POLO ensures chromosome bi-orientation by preventing and correcting erroneous chromosome–spindle attachments

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Summary

Correct chromosome segregation during cell division requires bi-orientation at the mitotic spindle. Cells possess mechanisms to prevent and correct inappropriate chromosome attachment. Sister kinetochores assume a 'back-to-back' geometry on chromosomes that favors amphitelic orientation but the regulation of this process and molecular components are unknown. Abnormal chromosome–spindle interactions do occur but are corrected through the activity of Aurora B, which destabilizes erroneous attachments. Here, we address the role of *Drosophila* POLO in chromosome–spindle interactions and show that, unlike inhibition of its activity, depletion of the protein results in bipolar spindles with most chromosomes forming stable attachments with both sister kinetochores bound to microtubules from the same pole in a syntelic orientation. This is partly the result of impaired localization and activity of Aurora B but also of an altered centromere organization with abnormal distribution of centromeric proteins and shorter interkinetochore distances. Our results suggests that POLO is required to promote amphitelic attachment and chromosome bi-orientation by regulating both the activity of the correction mechanism and the architecture of the centromere.

Key words: Cell cycle, *Drosophila melanogaster*, Kinetochore, Mitosis, Polo

Introduction

Distribution of sister chromatids during mitosis requires chromosome bi-orientation on the spindle with each sister kinetochore attached to microtubules from different poles in an amphitelic configuration. Abnormal kinetochore–microtubule interactions are destabilized by a correction mechanism that relies on Aurora B kinase (reviewed by Tanaka and Desai, 2008). At incorrectly attached kinetochores Aurora B phosphorylates Ndc80 preventing the stabilization of the attachment (Liu et al., 2009; Welburn et al., 2010). However, the structure of mitotic centromeres with the resulting geometry of sister kinetochores in a 'back-to-back' configuration might also play an essential role in this process (Ostergren, 1951), because after end-on attachment of one kinetochore, the unattached sister is oriented to microtubules from the opposite pole. The extent to which kinetochore geometry contributes to amphitelic attachment and chromosome bi-orientation is still under debate (Dewar et al., 2004, Loncarek et al., 2007).

POLO kinase was first identified in *Drosophila melanogaster* (Sunkel and Glover, 1988) and subsequently shown to be a highly conserved key regulator of mitotic progression (Archambault and Glover, 2009). POLO (Moutinho-Santos et al., 1999) and its human homologue Plk1 (Petronczki et al., 2008) localize to kinetochores from early prometaphase to late mitosis, and *polo* mutations are associated with abnormal chromosome segregation (Donaldson et al., 2001; Sunkel and Glover, 1988). Plk1 has been implicated in the regulation of kinetochore attachment to microtubules through its phosphorylation of BubR1 (Elowe et al., 2007), in the establishment of tension across sister kinetochores (Elowe et al., 2007; Wong and Fang, 2007) and in proper chromosome congression (Matsumura et al., 2007; Watanabe et al., 2008). Also, upon inactivation of Plk1 normal end-on attachments are lost and mostly lateral contacts are observed (Lenart et al., 2007). Nevertheless how POLO kinases regulate chromosome attachment remains largely unknown. We have used high-resolution microscopy of *Drosophila* S2 cells to characterize in detail the attachment of chromosomes to the spindle after POLO depletion.

Results and Discussion

Absence of Polo results in syntelic attachment of chromosomes

To determine the nature of chromosome–spindle interactions without POLO, cells were treated with specific double-stranded RNA (dsRNA) leading to rapid and effective depletion of the kinase (Fig. 2A) without affecting the cell culture growth (Fig. 2B). Analysis of the mitotic phenotype showed that, unlike control cells in which amphitelic attachment of sister kinetochores occurred (Fig. 1A, inset), in POLO-depleted cells kinetochores were located at the exterior of the area occupied by chromosomes (Fig. 1B–D) and showed syntelic attachment (Fig. 1B,C, insets). This orientation was further confirmed by the presence of the cohesion subunit Scc1 (also known as DRad21 and Rad21) between sister chromatids (Fig. 1F,F′). Occasionally, chromosomes establishing lateral contacts with microtubules could be found (Fig. 1D). Quantitative analysis revealed that in the absence of POLO most chromosomes are syntelic (supplementary material Fig. S1). This is in contrast to

