

Cell division: control of the chromosomal passenger complex in time and space

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Abstract The ultimate goal of cell division is equal transmission of the duplicated genome to two new daughter cells. Multiple surveillance systems exist that monitor proper execution of the cell division program and as such ensure stability of our genome. One widely studied protein complex essential for proper chromosome segregation and execution of cytoplasmic division (cytokinesis) is the chromosomal passenger complex (CPC). This highly conserved complex consists of Borealin, Survivin, INCENP, and Aurora B kinase, and has a dynamic localization pattern during mitosis and cytokinesis. Not surprisingly, it also performs various functions during these phases of the cell cycle. In this review, we will give an overview of the latest insights into the regulation of CPC localization and discuss if and how specific localization impacts its diverse functions in the dividing cell.

Introduction

During cell division, an exact copy of the genome is transmitted from mother cell to daughter cells. This requires equal segregation of the duplicated chromosomes (sister chromatids) during mitosis followed by cytoplasmic division to form two separate cells. A prerequisite for faithful segregation of the chromosomal content is bi-orientation of the sister chromatids on the mitotic spindle. This is achieved when two sister chromatids bind microtubules emanating from opposite poles of the cell (a state called amphitelic attachment). As long as this attachment state has not been reached for all chromosomes, the mitotic checkpoint is active and prevents

progression of the cell cycle into anaphase. If the mitotic checkpoint fails, cells enter anaphase prematurely with unattached or aberrantly attached kinetochores (multi-protein structures that assemble at centromeres and that form the microtubule attachment sites of the chromosomes), resulting in chromosome segregation errors. Generally, when the mitotic checkpoint is completely inactive, the extent of chromosome segregation errors is too severe to be compatible with cell survival (Kops et al. 2005). A weakened checkpoint, on the other hand, is thought to result in infrequent losses and gains of chromosomes (known as chromosomal instability) that can be compatible with life. However, the latter situation gives rise to aneuploidy (a state in which a cell contains a chromosome number deviating from a multiple of a haploid chromosome content) and may predispose to cancer (Kops et al. 2005; Ricke and van Deursen 2013; Thompson et al. 2010).

A major regulator of mitosis and cytokinesis is the evolutionarily conserved chromosomal passenger complex (CPC) consisting of the enzymatic core Aurora B kinase (AURKB), the scaffold protein inner centromere protein (INCENP), and two other non-enzymatic subunits Survivin (BIRC5) and Borealin (CDCA8). The Baculovirus Inhibitor of Apoptosis Protein repeat (BIR) protein Survivin, together with Borealin, binds the N-terminal part of INCENP, while Aurora B interacts with the C-terminal IN-box of INCENP. The N- and C-terminal regions of INCENP are separated by a large unstructured region that harbors a Heterochromatin Protein 1 (HP1) binding motif, multiple (potential) Cdk1 phosphorylation sites, and a predicted coiled-coil domain (Fig. 1) (Ainsztein et al. 1998; Carmena et al. 2012b; Dephoure et al. 2008; Hegemann et al. 2011; Honda et al. 2003; Jeyaprakash et al. 2007; Kaitna et al. 2000; Mackay et al. 1993, 1998; Malik et al. 2009; Nousiainen et al. 2006; Olsen et al. 2010). Because protein interactions within the CPC support protein stability of the individual CPC subunits, knockdown or depletion of any

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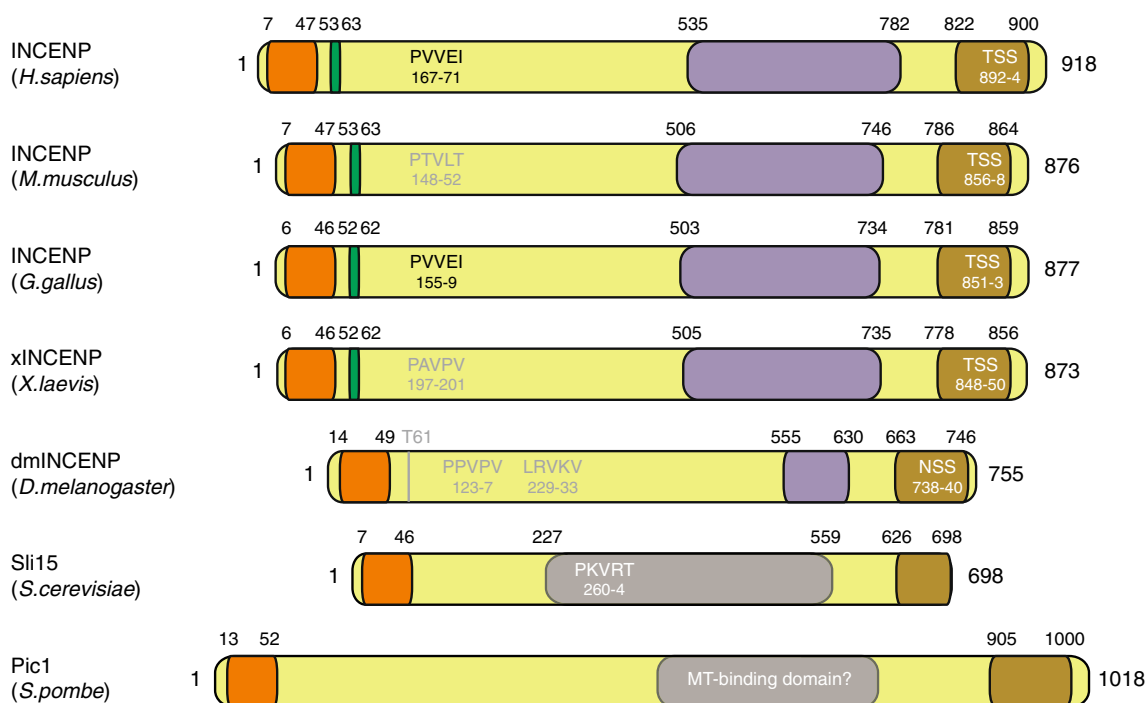


