 depletion (Fig. 4, D–G; and Fig. S3 D). We conclude that the anaphase promotion function of BUB-1–BUB-3, while largely independent of BUB-1 kinase activity, is dependent on a properly structured BUB-1 kinase domain.

BUB-1 depletion or blocking BUB-1/BUB-3 kinetochore targeting using Δ85-505 KNL-1 led to significant chromosome segregation errors and 100% embryonic lethality; in contrast, no segregation errors were observed in the K718R;D847N mutant, which exhibited normal embryonic viability in the bub-1Δ background (Figs. 4 C and 5 B). This conclusion was further supported by localization analysis of HCP-1—one of two functionally redundant C. elegans proteins with weak similarity to CENP-F (Moore et al., 1999; Cheeseman et al., 2005). Consistent with prior work (Encalada et al., 2005), HCP-1 was largely delocalized from kinetochores after BUB-1 depletion; in contrast, HCP-1 was normally localized in the K718R;D847N mutant (Figs. 5 C and S3 H). Load-bearing kinetochore–microtubule attachments were also normal in the K718R;D847N mutant. In the one-cell embryo, quantitative analysis of spindle elongation functions as a measure for the formation of load-bearing kinetochore–microtubule attachments, as the cortex generates pulling forces on astral microtubules that are resisted by kinetochore–microtubule attachments in the spindle (Oegema et al., 2001). Tracking spindle pole separation after NEBD revealed delayed load-bearing attachment formation after BUB-1 depletion (Fig. 5 D, left; the delay is evident in the “bump,” which indicates premature separation of the spindle poles followed by recovery to control metaphase spindle length), a nearly identical profile was observed in the Δ85-505 KNL-1 mutant that prevents BUB-1/BUB-3 kinetochore recruitment (Fig. 5 D, left). In contrast, the K718R;D847N mutant did not delay attachment formation; instead, there was an extended plateau at metaphase spindle length (Fig. 5 D, right). Thus, K718R;D847N BUB-1, while delaying anaphase onset to nearly the same extent as BUB-1 depletion, does not exhibit the chromosome segregation defects observed after BUB-1 depletion.

Conclusion

Here we describe a new function for kinetochore-localized BUB-1/BUB-3 complex in promoting anaphase onset, which adds to its known roles in checkpoint signaling and chromosome alignment. The conclusion that this new function is independent of checkpoint signaling was supported by four perturbations that inhibit checkpoint signaling: co-depletion of MAD-2, deletion of bub-3, removal of the docking site on kinetochores for BUB-1/BUB-3, and the K718R;D847N mutant in the BUB-1 kinase domain. Comparison of BUB-1 kinase active site mutants revealed that the anaphase promotion function resides in the kinase domain but is largely independent of kinase activity. Perturbation of BUB-1/BUB-3 delays but does not block anaphase onset, indicating that BUB-1/BUB-3’s anaphase promotion activity functions in parallel to other mechanisms triggering anaphase onset. These parallel mechanisms likely involve many complex phosphorylation events on APC/C, primarily dependent on Cdk1 (Fig. 5 E; Kraft et al., 2003; Labit et al., 2012).

A role for BUB-1/BUB-3 in promoting anaphase appears counterintuitive, given the long-standing focus on BUB-1/BUB-3 as the kinetochore scaffold for the checkpoint signal that inhibits anaphase onset. We speculate that conversion of a key kinetochore-localized negative regulator of anaphase into a positive promoter once necessary conditions, e.g., proper kinetochore-microtubule attachment, have been met, could aid coupling of chromosome alignment to activation of separase and sister separation (Fig. 5 E). Our inability to monitor APC/C activation leaves open the question as to the mechanism upstream of separase activation in which BUB-1/BUB-3 acts to promote anaphase onset. An appealing possibility is that kinetochore-localized BUB-1/BUB-3 promotes APC/C activation (Fig. 5 E, top right). Bub1 is known to bind the APC/C activator Cdc20, and, while prior work has focused on the significance of this interaction in checkpoint signaling and Bub1 degradation (Tang et al., 2004; Kang et al., 2008; Di Fiore et al., 2015), our results raise the possibility that Cdc20 binding could contribute positively to APC/C activation. An alternative possibility is that BUB-1/BUB-3 functions in a pathway, such...
as activation of a phosphatase, that acts in parallel with APC/C to promote anaphase onset (Fig. 5 E, bottom right). Future work is needed to distinguish between these possibilities.