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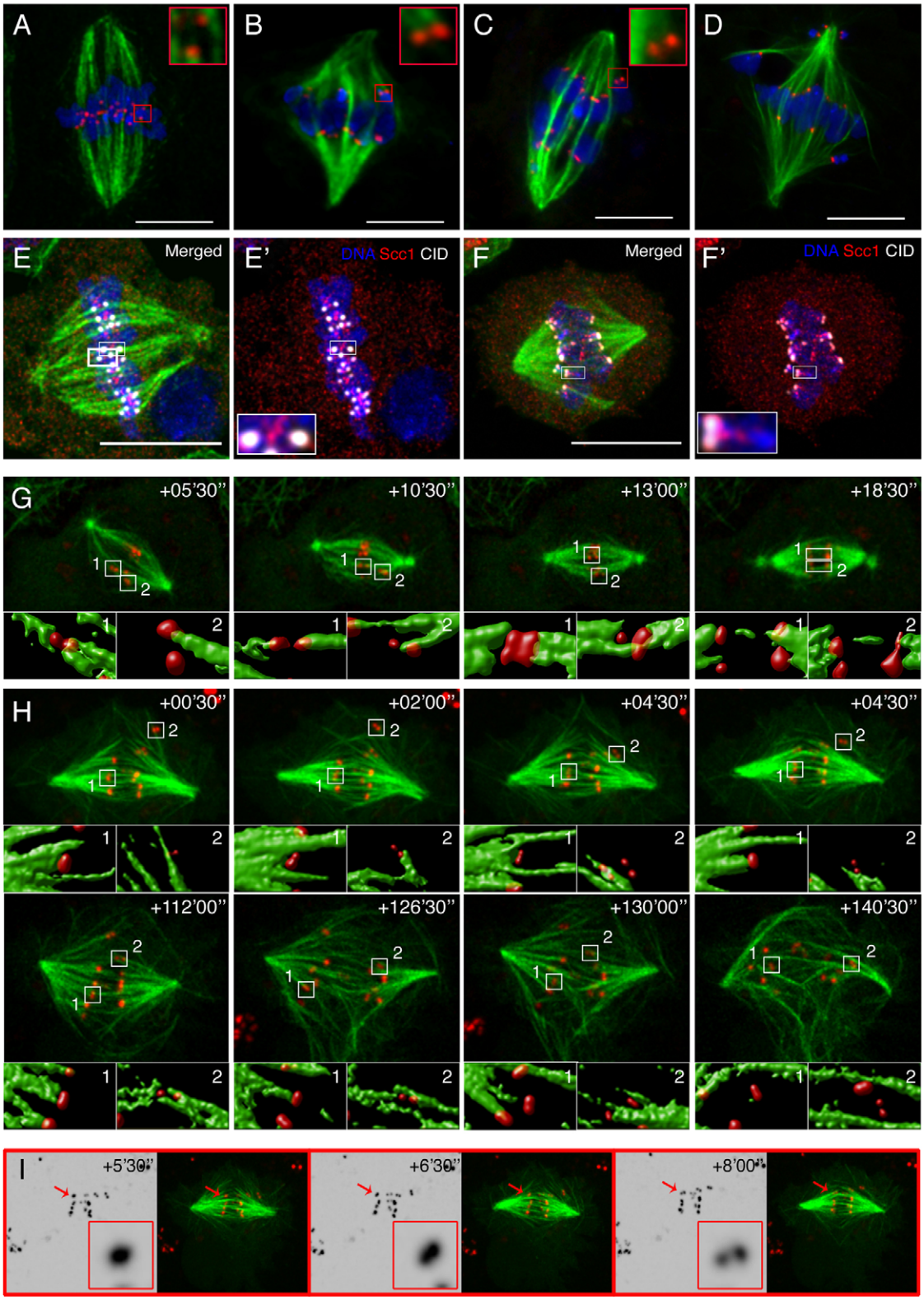
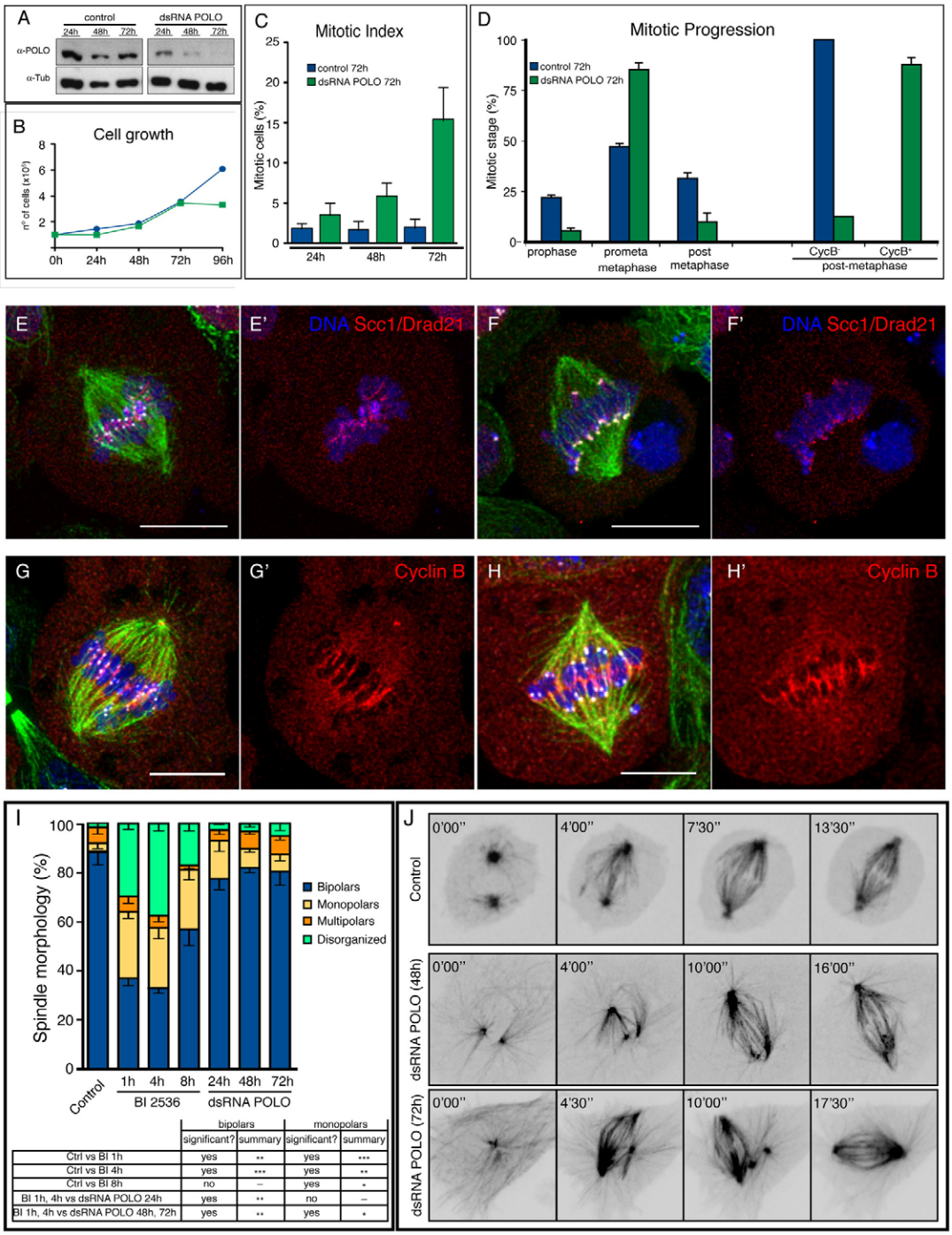


Fig. 1. Chromosomes attach to the mitotic spindle with syntelic orientation after POLO depletion. (**A–D**) Immunolocalization of α-tubulin (green) and CID (red) in (A) control and (B–D) POLO-depleted S2 prometaphase cells. (**E–F′**) Immunolocalization of Scc1 (red), α-tubulin (green) and CID (white) in control (E,E′) and POLO-depleted S2 cells (F,F′). Insets: high magnification images of selected regions. DNA is shown in blue. Scale bars: 10 μm. (**G–I**) 4D fluorescence microscopy of S2 cells expressing CID–mCherry (red) and GFP–α-tubulin (green). (G) Control cell (see also supplementary material Movie 1). (**H**) POLO-depleted cell (see also supplementary material Movie 2) (**I**) Selected stills from the time-lapse series; black and white insets: higher magnification of the CID–mCherry fluorescence signal for the kinetochore pair indicated by the arrow.