Fig. 1 Schematic depiction of INCENP in humans and the most commonly used model organisms to study the function of the CPC. The CEN-box is shown in orange (Jeyaprasath et al. 2007; Mackay et al. 1998). The green box is the β -tubulin binding region (which includes the Cdk1-phosphorylated Threonine-59; amino acid number in *Homo sapiens*) (Wheatley et al. 2001). Note that *Drosophila* INCENP contains a potential N-terminal Cdk1 site (TP) but not a recognizable β -tubulin binding region. The amino acids shown to be responsible for binding to HP1 (PxVxL/D) are depicted in black (Kang et al. 2011; Nozawa et al. 2010). Gray and white motifs are potential HP1 binding sites. Note that although Sli15 has a potential HP1 binding site, budding yeast lacks an HP1 homolog (Hickman et al. 2011). The domain responsible for central

spindle localization of human INCENP is shown in purple (Vader et al. 2007). This domain has a predicted coiled-coil structure conserved from *Xenopus* to humans (Mackay et al. 1993). Therefore, these coiled-coil domains are also depicted in purple. *Drosophila* INCENP has a short coiled-coil domain (shown in purple as well), the function of which has not been studied yet. Moreover, Sli15 contains a microtubule-binding domain that is not a coiled-coil (shown in gray) (Kang et al. 2001). For Pic1, no data are available on microtubule binding. The brown region is the IN-box including the TSS motif (if indicated), which binds and activates Aurora B and is itself phosphorylated by Aurora B (Adams et al. 2000; Kaitna et al. 2000; Xu et al. 2009)

CPC member as well as (chemical) inhibition of Aurora B in either fungi, fly, worm, frog, or mammalian cells gives rise to very similar phenotypes (Honda et al. 2003; Klein et al. 2006; Vader et al. 2006a). Disturbance of CPC function results in chromosome congression and segregation defects due to stabilization of incorrect kinetochore–microtubule attachments, an impaired function of the mitotic checkpoint and improper spindle formation. Moreover, cytokinesis is also impaired and cells that exit mitosis without a functional CPC are tetraploid and eventually die or senesce. The severity of these defects seems to depend on the level of knockdown or kinase inhibition; certain functions of the CPC are already disturbed when the complex is only partially inhibited (e.g., correction of merotelic attachments—see below), while others (e.g., its function in the mitotic checkpoint) may require complete inhibition (Adams et al. 2001; Biggins and Murray 2001; Carvalho et al. 2003; Cimini et al. 2006; Ditchfield et al. 2003; Gassmann et al. 2004; Giet and Glover 2001; Girdler et al. 2006; Hauf et al. 2003; Honda et al. 2003; Kallio et al. 2002; Lens et al. 2003; Santaguida et al. 2011; Schumacher

et al. 1998; Speliotes et al. 2000; Tanaka et al. 2002; Xu et al. 2010). Importantly, Aurora B heterozygous knockout mice have an increased cancer incidence underscoring the essential role of the CPC in maintaining chromosomal stability (Fernandez-Miranda et al. 2011).

Mammalian Aurora B is closely related to members of the AGC (cAMP-dependent, cGMP-dependent, protein kinase C) family of Serine/Threonine kinases, and the Aurora family also comprises Aurora A and Aurora C (Gold et al. 2006; Vader and Lens 2008). These kinases share a common consensus phosphorylation motif ([R/K]x[S/T] Φ , in which x can be any amino acid and Φ is a hydrophobic residue) and substrate specificity is mainly achieved by specific kinase distribution patterns within cells or between cells: Aurora A localizes to the mitotic spindle, centrosomes, and midbody, while Aurora B is present at chromosomal arms, inner centromeres, the central spindle, and the midbody (Alexander et al. 2011; Cheeseman et al. 2002). Aurora C behaves very similar to Aurora B with respect to localization and function, but is mainly expressed in germ

cells and in the morula (Avo Santos et al. 2011; Fernandez-Miranda et al. 2011; Sasai et al. 2004; Schindler et al. 2012; Slattery et al. 2009; Yanai et al. 1997). The differential mitotic localization of Aurora A and Aurora B is dictated by a distinct repertoire of interaction partners. Aurora A localizes to centrosomes through its interaction with Ajuba, while its association with the mitotic spindle depends on binding to TPX2 (Hirota et al. 2003; Kufer et al. 2002). Aurora B localization is mediated by its interaction with INCENP that in turn binds to HP1, implicated in chromosomal arm localization, and to Survivin and Borealin required for centromere localization (see below) (Ainsztein et al. 1998; Nozawa et al. 2010). Interestingly, just a single point mutation (G198N) in Aurora A changes its binding preference for TPX2 into a preference for INCENP and rescues the loss of Aurora B in chromosome alignment and mitotic checkpoint function (Fu et al. 2009; Hans et al. 2009). This underscores that substrate specificity of the Aurora A and Aurora B kinases is predominantly dictated by specific localization of these kinases. Indeed, the prevailing view is that the non-enzymatic subunits of the CPC activate Aurora B and guide it to its different locations in the dividing cell to encounter and phosphorylate substrates and hence to execute its local functions (Carmena et al. 2012b; Vader et al. 2006b).