Materials and methods

C. elegans strains

C. elegans strains used in this study are listed in Table S1 and were maintained at 20°C. RNAi-resistant bub-1 and knl-1 transgenes, together with information on their recoded regions, have been described previously (Espeut et al., 2012; Moyle et al., 2014). For the RNAi-resistant bub-1 transgenes, the short (44-bp) intron 5 was deleted and 122 bp of exon 5 and all of exon 6 were recoded. For RNAi-resistant knl-1 transgenes, exon 4 was recoded. The bub-1 transgenes were transferred into pCFJ151 (Frøkjær-Jensen et al., 2008), a vector used to generate insertion at the attTi5605 locus on Chr II, before injection into strain EG4322. The presence of transgenes and of engineered mutations was confirmed by PCR and sequencing. For the separase sensor, the CPAR-1 N-tail (2–159 aa) and HCP-3 histone-fold domain (187–288 aa) were amplified from genomic DNA and fused together. GFP was inserted after the start codon and followed by a GGRAGSGGRAGSGGRAGS linker, inserted into pCFJ151, and injected into the strain EG6429. GFP::H2b; α-tubulin::GFP, mCherry::H2b, BUB-1::GFP, and GFP::HCP-1 markers were transferred into transgenic strains by mating before analysis.

RNA-mediated interference (RNAi)

Double-stranded RNAs (dsRNAs) used in this study are listed in Table S2. For imaging, the dsRNA was injected into L4 worms and incubated for
38–43 h at 20°C. For double and triple RNAi, RNAs were mixed in equal ratios, with final concentrations of >0.7 mg/ml for each dsRNA.

**Immunoblotting**

For immunoblotting, worms from an NGM+OP50 agar plate were washed with M9 (22 mM KH$_2$PO$_4$, 42 mM Na$_2$HPO$_4$, 86 mM NaCl, and 1 mM MgSO$_4$•7H$_2$O) supplemented with 0.1% Triton X-100. After adding 100 µl of sterile glass beads to the ~100 µl of pelletted worms and 50 µl of 4x sample buffer (40% glycerol, 2.40 mM Tris-HCl, pH 6.8, 8% SDS, 0.04% bromophenol blue, and 5% β-mercaptoethanol), samples were boiled and vortexed. For immunoblotting after RNAi, L4 worms were injected with dsRNA and incubated for 40–42 h at 20°C. Worms were transferred into 500 µl of M9 and washed with 1 ml of M9 + 0.1% Triton X-100. 4x sample buffer was added and worms were lysed in an acquired seic water bath for 10 min at 70°C; the final sample was comprised of 1 worm/µl. Samples were loaded onto 8% SDS gels, transferred to nitrocellulose, probed with 1 µg/ml affinity-purified anti-BUB-1 (rabbit; antigen was BUB-1 [aa 287–667]; Desai et al., 2003) and anti-KNL-1 (rabbit; antigen was KNL-1 [aa 8–256]; Desai et al., 2003), anti-BUB-3 (rabbit; antigen was BUB-3 [aa 189–432]; Essex et al., 2009), anti-SGO-1 (rabbit; antigen was SGO-1 [aa 120–308]; generated in the study), or anti-α-tubulin (mouse monoclonal DM1α; Sigma-Aldrich) antibodies.

**Imaging and quantification**

For imaging of one- and two-cell embryos, hermaphrodite adult worms were dissected into M9 buffer (22 mM KH$_2$PO$_4$, 42 mM Na$_2$HPO$_4$, 86 mM NaCl, and 1 mM MgSO$_4$•7H$_2$O), and embryos were transferred to 2% agarose pads positioned on a microscope slide and covered with an 18 × 18 mm coverslip.