	bipolars		monopolars	
	significant?	summary	significant?	summary
Ctrl vs BI 1h	yes	**	yes	***
Ctrl vs BI 4h	yes	***	yes	**
Ctrl vs BI 8h	no	–	yes	*
BI 1h, 4h vs dsRNA POLO 24h	yes	**	no	–
BI 1h, 4h vs dsRNA POLO 48h, 72h	yes	**	yes	*

Fig. 2. POLO-depleted cells arrest with bipolar spindles. Monitoring of (**A**) POLO depletion, (**B**) cell culture growth, (**C**) mitotic index and (**D**) phenotypic analysis. Upon dsRNA treatment, POLO depletion was >55% at 24 hours, >85% at 48 hours and >95% at 72 hours, as determined by quantitative immunoblotting. (**E–H′**) Immunolocalization of Scc1 (Scc/Drad21; E–F′; red) and cyclin B (G–H′; red) in control (E,E′ and G,G′) and in POLO-depleted cells (F,F′ and H,H′); α-tubulin is green; CID is white; DNA is blue. Scale bars: 10 μm. (**I**) Quantitative analysis of spindle morphology upon POLO inhibition and depletion. (**J**) Stills from time-lapse analysis of spindle assembly in control and POLO-depleted S2 cells (supplementary material Movies 3, 4 and 5, respectively).

what happens when the kinase activity is inhibited with BI2536, a specific Plk1 inhibitor (Lenart et al., 2007). Syntelic attachment of chromosomes due to the absence of POLO function has not been described previously but it was reported that *polo*^{9} mutant neuroblasts, a strong hypomorphic allele, arrest after centromere separation (Donaldson et al., 2001). Analysis of chromosome attachment in these neuroblasts also revealed the presence of syntelic chromosomes rather than centromere separation (supplementary material Fig. S2). We analyzed this phenotype further using three-dimensional time-lapse (4D) microscopy of S2 cells stably expressing GFP–tubulin and centromere identifier protein (CID)–mCherry (Fig. 1G–I). In controls, rapid

congression of amphitelic chromosomes followed nuclear envelope breakdown (Fig. 1G, also see supplementary material Movie 1). These attachments remained stable throughout prometaphase and metaphase (Fig. 1G, inset 1) and occasional monoriented chromosomes were rapidly became amphitelic (Fig. 1G, inset 2). Hence, in S2 cells, amphitelic attachment is highly efficient and syntelic chromosomes were not detected. However, POLO-depleted cells showed a very prolonged prometaphase arrest (supplementary material Movie 2) during which the majority of chromosomes maintained persistent syntelic attachment (Fig. 1H, inset 1). Consistently, very intense CID dots were seen attached to microtubules of a single spindle pole that invariably resolved into two kinetochores (Fig. 1I). Occasionally, chromosomes showing lateral interactions with the spindle were also observed (Fig. 1H, inset 2). These results show that depletion of POLO either by dsRNAi or by mutation leads to stable syntelic attachment, in contrast to inhibition of the kinase that results mainly in unattached and lateral-interacting chromosomes, as has been described for human cells (Lenart et al., 2007).

POLO depletion causes a robust prometaphase arrest with bipolarly assembled spindles

As previously documented (Bettencourt-Dias et al., 2004; Donaldson et al., 2001), POLO depletion caused a strong prometaphase arrest (Fig. 2C,D), with Scc1 present between sister chromatids (Fig. 2F,F′) and high cyclin B levels (Fig. 2H,H′). Interestingly, most (80%) POLO-depleted cells had bipolar spindles, even after long periods of dsRNA treatment (Fig. 2I). Although we cannot rule out that this phenotype arose from partial depletion of POLO, our results are in agreement with descriptions of phenotypes after severe reduction of POLO levels either by mutation (Donaldson et al., 2001) or by RNAi in cultured cell lines (Bettencourt-Dias et al., 2004). Yet, POLO kinases have been implicated in centrosome maturation, and impaired POLO kinase activity has been shown to lead to the formation of monopolar spindles (Lenart et al., 2007; Llamazares et al., 1991; Santamaria et al., 2007; Sunkel and Glover, 1988). Given that in the absence of POLO, chromosomes attach syntelically to bipolar spindles, we hypothesized that this particular orientation could be the outcome of bipolarization of a previously assembled monopolar structure, similar to the situation in monastrol washout experiments (Lampson et al., 2004). Thus, we followed in vivo spindle assembly in POLO-depleted cells expressing GFP–tubulin (Fig. 2J). We found that upon nuclear envelope breakdown either after 48 hours or 72 hours of POLO depletion, irrespectively of the presence of robust microtubule-organizing centers (supplementary material Movies 3–5), microtubules rapidly organized into bipolar structures, showing that S2 cells without POLO rarely form monopolar spindles. In agreement with this, a previous study on Plk1-depleted HeLa cells found a substantial proportion of bipolar spindles (38%) and only 18% of monopolar structures (Sumara et al., 2004). Our results confirm the previous suggestion (McInnes et al., 2006) that particular aspects of the phenotype are different depending on whether the catalytic activity of POLO is impaired or the protein is depleted.