Aurora B and INCENP are first seen in late S phase nuclei at pericentromeric regions and near nucleoli, concomitant with an increase in their protein levels. Aurora B remains pericentromeric until late G2 when the kinase becomes more diffusely localized on chromatin (Fig. 2) (Andersen et al. 2005; Bischoff et al. 1998; Cooke et al. 1987; Hayashi-Takanaka et al. 2009; Monier et al. 2007; Terada 2006; Zeitlin et al. 2001b). Co-localization of Aurora B and Survivin at pericentromeric regions in G2 has been observed, yet the precise localization of Borealin in interphase cells has not been studied carefully (Monier et al. 2007; Zeitlin et al. 2001b). The presence of Aurora B at pericentromeres coincides with phosphorylation of Serine-10 of Histone H3 (H3-S10) at these foci indicating that the kinase is already active in late S phase. Although its function in interphase is unknown, transient inhibition of Aurora B kinase activity in interphase gives rise to segregation defects in anaphase, suggesting that already before mitosis Aurora B may regulate proper kinetochore formation or function (Emanuele et al. 2008; Hayashi-Takanaka et al. 2009).

In early prophase, Aurora B and the other passenger proteins are briefly present on chromosomal arms (Fig. 2). Here, the CPC appears to contribute to sister chromatid resolution (Dai et al. 2006; Gimenez-Abian et al. 2004; Losada et al. 2002; Nishiyama et al. 2013). The CPC acts in concert with Cdk1 to phosphorylate the cohesin-stabilizing protein Sororin (Dreier et al. 2011; Nishiyama et al. 2013). These phosphorylation events cause dissociation of Sororin from the cohesin

subunit precocious dissociation of sisters protein 5 (Pds5) leading to Wapl-mediated release of acetylated cohesin from chromosome arms and loss of cohesion (Dreier et al. 2011; Kueng et al. 2006; Nishiyama et al. 2013). In addition, the CPC may enhance chromosome compaction by loading of Condensin I onto chromosomal arms (Lipp et al. 2007; Takemoto et al. 2007). However, unlike fission yeast Bir1p mutants that display impaired chromosome condensation, inhibition of Aurora B or depletion of CPC subunits does not have a pronounced effect on chromosome condensation at the onset of mitosis in mammalian cells (Carvalho et al. 2003; Ditchfield et al. 2003; Hauf et al. 2003; Lens et al. 2003; Morishita et al. 2001; Rajagopalan and Balasubramanian 2002).

During (pro)metaphase, when the CPC becomes concentrated at the inner centromere (Fig. 2), the CPC ensures chromosome bi-orientation by detaching erroneous kinetochore–microtubule attachments (a process dubbed “error correction”) (Gassmann et al. 2004; Zeitlin et al. 2001a; Hauf et al. 2003; Tanaka et al. 2002). These erroneous attachments can be monotelic (a condition in which only one sister kinetochore in a pair of sister kinetochores is bound by microtubules), syntelic (attachment in which two sister kinetochores are bound by microtubules from the same spindle pole), and merotelic (attachment in which a single kinetochore is bound to microtubules emanating from both spindle poles). Furthermore, at this stage, the CPC supports proper functioning of the mitotic checkpoint through kinetochore recruitment and activation of the mitotic checkpoint kinase Mps1 (Biggins and Murray 2001; Kallio et al. 2002; Santaguida et al. 2011; Saurin et al. 2011). At anaphase onset, the CPC transfers to the cleavage furrow and to the overlapping ends of the midzone microtubules (Fig. 2). Here, it contributes to furrow ingression, central spindle formation, and to axial shortening of chromosomal arms (Douglas et al. 2010; Miyauchi et al. 2007; Mora-Bermudez et al. 2007; Neurohr et al. 2011; Petronczki et al. 2007; Xu et al. 2009). Finally, when present at the midbody, the CPC supports timely abscission (Carlton et al. 2012; Norden et al. 2006; Steigemann et al. 2009).

This review focuses on the regulation of CPC localization in mitosis and during cytokinesis, and discusses recent findings that both support and challenge the idea that precise localization of the CPC is key to its proper function before and after anaphase onset.

Centromere recruitment of the CPC

Inner centromere localization of the CPC depends on Survivin and Borealin interacting with the N-terminus of INCENP in a three-helical bundle arrangement (Jeyapakash et al. 2007; Klein et al. 2006; Vader et al. 2006a). Survivin interacts with