Imaging of strains expressing GFP::H2B;γ-tubulin::GFP was performed on a deconvolution microscope (DeltaVision; Applied Precision/GE Healthcare) controlled by a sofWox workstation (DeltaVision; Applied Precision/GE Healthcare) equipped with a charge-coupled device camera (CoolSNAP; Roper Scientific) with 5 × 2 µm z stacks, 2 × 2 binning, and a 60X 1.3 NA Plan-Apochromat objective lens (Olympus) at 10s intervals for 100-ms exposure at 18°C. Acquired sequences were processed (CoolSNAP; Roper Scientific) with 5 × 2 µm z stacks, 2 × 2 binning, and an 18 nm coverslip.

Imaging of strains expressing GFP::BUB-3 localizes only to the spindle pole and measuring the distance between them from NEBD onwards. NEDB was scored as the frame where free histone signal in the nucleus equilibrates with the cytoplasm, which is just before abrupt chromosome movement starts. Anaphase onset was scored as the first frame with visible separation of sister chromatids. Lagging chromatin was scored as visible threads of GFP::H2B signal between separating chromatid masses. For all other strains, images were acquired on a spinning disc confocal system (Revolution XD Confocal System; Andor Technology) controlled by iQ software (Andor Technology) and a spinning disc confocal scanner (CSU-10; Yokogawa Electric Corporation) mounted on an inverted microscope (TE2000-E; Nikon) equipped with 100× or 60× 1.4 NA Plan-Apochromat lenses, and outfitted with an electron multiplication back-thinned microscope (TE2000-E; Nikon) equipped with 100× or 60× 1.4 NA Plan-Apochromat lenses, and outfitted with an electron multiplication back-thinned microscope (DeltaVision; Applied Precision/GE Healthcare) equipped with a charge-coupled device camera (CoolSNAP; Roper Scientific) with 5 × 2 µm z stacks, 2 × 2 binning, and an 18 nm coverslip.

To monitor BUB-1::GFP localization in the KNL-1::mCh mutant, a 5 × 2 µm z series, with 1 × 1 binning, was acquired every 20 s, with 200ms and 300-ms exposure times for GFP and mCherry. For the sepa-

**Online supplemental material**

Fig. S1 shows two additional examples of bub-1(RNAi) and characterization of the bub-3(ok3437) mutant. Fig. S2 shows the checkpoint signaling defect of KNL-1(∆85-505) and the lack of an effect of the separase sensor on fertility, viability, or NEBD-anaphase onset duration. Fig. S3 shows characterization of the bub-1(ok3383) mutant, BUB-1 kinase domain variants, and the sgo-1(∆m2442) mutant. Table S1 lists C. elegans strains used in this study and Table S2 lists oligos used to generate dsRNAs. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201412035/DC1.

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**References**

Bollen, M., D.W. Gerlich, and B. Lesage. 2009. Mitotic phosphatases: from entry guards to exit guides. Trends Cell Biol. 19:531–541. http://dx.doi.org/10.1016/j.tcb.2009.06.005

Chang, L., and D. Barford. 2014. Insights into the anaphase-promoting complex: a molecular machine that regulates mitosis. Curr. Opin. Struct. Biol. 29:1–9. http://dx.doi.org/10.1016/j.sbi.2014.08.003

Cheeseeman, I.M., and A. Desai. 2005. Molecular architecture of the kinetochore-microtubule interface. Nat. Rev. Mol. Cell Biol. 6:33–46. http://dx.doi.org/10.1038/nrm2130

Cheeseeman, I.M., I. MacLeod, J.R. Yates III, K. Oegema, and A. Desai. 2005. The CENP-F-like proteins HCP-1 and HCP-2 target CLASP to kinetochores to mediate chromosome segregation. Curr. Biol. 15:771–777. http://dx.doi.org/10.1016/j.cub.2005.03.018

Desai, A., S. Rybina, T. Müller-Reichert, A. Shevchenko, A. Shevchenko, A. Hyman, and K. Oegema. 2003. KNL-1 directs assembly of the microtubule-binding interface of the kinetochore in C. elegans. Genes Dev. 17:2421–2435. http://dx.doi.org/10.1101/gad.112630