POLO-depleted cells have an impaired correction mechanism

The accuracy of chromosomal attachments is partly ensured by the activity of a correction mechanism that requires localization and activity of Aurora B at centromeres (reviewed by Ruchaud et al., 2007). In control cells, Aurora B localizes to centromeres from early mitosis to metaphase (Fig. 3A′,A″) but after POLO depletion becomes distributed over the chromatin (Fig. 3B′,B″). Similar results were obtained for *Drosophila* innercentromere protein (INCENP), another subunit of the complex (supplementary material Fig. S3). This localization pattern could result from either a failure in recruitment or in the maintenance of the Chromosomal Passenger Complex (CPC) at the centromeres. Ndc80 is a substrate of Aurora B and a conserved kinetochore protein that shows reduced affinity for microtubule binding when phosphorylated (Cheeseman et al., 2006). To determine whether Ndc80 phosphorylation is Aurora B dependent in S2 cells, we analyzed Ndc80 phosphorylation status after treatment with the specific inhibitor binucleine 2 (Smurnyy et al., 2010) and after Aurora B depletion by dsRNA (Fig. 3C). Western blot analysis detected both unphosphorylated and phosphorylated Ndc80, the latter as a slower migrating band that was virtually absent upon phosphatase treatment (Fig. 3C, upper panel). Likewise, predominantly unphosphorylated Ndc80 was detected in extracts prepared from cultures treated with either binucleine 2 or after Aurora B depletion (Fig. 3C, lower panel) strongly suggesting that Ndc80 is an Aurora B substrate in these cells. We also observed a prominent decrease in phosphorylated Ndc80 levels in the absence of POLO (Fig. 3C), indicating that Aurora B activity was impaired in these cells. Aurora B activity was lower not only at kinetochores and/or centromeres, since histone H3 phosphorylation at S10, a well-known chromatin substrate of Aurora B (Giet and Glover, 2001), is also found to be diminished upon POLO depletion (supplementary material Fig. S4).

The syntelic phenotype observed after POLO depletion could be exclusively caused by an impaired correction mechanism. However, in vivo analysis of Aurora B-depleted cells treated with MG132 to prevent quick mitotic exit (Adams et al., 2001), showed low levels (28%) of syntelic attachment (Fig. 3D–F; see also supplementary material Movies 6, 7). Aurora B was severely depleted after dsRNA treatment (95%; Fig. 3C) and mitotic abnormalities, including chromatin condensation defects and cytokinesis failure, were observed (supplementary material Figs S4, S5) (Adams et al., 2001; Giet and Glover, 2001). Nevertheless, in the absence of POLO most chromosomes (93%) were syntelic (Fig. 3F). Therefore, we conclude that impairment of Aurora B activity accounts for only a small proportion of persistent syntelic chromosomes, and is unlikely to be the unique source of abnormal chromosome attachments in POLO-depleted cells.

POLO is necessary for proper centromere architecture

Kinetochores assemble on the surface of centromeres assuming a ‘back-to-back’ geometry thought to favor amphitelic attachment (Tanaka, 2010; Loncareck et al., 2007). To analyze centromere architecture in POLO-depleted cells, we performed immunolocalization to identify Mei-S332, the *Drosophila* homologue of shugoshin, which accumulates between sister kinetochores early in mitosis (Moore et al., 1998) (Fig. 4A,A′). In POLO-depleted cells Mei-S332 was found at the centromeres but it was displaced, partially overlapping the CID staining (Fig. 4B,B′). Fluorescence intensity profiles of Mei-S332 in relation to the kinetochore-assembly-site marker CID, revealed that, whereas in controls Mei-S332 accumulated mostly