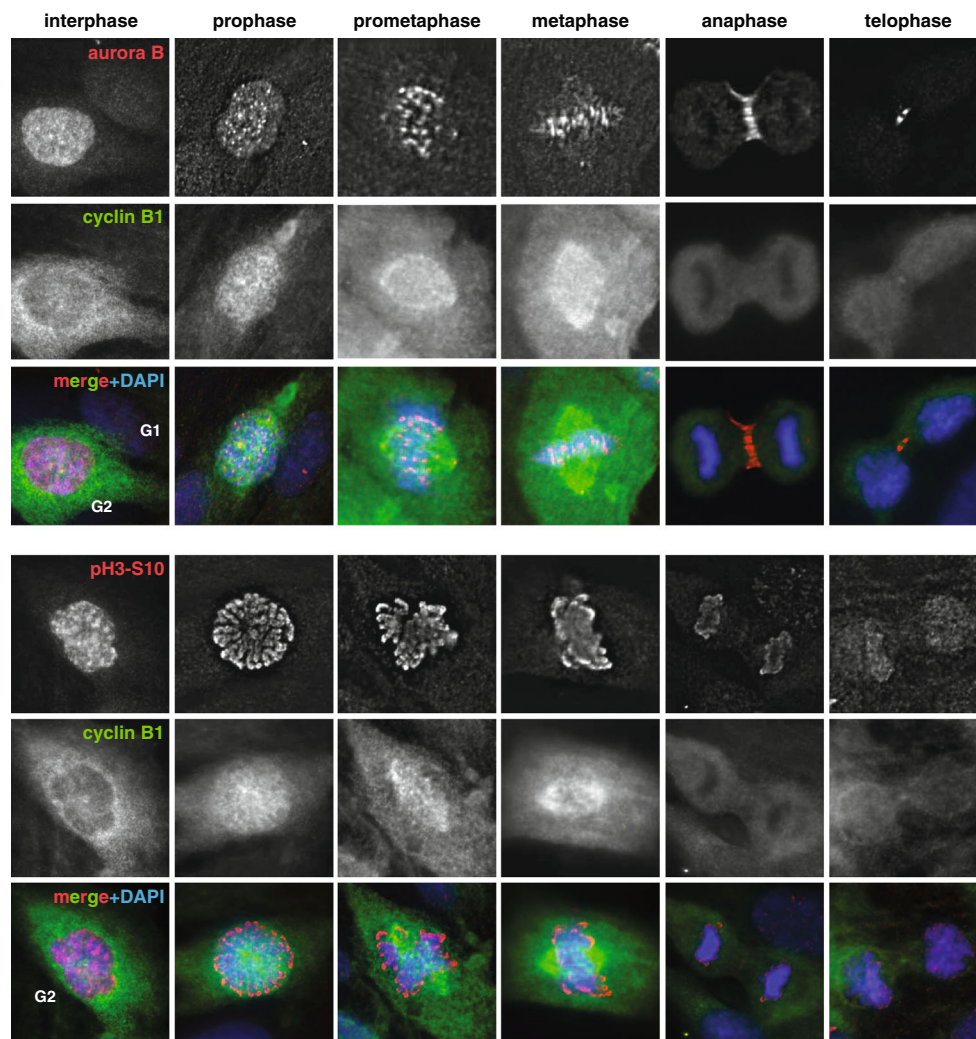


Fig. 2 Localization of Aurora B and Histone H3 phosphorylated on Serine-10 in different phases of the cell cycle. Asynchronously growing RPE1 cells were stained using antibodies against Aurora B (A300-431A, Bethyl Laboratories) or phospho-Histone H3S10 (06-570, Merck-Millipore) and Cyclin B1 (sc-245, Santa Cruz Biotechnology). Cyclin B1 staining was used to determine cell cycle stage. Aurora B levels are higher in G2 cells than in G1 cells. In late G2 (when cytoplasmic levels of Cyclin B1 are high), Aurora B localizes diffusely in the nucleus and only in prophase Aurora B starts to accumulate clearly on centromeres, where

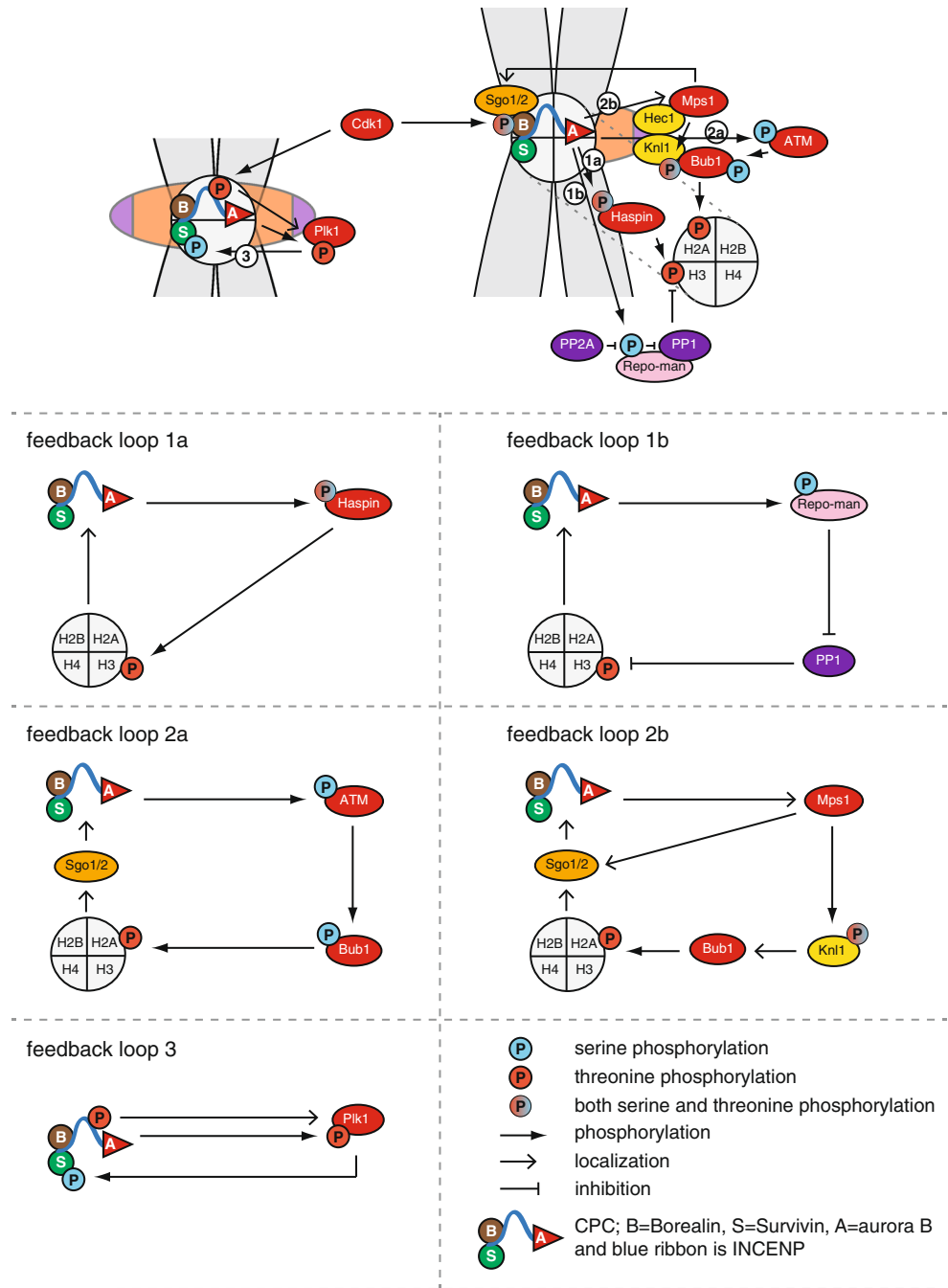
it stays during prometaphase and metaphase. When cells enter anaphase, Aurora B moves to the midzone and subsequently, in telophase, it localizes to the midbody. Phosphorylation of Histone H3-S10 increases dramatically when cells enter mitosis and stays high until cells enter anaphase. All images were acquired on a deconvolution system (DeltaVision RT; Applied Precision) with a 100x/1.40 NA U Plan S Apochromat objective (Olympus) using softWoRx software (Applied Precision). Images are average intensity projections of stacks except for the DAPI images (maximum intensity)