Di Fiore, B., N.E. Duvey, A. Hagtung, D. Izawa, J. Mansfeld, T.J. Gibson, and J. Pines. 2015. The ARFBA spindle forms APCC activators and is shared by APCC substrates and regulators. Dev. Cell. 32:358–372. http://dx.doi.org/10.1016/j.devcel.2015.01.003

Encalada, S.E., J. Willis, R. Lyczak, and B. Bowerman. 2005. A spindle checkpoint functions during mitosis in the early Caenorhabditis elegans embryo. Mol. Biol. Cell. 16:1096–1107. http://dx.doi.org/10.1091/mbc.E04-08-0712

Espeut, J.D.K. Chee, L. Krennig, K. Oegema, and A. Desai. 2012. Microtubule binding by KNL-1 contributes to spindle checkpoint silencing at the kinetochore. J. Cell Biol. 196:469–482. http://dx.doi.org/10.1083/jcb.201111107

Essex, A., A. Dammermann, L. Lewellyn, K. Oegema, and A. Desai. 2009. Systematic analysis in Caenorhabditis elegans reveals that the spindle checkpoint is composed of two largely independent branches. Mol. Biol. Cell. 20:1252–1267. http://dx.doi.org/10.1091/mbc.E08-10-1047

Felix, M.A., J.C. Labbé, M. Doréé, T. Hunt, and E. Karsent. 1990. Triggering of cyclin degradation in interphase extracts of amphibian eggs bycdc-2 kinase. Nature. 346:379–382. http://dx.doi.org/10.1038/346379a0

Fernius, J., and K.G. Hardwick. 2007. Bub1 kinase targets Sgo1 to ensure efficient chromosome biorientation in budding yeast mitosis. PLoS Genet. 3:e213. http://dx.doi.org/10.1371/journal.pgen.0030213

Frokjaer-Jensen, C., M.W. Davis, C.E. Hopkins, B.J. Newman, J.M. Thummler, S.P. Olsen, M. Grunnet, and E.M. Jorgensen. 2008. Single-copy inser-

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GLE2p-binding sequence (GLEBS)-containing proteins. *J. Biol. Chem.* 276:26559–26567. http://dx.doi.org/10.1074/jbc.M101083200

Wang, R., Z. Kaul, C. Ambardekar, T.G. Yamamoto, K. Kavdia, K. Kodali, A.A. High, and R. Kitagawa. 2013. HECT-E3 ligase ETC-1 regulates securin and cyclin B1 cytoplasmic abundance to promote timely anaphase during meiosis in *C. elegans*. *Development*. 140:2149–2159. http://dx.doi.org/10.1242/dev.090688

Warren, C.D., D.M. Brady, R.C. Johnston, J.S. Hanna, K.G. Hardwick, and F.A. Spencer. 2002. Distinct chromosome segregation roles for spindle checkpoint proteins. *Mol. Biol. Cell*. 13:3029–3041. http://dx.doi.org/10.1091/mbc.E02-04-0203

Wieser, S., and J. Pines. 2015. The biochemistry of mitosis. *Cold Spring Harb. Perspect. Biol.* 7:a015776. http://dx.doi.org/10.1101/cshperspect.a015776

Yamagishi, Y., T. Honda, Y. Tanno, and Y. Watanabe. 2010. Two histone marks establish the inner centromere and chromosome bi-orientation. *Science*. 330:239–243. http://dx.doi.org/10.1126/science.1194498

Yamagishi, Y., C.-H. Yang, Y. Tanno, and Y. Watanabe. 2012. MPS1/Mph1 phosphorylates the kinetochore protein KNL1/Spc7 to recruit SAC components. *Nat. Cell Biol.* 14:746–752. http://dx.doi.org/10.1038/ncb2515

Yudkovsky, Y., M. Shteinberg, T. Listovsky, M. Brandeis, and A. Hershko. 2000. Phosphorylation of Cdc20/fizzy negatively regulates the mammalian cyclosome/APC in the mitotic checkpoint. *Biochem. Biophys. Res. Commun.* 271:299–304. http://dx.doi.org/10.1006/bbrc.2000.2622