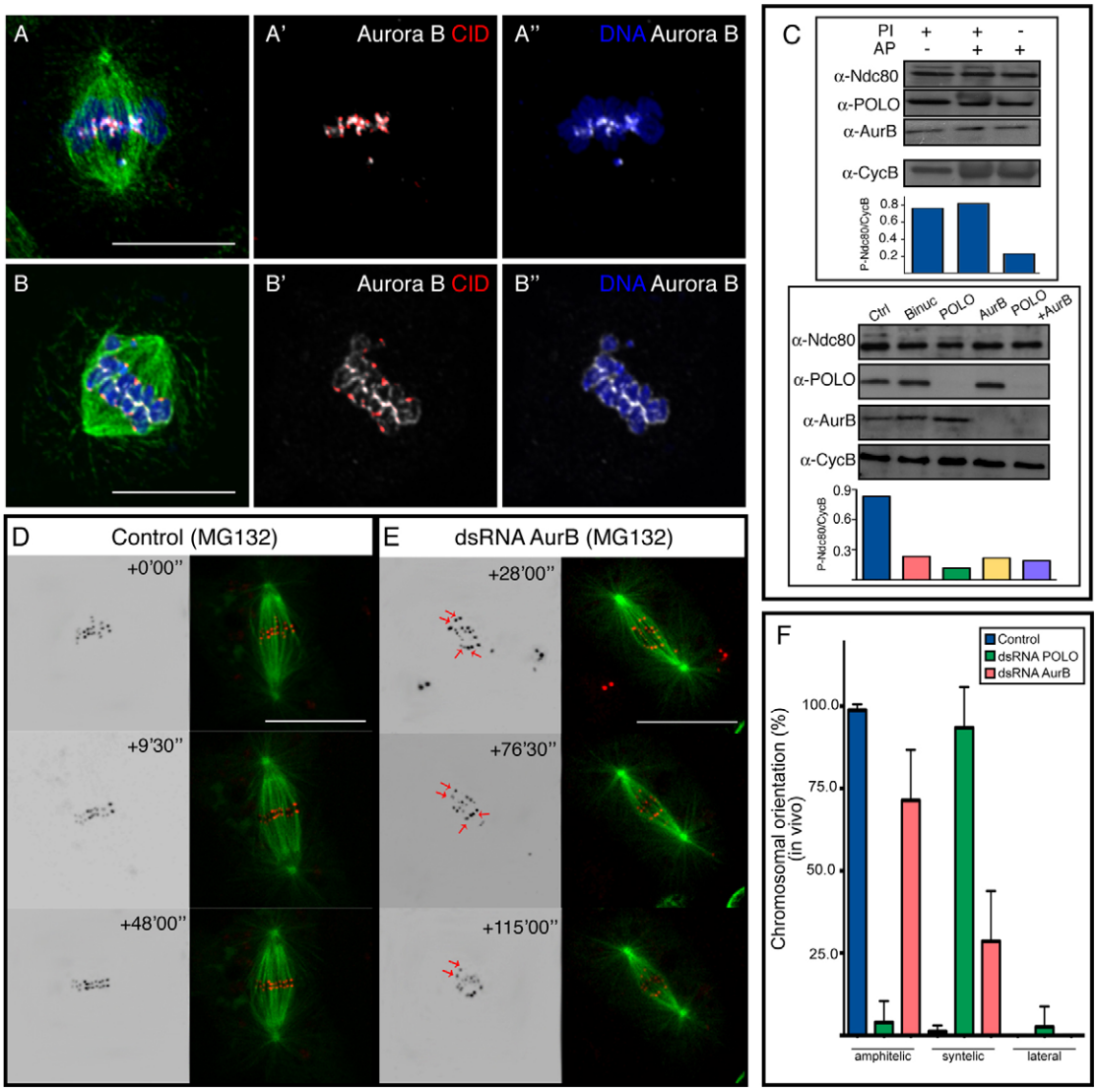


Fig. 3. Aurora B localization and activity are affected in POLO-depleted cells. (**A**,**B**) Immunolocalization of Aurora B (white) at prometaphase in control cells (A) and after POLO depletion (B); DNA is blue, α-tubulin is green and CID is red. Scale bars: 10 μm. (**C**) Upper panel: immunoblot of total extracts prepared from control cells in the presence (+) and absence (–) of phosphatase inhibitors (PI) and alkaline phosphatase (AP). Lower panel: immunoblot of control, binucleine-2-treated, POLO-depleted, Aurora B (AurB)-depleted and POLO+AurB-depleted cell extracts; specific antibodies against Ndc80, POLO, AurB and cyclin B were used. Graphs: quantitative immunoblotting of relative intensity of phosphorylated Ndc80 normalized to cyclin B levels. (**D**,**E**) Selected frames from 4D microscopy analysis of GFP–Tub::CID–mCherry in (D) control and (E) AurB-depleted cells (see also supplementary material Movies 6 and 7, respectively). Red arrows indicate syntelic kinetochore pairs. (**F**) In vivo chromosome orientation, determined over time using 4D microscopy, expressed as the average percentage of the total chromosome number in each cell (control cells, $n=5$; POLO-depleted cells, $n=10$; AurB-depleted cells, $n=7$).

between sister kinetochores (Fig. 4A′′,A′′′), in POLO-depleted chromosomes Mei-S332 had a much wider distribution (Fig. 4B′,B′′′). These results suggest that POLO depletion displaces intracentromeric proteins, indicative of an abnormal centromeric architecture.

Previous studies have shown that syntelic attachments result in centromeric distortion, leading to a 'side-by-side' configuration of sister kinetochores with shorter interkinetochore distances (Loncarek et al., 2007). In POLO-depleted cells, alteration of the structure of the centromere could be either the primary cause of syntelic attachments or, more probably, a consequence. To distinguish between these two possibilities, we quantified interkinetochore distances in chromosomes from POLO-depleted cells that were unable to interact with microtubules because of depletion of Ndc80 and Nuf2, two essential kinetochore components for microtubule attachment (reviewed by Orr et al., 2010). Absence of the Ndc80 complex alone leads to failure in chromosome congression and kinetochores predominantly establish lateral contacts with microtubules (Fig. 4C). As expected, after depletion of Ndc80+Nuf2 and POLO, syntelic attachments were lost and mainly lateral interactions and unattached chromosomes were present (Fig. 4C). However, although interkinetochore distances in chromosomes of control and Ndc80+Nuf2-depleted cells were similar, in POLO-depleted cells these were substantially shorter, regardless of the presence of the Ndc80 complex (Fig. 4E,F).