histone H3 phosphorylated on Threonine-3 (H3-T3) by the kinase Haspin, while Borealin binds to the Bub1-dependent histone H2A Threonine-120 (H2A-T120) phosphorylation site via the Shugoshin proteins (Shugoshin-1 and Shugoshin-2 in human cells, Shugoshin-2 in fission yeast; Fig. 3) (Kelly et al. 2010; Wang et al. 2010; Yamagishi et al. 2010; Kawashima et al. 2010; Tsukahara et al. 2010). The inner centromere is the site where these two histone phosphorylation marks seem to overlap providing an explanation as to why the CPC concentrates at this site (Yamagishi et al. 2010). Moreover, the observation that the interaction between Borealin and Shugoshin requires the phosphorylation of Borealin by Cdk1 may explain why centromere

accumulation of the CPC starts in late prophase (Fig. 2) (Tsukahara et al. 2010).

Interestingly, in fission yeast, the Survivin homolog Bir1p mediates both the interaction with phosphorylated H3-T3 and the interaction with Shugoshin-2 required for CPC centromere recruitment (Tsukahara et al. 2010; Yamagishi et al. 2010). Recently, mammalian Survivin was also found to directly interact with Shugoshin-1 via a stretch of amino acids (AKER) in Shugoshin-1 that resembles the phosphorylated N-terminus of histone H3 (ARTphK) (Jeyaprakash et al. 2011). Since these interactions depend on the same domain in Survivin, they are thought to be mutually exclusive, and it was proposed that Survivin binds to Shugoshin-1 before H3-

Fig. 3 Feedback loops that regulate accumulation of the CPC on the inner centromere. The numbers in white circles in the summarizing image at the top indicate the different feedback loops. These loops are clarified in the lower part of the figure. Loop 1a covers the Aurora B-Haspin circuit and loop 1b the Aurora B-Repo-Man-PP1 loop. Loop 2a involves Aurora B-ATM-Bub1 and loop 2b Aurora B-Mps1-Bub1. Loop 3 involves INCENP/Aurora B-Plk1. See main text for details. Kinases are shown in red and phosphatases in purple



T3 is phosphorylated. Upon H3-T3 phosphorylation, Survivin interacts with the phosphorylated histone, while Borealin binds Shugoshin (Jeyaprakash et al. 2011).

Feedback loops regulating centromeric localization of the CPC

Since Haspin and Bub1 generate the CPC centromere docking sites, upstream regulators of Haspin and Bub1 localization and activity also control CPC localization and activity. These

signaling cascades are in turn controlled by Aurora B kinase activity suggesting the existence of extensive feedback mechanisms (Fig. 3).

Feedback between Haspin and Aurora B

The crystal structure of Haspin suggests that the activation loop in the kinase domain naturally adopts an active conformation and, unlike other kinases, it does not contain any phosphorylatable residues in its activation loop (Villa et al. 2009). This most likely explains why Haspin isolated from

interphase or mitotic cells is equally active in vitro (Villa et al. 2009; Wang et al. 2011). Still, phosphorylation of H3-T3 occurs only in mitosis indicating that the cellular activity of Haspin is regulated (Dai and Higgins 2005; Wang et al. 2011). Indeed, Haspin is phosphorylated on multiple serine and threonine residues by Aurora B in mitosis, and mutation of these sites affects the cellular activity of Haspin towards H3-T3 (Wang et al. 2011). In line with the phenotype induced by a non-phosphorylatable mutant of Haspin, Aurora B inhibition in mitotic cells strongly reduces H3-T3 phosphorylation, suggesting that Aurora B enhances its own recruitment to centromeres by regulating Haspin. The mechanism by which Aurora B phosphorylation affects Haspin function is currently not known, but it could be mediated through release of an inhibitory factor or supporting substrate binding (Wang et al. 2011).

Additionally, posttranslational modification of Haspin substrates or the activity of phosphatases could also affect Haspin-dependent substrate phosphorylation. In line with this, methylation of H3-K4, which neighbors H3-T3, inhibits phosphorylation on H3-T3 by Haspin, at least in vitro (Eswaran et al. 2009). Moreover, H3-T3 is dephosphorylated by the PP1 γ -Repo-Man phosphatase and this was shown to restrict H3-T3 phosphorylation to centromeres in prometaphase and metaphase (Qian et al. 2011). However, the activity of PP1 γ -Repo-Man needs to be restrained in mitosis to allow H3-T3 phosphorylation in the first place. Recent work suggests a role for Aurora B in this regulation. Aurora B was found to phosphorylate Serine-893 in Repo-Man preventing its chromosomal targeting and thereby supporting phosphorylation of H3-T3. Repo-Man not only binds PP1 γ but it also interacts with PP2A and the latter phosphatase in turn counteracts the phosphorylation of Serine-893 by Aurora B (Qian et al. 2013). This thus implies that the positive feedback between Haspin and Aurora B and feedback between Aurora B and its counteracting phosphatases determine the level of phosphorylated H3-T3 at the centromere and thereby the centromeric accumulation of the CPC (Fig. 3, feedback loop 1a and 1b).