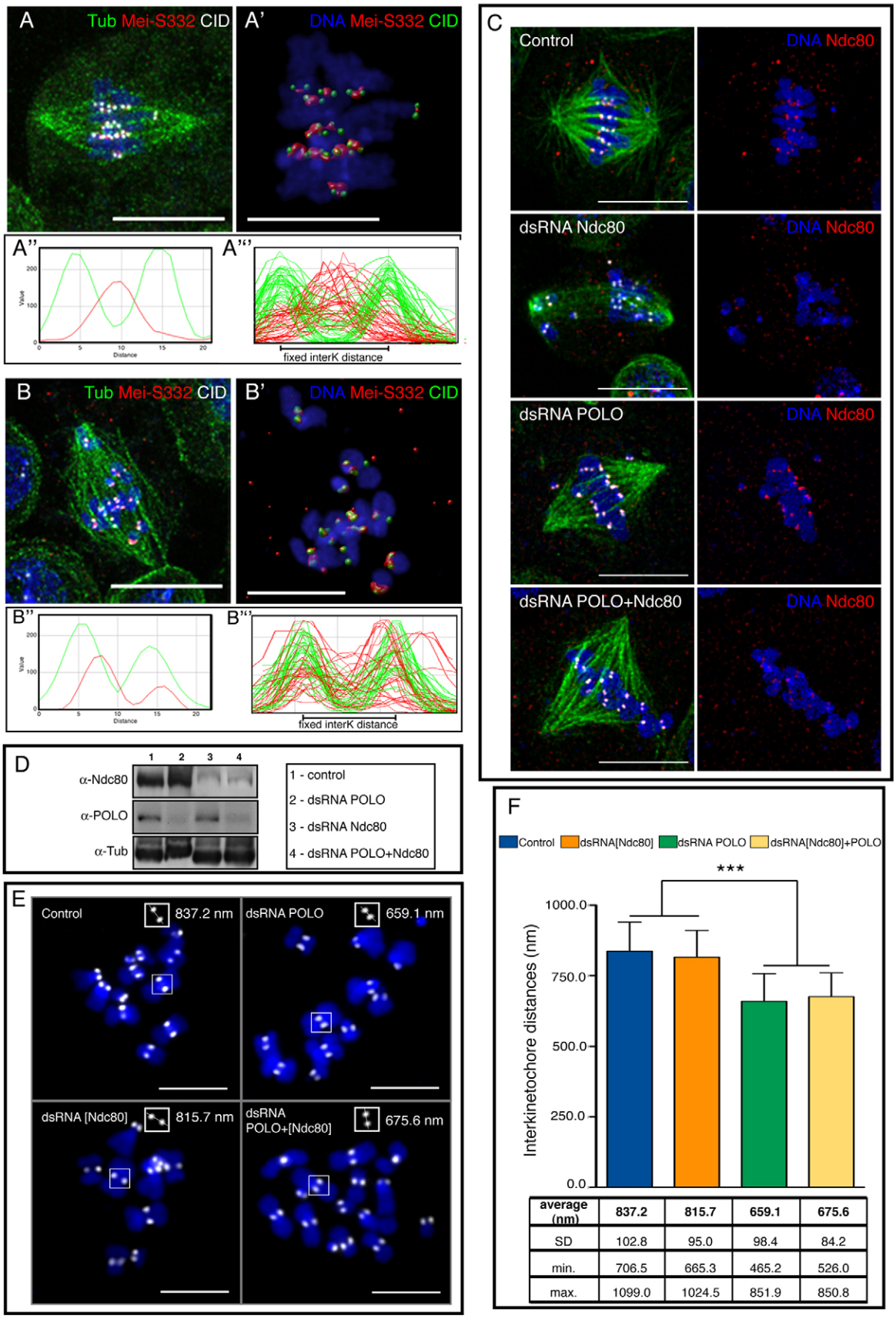


Fig. 4. See next page for legend.

These results show that the shorter interkinetochore distance observed in syntelic chromosomes after POLO depletion is independent of microtubule attachment. These data suggest that POLO is required to maintain a normal interkinetochore distance, independently of spindle microtubule attachment.

POLO kinase is key to ensuring chromosome bi-orientation

Chromosome bi-orientation relies upon a correction mechanism, which actively promotes the destabilization of chromosome attachment errors, and a prevention mechanism, which acts to reduce inaccurate chromosome orientations. Our findings show that POLO kinase is a major regulator of proper chromosome attachments and bi-orientation by intervening in both mechanisms. On the one hand, we found that POLO is essential for the centromeric localization and activity of Aurora B, the key kinase in the correction mechanism (reviewed by Lampson and Cheeseman, 2010), so that without POLO destabilization of improper attachments does not take place. A recent study (Foley et al., 2011) also showed that both Plk1 and Aurora B kinases have a role in the destabilization of the of kinetochore attachment to the microtubule that is counter balanced by the centromeric localization and activity of the B56-PP2A phosphatase. On the other hand, we observed that depletion of POLO causes the displacement of proteins within the centromere and considerable shortening of interkinetochore distances, indicating that this kinase is essential for the architecture of the centromere and, therefore, the spatial organization of sister kinetochores. In addition, it is also well established that POLO-like kinases regulate resolution of chromosome arms (reviewed by Archambault and Glover, 2009), the decatenation enzyme topoisomerase II (Wang et al., 2010, Kurasawa et al., 2010) and, more recently, the condensin II complex (Abe et al., 2011). Therefore, it is plausible that alteration of the chromosomal topology caused by lack of POLO function could affect centromeric architecture.

The observation that POLO is necessary for proper chromosome attachment because it participates directly in both error-prevention and error-correction mechanisms has important implications for how bi-orientation is achieved, and sheds new light on the extent to which the geometry of the kinetochore pair contributes to the accuracy of chromosome attachments (Dewar et al., 2004; Sakuno et al., 2009). Our results suggest a major contribution of the prevention mechanism in avoiding attachment errors in metazoan cells.

Fig. 4. POLO depletion affects centromeric architecture and results in shortened interkinetochore distances. (**A**,**B**) Immunolocalization of Mei-S332 in control (A) and POLO-depleted (B) cells. (**A′**,**B′**) Surface rendered images of the chromosomes shown in A and B. (**A″**,**B″**) Representative and (**A‴**,**B‴**) superimposed fluorescence profiles; line scans were traced from the center of the pairs of CID staining (green) and over the Mei-S332 fluorescence signal (red); at least three kinetochore pairs per cell were analyzed (control, $n=6$; POLO-depleted cells, $n=8$). (**C**) Immunolocalization of Ndc80 (red), α-tubulin (green), CID (white) and DNA (blue) in control, Ndc80+Nuf2-depleted (labeled as Ndc80), POLO-depleted and Ndc80+Nuf2+POLO-depleted cells. Scale bars: 10 μm. (**D**) Western blot showing Ndc80 and POLO depletion levels: >75% in Ndc80+Nuf2 dsRNA-treated cells, >95% in POLO dsRNA-treated cells, >85% and >95% in Ndc80+Nuf2+POLO dsRNA-treated cells. (**E**) Chromosomal spreads. Boxed regions show examples of the distance measured for F. Scale bars: 5 μm. (**F**) Average interkinetochore distances; control and Ndc80+Nuf2-depleted cells, $n=30$; POLO- and Ndc80+Nuf2+POLO-depleted cells, $n=50$. Bars represent the standard deviation; $P<0.001$.

Materials and Methods

Cell culture and western blotting

Drosophila S2 cells were cultured according to the method of Maiato et al. (Maiato et al., 2003). For western blot analysis total protein extracts from 1×10^6 cells were used. BI2536 (Boehringer Ingelheim) was used at 50 nM, binucleine 2 (Sigma-Aldrich) at 40 μM and MG1323 at 20 μM (Calbiochem). Ndc80 phosphorylation status was determined in the presence of phosphatase inhibitors (Sigma-Aldrich) after incubation with 20 IU alkaline phosphatase (Fermentas, FastAP) for 1 hour at 37℃. A GS800 densitometer and Quantity One© software (Bio-Rad) were used for quantitative immunoblotting.

Double-stranded RNA interference

RNA interference (RNAi) was performed as described previously (Maiato et al., 2003). A 740 bp *Eco*RI–*Bam*HIII POLO cDNA fragment (LD11851) containing the 5′ UTR region was cloned into both pSPT18 and pSPT19 expression vectors (Roche). RNA was synthesized using a T7 Megascript kit (Ambion). Aurora B dsRNA was synthesized from the pOT2 LD39409 using a T7/SP6 Megascript kit (Ambion). 15 μg of each dsRNA were used.