Finally, the localization of Haspin is also expected to affect the level of centromeric H3-T3 phosphorylation. GFP-tagged Haspin is found on chromatin, but due to the lack of specific antibodies, the exact localization pattern of the endogenous mammalian kinase and the factors that regulate its localization are not known (Dai et al. 2005). In *Schizosaccharomyces pombe*, the Haspin homolog Hrk1 is recruited to chromatin in mitosis in a Pds5-dependent manner. Pds5 is a cohesin-associated protein implicated in sister chromatid cohesion (Hartman et al. 2000; Panizza et al. 2000; Sumara et al. 2000). Currently, there is no clear evidence that human Pds5A or Pds5B is enriched in centromeric regions, but chromosome spreads derived from Pds5B-depleted cells show an increased frequency of cells with reduced centromere localization of Aurora B (Losada et al. 2005). Moreover, Pds5B knockout mouse embryonic fibroblasts display centromere cohesion

defects, reduced H3-T3 phosphorylation, and impaired centromeric accumulation of Aurora B (A. Losada, personal communication). This suggests that in mammalian cells Pds5B may be responsible for Haspin recruitment.

Recruitment of Haspin to cohesin-rich areas could be an interesting way to regulate centromere-specific CPC recruitment, since centromeres are the regions where the two sister chromatids are held together by cohesin until anaphase. Cohesin is removed from the chromosomal arms during prophase by the so-called prophase pathway (Hauf et al. 2005; Sumara et al. 2000). Centromeric cohesin is protected from the prophase pathway by Bub1-dependent recruitment of Shugoshin to centromeres. (McGuinness et al. 2005; Salic et al. 2004; Tang et al. 2004). Since the Shugoshin proteins in turn recruit the CPC via Borealin, this may create the ideal setting for feedback loops between Bub1, Aurora B, and Haspin to ensure centromeric accumulation of the CPC at the end of prophase.

Feedback between Bub1 and Aurora B

Bub1 kinase activity is required for H2A-T120 phosphorylation, but little is known about direct activation of Bub1 kinase by upstream kinases and whether these inputs affect H2A-T210 phosphorylation (Kawashima et al. 2010; Ricke et al. 2012). The DNA damage kinase ATM, which appears to have a DNA damage-independent role during mitosis, was shown to phosphorylate Bub1 and support Bub1-dependent H2A phosphorylation. Interestingly, the mitotic activity of ATM requires phosphorylation of Serine-1403 by Aurora B implying feedback between Bub1 and Aurora B via ATM (Fig. 3, feedback loop 2a) (Matsuoka et al. 2007; Yang et al. 2011).

Besides direct regulation of kinase activity, kinetochore localization of Bub1 is also essential for H2A phosphorylation (Kawashima et al. 2010; Ricke et al. 2012; Yamagishi et al. 2010). Kinetochore localization of Bub1 depends on Mps1 activity both in yeast and human cells (London et al. 2012; Maciejowski et al. 2010; Shepperd et al. 2012; Sliedrecht et al. 2010; van der Waal et al. 2012; Yamagishi et al. 2012). In yeast, this recruitment is mediated via phosphorylation of the kinetochore protein Spc105/Knl1 by Mps1, which enhances binding of Bub1 to Knl1 (London et al. 2012; Shepperd et al. 2012; Yamagishi et al. 2012). In mammalian cells, however, also Bub3 may contribute to Bub1 kinetochore localization (Krenn et al. 2012; Taylor et al. 1998; Meraldi et al. 2004).

Given its role in Bub1 kinetochore recruitment, it is not surprising that Mps1 inhibition delays the centromeric accumulation of the CPC. Yet, Mps1 not only supports CPC recruitment via kinetochore localization of Bub1 but also via a process that appears to act more directly on the localization of Shugoshin-1, downstream of Bub1 (van der Waal et al. 2012). Since active Aurora B in turn is required for kinetochore localization of both Mps1 and Bub1, and phosphorylation of

H2A-T120, positive feedback appears to regulate this CPC recruitment pathway as well (Fig. 3, feedback loop 2b) (Hauf et al. 2003; Santaguida et al. 2011; Saurin et al. 2011; Vazquez-Novelle and Petronczki 2010; van der Waal et al. 2012). The effect of Aurora B inhibition on Bub1 kinetochore localization and phosphorylation of H2A might in part be due to impaired Ndc80/Hec1-dependent kinetochore recruitment and activation of Mps1 (Nijenhuis et al. 2013; Santaguida et al. 2011; Saurin et al. 2011).

Feedback between Plk1 and the CPC

Another feedback loop involved in CPC centromere recruitment, in particular to misaligned chromosomes, involves Plk1 (Fig. 3, feedback loop 3). In nontransformed diploid RPE1 cells and primary fetal fibroblasts, Aurora B is enriched on misaligned chromosomes in a Plk1- and Aurora B-dependent manner (Salimian et al. 2011). Although levels of phosphorylated H2A-T120 are clearly increased on these misaligned chromosomes, inhibition of Plk1 does not affect phosphorylation of either of the two centromere recruitment marks (H3-T3 and H2A-T120). Survivin, however, may be more directly involved because Plk1 can phosphorylate Serine-20 in Survivin, which appears to be required for Aurora B activation (Chu et al. 2011). INCENP in turn, while phosphorylated on Threonine-388 by Cdk1, mediates kinetochore localization of Plk1 (Goto et al. 2006). Moreover, in *Drosophila* cells, Aurora B was shown to directly phosphorylate Polo in its activation loop at centromeres in early mitosis (Carmena et al. 2012a). Also in human cells, phosphorylation of the T-loop residue Threonine-210 in Plk1 is reduced in cells from which Aurora B or INCENP has been depleted, suggesting that this is a conserved mechanism (Carmena et al. 2012a).

Overall, these data indicate extensive feedback between the CPC, Haspin, Bub1, Mps1, and Plk1 to ensure rapid accumulation of the CPC at centromeres when cells enter mitosis and kinetochore-microtubule attachments are being established. In addition, these feedback loops may warrant high CPC levels on aberrantly attached chromosomes where efficient error correction is particularly needed.