Immunofluorescence in *Drosophila* S2 cells

For immunostaining, cells were fixed in 3.7% formaldehyde (Sigma) in PHEM (60 mM PIPES, 25 mM HEPES pH 7.0, 10 mM EGTA, 4 mM $MgSO_4$) for 12 minutes and then detergent-extracted with 0.5% Triton X-100 in PBS three times for 5 minutes each. When required, cultured cells were incubated with 25 μM colchicine for 1 hour. Immunostaining was performed as described previously (Coelho et al., 2008). Slides were mounted in Vectashield containing DAPI (Vector Laboratories) for observation in a Zeiss Axiovert 200M microscope (Zeiss, Germany) or in a Leica SP2AOBS SE scanning confocal microscope (Leica Microsystems). Deconvolution of data stacks was done with Huygens Essential, version 3.0.2p1 (Scientific Volume Imaging, Hilversum, The Netherlands). Fluorescence intensity profiles were traced using an RGB profiler plugin of ImageJ1.45h Software (NIH). The relative intensities of phosphorylated histone H3 fluorescence were determined on maximum projected images acquired with fixed exposure acquisition settings; DAPI staining was used to define a selected area, and mean pixel intensities were determined (ImageJ).

Antibodies

The following antibodies were used: anti-α-tubulin (α-Tub; DM1A, Sigma) mouse monoclonal, 1:10,000 for western blotting (WB); anti-α-Tub (B152, Sigma) mouse monoclonal, 1:5000 for immunofluorescence (IF); mouse anti-POLOMA294 1:80 [(Llamazares et al., 1991); WB]; anti-CID, 1:1000 rat polyclonal (C.E.S., unpublished data); anti-Scc1/DRad2, 1:1500 guinea pig polyclonal (a gift from Stefan Heidman); anti-Cyc B rabbit polyclonal, 1:6000 (WB) and 1:3000 (IF; Christian Lehner); anti-Aurora B (Rb2), 1:500 (WB) rabbit polyclonal (a gift from David Glover); anti-Aurora B (Rb963), 1:500 and anti-INCENP (Rb801) 1:1500, rabbit polyclonals (gifts from Mar Carmena); anti-Mei-S322 guinea pig polyclonal, 1:10,000 (a gift from Terry Orr-Weaver); anti-Ndc80 (Rb272), 1:5000 (WB) and 1:200 (IF) rabbit polyclonal (gifts from Michael Goldberg); anti-pSer10H3 (Upstate) rabbit polyclonal, 1:250 (WB) and 1:10,000 (IF). Anti-HRP mouse and rabbit secondary antibodies, 1:5000 (Amersham) were visualized using the ECL system (GE Healthcare). Secondary antibodies with conjugated fluorescent dyes from the Alexa series (Invitrogen) were used according to manufacturer's instructions.

Time-lapse fluorescence imaging

Time-lapse analysis of mitosis was done on S2 cells stably expressing CID–mCherry and GFP–α-Tub (Coelho et al., 2008). Control, POLO- or Aurora B RNAi-treated cells were plated on MatTek glass bottom dishes (MatTek Corporation) coated with 0.25 mg/ml concanavalin A (Sigma). 4D fluorescence microscopy datasets were collected every 30 seconds with 0.5 μm z-steps covering the entire cell volume using a 100˚, 1.4 NA plan-apochromatic objective at 25℃ with a spinning disk confocal system, with or without an EMCCD iXonEM+ camera, 488 nm and 561 nm laser lines, and a Yokogawa CSU-22 unit on an inverted microscope (IX81; Olympus), driven by Andor IQ live-cell imaging software (www.andor.com). For deconvolution, Huygens Essential was used. Image sequence analysis and video assembly was done with ImageJ.

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References

Abe, S., Nagasaka, K., Hirayama, Y., Kozuka-Hata, H., Oyama, M., Aoyagi, Y., Obuse, C. and Hirota, T. (2011). The initial phase of chromosome condensation requires Cdk1-mediated phosphorylation of the CAP-D3 subunit of condensin II. *Genes Dev*. **25**, 863-874.

Adams, R. R., Maiato, H., Earnshaw, W. C. and Carmena, M. (2001). Essential roles of Drosophila innercentromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphasechromosome alignment, kinetochore disjunction, and chromosome segregation. *J. Cell Biol.* **153**, 865-880.

Archambault, V. and Glover, D. M. (2009). Polo-like kinases: conservation and divergence in theirfunctions and regulation. *Nat. Rev. Mol. Cell Biol.* **10**, 265-275.

Bettencourt-Dias, M., Giet, R., Sinka, R., Mazumdar, A., Lock, W. G., Balloux, F., Zafiropoulos, P. J., Yamaguchi, S., Winter, S., Carthew, R. W. et al. (2004). Genome-wide survey of protein kinases required for cell cycle progression. *Nature* **432**, 980-987.

Cheeseman, I. M., Chappie, J., Wilson-Kubalek, E. and Desai, A. (2006). The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell* **127**, 983-997.

Coelho, P. A., Queiroz-Machado, J., Carmo, A. M., Moutinho-Pereira, S., Maiato, H. and Sunkel, C. E. (2008). Dual role of topoisomerase II in centromere resolution and aurora B activity. *PLoS Biol.* **6**, e207.

Dewar, H., Tanaka, K., Nasmyth, K. and Tanaka, T. U. (2004). Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. *Nature* **428**, 93-97.

Donaldson, M. M., Tavares, A., Ohkura, H., Deak, P. and Glover, D. M. (2001). Metaphase arrest with centromere separation in polo mutants of Drosophila. *J. Cell Biol.* **153**, 663-676.

Elowe, S., Hummer, S., Uldschmid, A., Li, X. and Nigg, E. A. (2007). Tension-sensitive Plk1 phosphorylation on BubR1 regulates the stability of kinetochore microtubule interactions. *Genes Dev.* **21**, 2205-2219.

Foley, E. A., Maldonado, M. and Kapoor, T. M. M. (2011). Formation of stable attachments between kinetochores and microtubules depends on the B56-PP2A phosphatase. *Nat. Cell Biol.* **13**, 1265-1271.

Giet, R. and Glover, D. M. (2001). Drosophila aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J. Cell Biol.* **152**, 669-682.

Hauf, S., Cole, R. W., LaTerra, S., Zimmer, C., Schnapp, G., Walter, R., Heckel, A., van Meel, J., Rieder, C. L. and Peters, J. M. (2003). The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J. Cell Biol.* **161**, 281-294.

Lampson, M. A. and Cheeseman, I. M. (2011). Sensing centromere tension: Aurora B and the regulation of kinetochore function. *Trends Cell Biol.* **21**, 133-140.