Is centromere localization of the CPC required for its function in (pro)metaphase?

In all commonly studied model organisms, Aurora B localization is confined to (inner) centromeres during prometaphase and metaphase. Moreover, the evolutionarily conserved meticulous mode of regulation of CPC recruitment to the inner centromere implies that centromeric accumulation of the CPC is crucial to Aurora B function. In fact, it is central to the prevailing model explaining how chromosome bi-orientation is achieved (Andrews et al. 2004; Liu et al. 2009; Tanaka et al.

2002). In this model, erroneous kinetochore–microtubule attachments that fail to generate (sufficient) tension across sister kinetochores are detached because kinetochore-localized microtubule binding proteins of the KMN network (consisting of the Knl1 complex, the Mis12 complex, and the Ndc80/Hec1 complex) are in close proximity of the centromere-localized kinase and are phosphorylated by Aurora B (Cheeseman et al. 2006; Welburn et al. 2010). Phosphorylation of Ndc80/Hec1 and Knl1 reduces their microtubule-binding affinity resulting in the detachment of kinetochore microtubules (Cheeseman et al. 2002, 2006; De Luca et al. 2006). Correct, amphitelic attachments that generate tension across sister kinetochores are stabilized because these microtubule-binding outer kinetochore substrates are pulled out of reach of Aurora B. This spatial restriction model thus relies on the strict localization of the CPC to inner centromeres.

Interestingly, recent work in budding yeast challenges this spatial restriction model. Yeast cells expressing an N-terminal deletion mutant of the INCENP homolog Sli15 (Sli15 Δ NT) that does not interact with Bir1 (budding yeast homolog of Survivin) and as a consequence fails to localize Ipl1 (budding yeast homolog of Aurora B) to centromeres are viable and capable of supporting proper chromosome segregation (Campbell and Desai 2013). Strikingly, even loss of viability and segregation defects caused by Bir1 deletion mutants or mutants of the centromere recruitment proteins Bub1 and Shugoshin-1 (the latter is not involved in cohesion protection in budding yeast mitosis) are suppressed by Sli15 Δ NT, strongly suggesting that centromere localization of the CPC is not required for cell viability or chromosome bi-orientation (Campbell and Desai 2013; Indjejan et al. 2005). Unlike wild-type Sli15, which localizes to centromeres, the Sli15 truncation mutant was found at the pre-anaphase spindle and weakly at kinetochores (Campbell and Desai 2013). Spindle localization of Sli15 before anaphase is prevented by both Cdk1- and Ipl1-dependent phosphorylation of the microtubule-binding domain in Sli15 (Nakajima et al. 2011; Pereira and Schiebel 2003). Mutation of these Cdk1 or Ipl1 phosphorylation sites into alanines causes both Sli15 and Ipl1 to prematurely localize to the pre-anaphase spindle, quite similar to Sli15 Δ NT (Campbell and Desai 2013; Nakajima et al. 2011). Interestingly, expression of such a non-phosphorylatable Sli15 mutant (Sli15-6A) partially rescued viability of Bir1 deletion mutants. This argues that mere clustering of Sli15/Ipl1 on the spindle might be sufficient to activate Ipl1 and to phosphorylate kinetochore substrates when inter-kinetochore tension is low. Furthermore, it implies that tension sensing cannot be explained by a change in distance between centromere-localized kinase and kinetochore-localized substrates (see below).

While centromeric localization of the CPC may not be important for proper chromosome segregation and viability in budding yeast, it remains to be determined if this also

applies to higher organisms. Expression of a Survivin BIR domain mutant (Survivin_{D72A/D73A}) that fails to localize the CPC to centromeres but does localize the complex to the central spindle in chicken DT40 cells rescues cytokinesis failure and survival of cells lacking endogenous Survivin (Yue et al. 2008). However, this mutant does not rescue a taxol-induced mitotic checkpoint response, a phenotype that is strongly correlated with an impaired chromosome segregation function of the CPC (Lens et al. 2006; Lens et al. 2003), and expression of a similar mutant (Survivin_{D70A/D71A}) in HeLa cells fails to rescue chromosome alignment defects caused by depletion of endogenous Survivin (Wang et al. 2010). Similarly, depletion or inhibition of Haspin, the kinase responsible for phosphorylation of H3-T3 required for centromeric CPC recruitment, also causes chromosome alignment defects, but not cytokinesis failure (Dai et al. 2005; De Antoni et al. 2012; Huertas et al. 2012; Wang et al. 2012). This suggests that centromeric localization of the CPC is needed for proper chromosome segregation in mammalian cells and that rescue of cytokinesis failure by Survivin BIR domain mutants might be the reason why cells expressing these mutants are viable.

Similar to Sli15 Δ NT, deletion of the first ~40–60 N-terminal amino acids in human or chicken INCENP (INCENP- Δ CEN) disturbs the interaction with Survivin and Borealin and disrupts CPC centromere localization (Ainsztein et al. 1998; Klein et al. 2006; Mackay et al. 1993; Vader et al. 2006a). However, in marked contrast to Sli15 Δ NT, these INCENP deletion mutants do not rescue Aurora B activity or mitotic checkpoint function in response to taxol in INCENP or Survivin-depleted cells (Vader et al. 2006a). Instead of localizing to the mitotic spindle, human and chicken INCENP- Δ CEN localize weakly and diffusely to chromatin (Mackay et al. 1998; Vader et al. 2006a). Similarly, expression of the Survivin-BIR domain mutants or inhibition of Haspin also results in diffuse localization of the CPC over chromosomal arms. Because antibody-mediated cross-linking of a *Xenopus* INCENP- Δ CEN mutant does rescue Aurora B activity in *Xenopus* egg extracts, one could argue that this weak chromatin association may cluster INCENP/Aurora B insufficiently (Kelly et al. 2007). Hence, it would be interesting to tether mammalian INCENP- Δ CEN to the mitotic spindle and test if this type of localization clusters the bimolecular complex sufficiently to support functional activity of Aurora B in human cells.