Lampson, M. A., Renduchitala, K., Khodjakov, A. and Kapoor, T. M. (2004). Correcting improper chromosome-spindle attachments during cell division. *Nat. Cell Biol.* **6**, 232-237.

Lenart, P., Petronczki, M., Steegmaier, M., Di Fiore, B., Lipp, J. J., Hoffmann, M., Rettig, W. J., Kraut, N. and Peters, J. M. (2007). The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. *Curr. Biol.* **17**, 304-315.

Liu, D., Vader, G., Vromans, M. J., Lampson, M. A. and Lens, S. M. (2009). Sensing chromosome biorientation by spatial separation of Aurora B kinase from kinetochore substrates. *Science* **323**, 1350-1353.

Llamazares, S., Moreira, A., Tavares, A., Girdham, C., Spruce, B. A., Gonzalez, C., Karess, R. E., Glover, D. M. and Sunkel, C. E. (1991). Polo encodes a protein kinase homolog required formitosis in Drosophila. *Genes Dev.* **5**, 2153-2165.

Loncarek, J., Kisurina-Evgenieva, O., Vinogradova, T., Hergert, P. J., LaTerra, S., Kapoor, T. M. and Khodjakov, A. L. (2007). The centromere geometry essential for keeping mitosis error free is controlled by spindle forces. *Nature* **450**, 745-749.

Kurasawa, Y. and Yu-Lee, L. Y. (2008). PICH and cotargeted Plk1 coordinately maintain prometaphase chromosome arm architecture. *Mol. Biol. Cell* **21**, 1188-1199.

Maiato, H., Sunkel, C. E. and Earnshaw, W. C. (2003). Dissecting mitosis by RNAi in Drosophila tissue culture cells. *Biol. Proced. Online* **5**, 153-161.

Matsumura, S., Toyoshima, F. and Nishida, E. (2007). Polo-like kinase 1 facilitates chromosome alignment during prometaphase through BubR1. *J. Biol. Chem.* **282**, 15217-15227.

McInnes, C., Mazumdar, A., Mezna, M., Meades, C., Midgley, C., Scaerou, F., Carpenter, L., Mackenzie, M., Taylor, P., Walkinshaw, M. et al. (2006). Inhibitors of Polo-like kinase reveal roles in spindle-pole maintenance. *Nat. Chem. Biol.* **2**, 608-617.

Moore, D. P., Page, A. W., Tang, T. T., Kerrebrock, A. W. and Orr-Weaver, T. L. (1998). The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. *J. Cell Biol.* **140**, 1003-1012.

Moutinho-Santos, T., Sampaio, P., Amorim, I., Costa, M. and Sunkel, C. E. (1999). In vivo localization of the mitotic POLO kinase shows a highly dynamic association with the mitotic apparatus during early embryogenesis in Drosophila. *Biol. Cell* **91**, 585-596.

Orr, B., Afonso, O., Feijao, T. and Sunkel, C. E. (2010). Driving chromosome segregation: lessons from the human and Drosophila centromere-kinetochore machinery. *Biochem. Soc. Trans.* **38**, 1667-1675.

Ostergren, G. (1951). The mechanism of co-orientation in bivalents and multivalents: The theory of orientation by pulling. *Hereditas* **37**, 85-156.

Petronczki, M., Lenart, P. and Peters, J. (2008). Polo on the rise - from mitotic entry to cytokinesis with Plk1. *Dev. Cell* **14**, 646-659.

Ruchaud, S., Carmena, M. and Earnshaw, W. C. (2007). Chromosomal passengers: Conducting cell division. *Nat. Rev. Mol. Cell Biol.* **8**, 798-812.

Sakuno, T., Tada, K. and Watanabe, Y. (2009). Kinetochore geometry defined by cohesion within the centromere. *Nature* **458**, 852-858.

Santamaria, A., Neef, R., Eberspacher, U., Eis, K., Husemann, M., Mumberg, D., Prechtl, S., Schulze, V., Siemeister, G., Wortmann, L. et al. (2007). Use of the novel Plk1 inhibitor ZK-Thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. *Mol. Biol Cell* **18**, 4024-4036.

Smurnyy, Y., Toms, A. V., Hickson, G. R., Eck, M. J. and Eggert, U. S. (2010). Binucleine 2, an isoform specific inhibitor of Drosophila Aurora B kinase, provides insights into the mechanism of cytokinesis. *ACS Chem. Biol.* **5**, 1015-1020.

Sumara, I., Giménez-Abián, J. F., Gerlich, D., Hirota, T., Kraft, C., de la Torre, C., Ellenberg, J. and Peters, J. M. (2004). Roles of polo-like kinase 1 in the assembly of functional mitotic spindles. *Curr. Biol.* **14**, 1712-1722.

Sunkel, C. E. and Glover, D. M. (1988). Polo, a mitotic mutant of Drosophila displaying abnormal spindle poles. *J. Cell Sci.* **89**, 25-38.

Tanaka, T. U. (2010). Kinetochore-microtubule interactions: Steps towards bi-orientation. *EMBO J.* **29**, 4070-4082.

Tanaka, T. U. and Desai, A. (2008). Kinetochore-microtubule interactions: The means to the end. *Curr. Opin. Cell Biol.* **20**, 53-63.

Wang, L. H., Mayer, B., Stemmann, O. and Nigg, E. A. (2010). Centromere DNA decatenation depends on cohesin removal and is required for mammalian cell division. *J. Cell Sci.* **123**, 806-813.

Watanabe, N., Sekine, T., Takagi, M., Iwasaki, J., Imamoto, N., Kawasaki, H. and Osada, H. (2009). Deficiency in chromosome congression by the inhibition of PLK1 polo box domain-dependent recognition. *J. Biol. Chem.* **284**, 2344-2353.

Welburn, J. P., Vleugel, M., Liu, D., Yates, J. R., 3rd, Lampson, M. A., Fukagawa, T. and Cheeseman, I. M. (2010). Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. *Mol. Cell* **38**, 383-392.

Wong, O. K. and Fang, G. (2007). Cdk1 phosphorylation of BubR1 controls spindle checkpoint arrestand Plk1-mediated formation of the 3F3/2 epitope. *J. Cell Biol.* **179**, 611-617.