Bi-orientation without centromere-localized CPC

If error-free chromosome segregation does not require centromere localization of the CPC, how can the CPC discriminate between correctly and incorrectly attached kinetochore microtubules? In other words: How is the lack of bi-orientation and inter-kinetochore tension translated into Aurora B-dependent error correction, and how is this error correction activity

silenced to allow the stabilization of amphitelically attached kinetochore microtubules?

One explanation is that tension sensing is an intrinsic property of the kinetochore (Campbell and Desai 2013). Indeed, kinetochores themselves can be stretched when attached to microtubules (intra-kinetochore stretching) (Akiyoshi et al. 2010; Maresca and Salmon 2009; Uchida et al. 2009). However, tension is thought to be the result of pulling forces exerted by amphitelically attached microtubules that are resisted by centromeric cohesin, a ring-shaped protein complex embracing the sister chromatids and holding them together until anaphase onset. Based on this definition, tension can only build up once chromosomes have bi-oriented, and this is usually visualized in cells by an increase in inter-kinetochore distance (Tanaka 2005). Because intra-kinetochore stretching is already observed in mono-oriented chromosomes, it is not correlated with bi-orientation or tension (Akiyoshi et al. 2010; Maresca and Salmon 2009; Uchida et al. 2009).

Although intra-kinetochore stretching may not be related to inter-kinetochore tension, *in vitro* force studies on purified budding yeast kinetochores demonstrated that the mere application of tension by a laser trap is sufficient to stabilize kinetochore–microtubule attachments similar to what was already proposed by Nicklas and colleagues based on micro-needle pulling experiments in grasshopper spermatocytes (Akiyoshi et al. 2010; Ault and Nicklas 1989; Nicklas and Koch 1969; Nicklas and Ward 1994). Although binding of the isolated budding yeast kinetochores to purified microtubules depended on Ndc80/Hec1 and Spc105/Knl1, the force-induced stabilization of attachments were unlikely due to reduced microtubule destabilizing activity of Ipl1, Mph1 (the budding yeast homolog of Mps1), or possibly other kinases because the *in vitro* laser trap experiments were performed in the absence of ATP (Akiyoshi et al. 2010; Maure et al. 2007). Instead, stabilization of kinetochore–microtubule attachments induced by mechanical tension was proposed to occur as a result of a change in the net balance between microtubule tip assembly (associated with a low kinetochore–microtubule detachment rate) and disassembly (associated with a high kinetochore–microtubule detachment rate) (Akiyoshi et al. 2010). In line with this, by using optical tweezers to pull on beads coated with the microtubule polymerizing protein XMAP215, Trushko and coworkers showed that the growth speed of microtubules can be increased by tension, most likely because tensile force enhances the microtubule polymerization activity of XMAP215 (Trushko et al. 2013). Interestingly, when purifying kinetochores Akiyoshi et al. copurified Stu2p, which is the budding yeast homolog of XMAP215, the microtubule polymerization activity of which does not depend on ATP (Brouhard et al. 2008; Akiyoshi et al. 2010).

When translated back into a cellular setting in which Aurora B is present, this could imply that even when Aurora

B is constitutively active (either at the centromere or at another site near the kinetochore), once chromosomes become bipolarly attached, the microtubule stabilizing effect of the mechanical force may simply outweigh the microtubule detaching activity of Aurora B kinase. If this model was true, then stable positioning of Aurora B at kinetochores using a Mis12-INCENP fusion protein should not affect the stability of amphitelicly attached kinetochore microtubules. Mis12-INCENP expressing cells are capable of chromosome alignment and increasing inter-kinetochore distances, suggesting chromosome bi-orientation can occur. However, (bipolar) attachments are not stabilized and cells are delayed in mitosis with chromosomes frequently falling out of the metaphase plate (Liu et al. 2009). Although the possibility that the artificially kinetochore-localized Aurora B prevents the establishment of proper attachments cannot be excluded, it may also suggest that the attachment status of the kinetochore (unattached or syntelically attached versus amphitelicly attached) is somehow translated into more or less Aurora B kinase activity or into more or less phosphorylation of Aurora B kinetochore substrates, via for instance recruitment and/or exchange of PP2A and PP1 phosphatases (Foley et al. 2011; Liu et al. 2010; Trinkle-Mulcahy et al. 2003). The question then remaining is how this is accomplished when the CPC is not localized at inner centromeres and the spatial restriction model cannot be applied? Work from De Luca et al. suggests that a very small, but active pool of Aurora B might reside at unattached kinetochores and diminishes upon microtubule attachment and establishment of tension (DeLuca et al. 2011). How this putative kinetochore-localized pool of active Aurora B is regulated remains to be determined, but it might, at least to some extent, explain why Aurora B detaches incorrectly attached kinetochore microtubules and not amphitelic attachments. In case of the latter, while the pool of active Aurora B at kinetochores becomes smaller, the levels of PP1 γ at kinetochores increase. These changes likely ensure a switch-like transition from an incorrectly attached kinetochore with phosphorylated substrates into an amphitelicly attached kinetochore with dephosphorylated substrates (Liu et al. 2010; Trinkle-Mulcahy et al. 2003). Interestingly, also in the Sli15 Δ NT budding yeast strain, a pool of Ipl1 was found at kinetochores. Since a Sli15 mutant that prematurely localizes to the pre-anaphase spindle can only partially rescue the segregation defects of a Bir1 mutant, this kinetochore-bound pool may explain the superior rescue activity of Sli15 Δ NT.

What is the function of centromere-localized Aurora B?

If centromere localization of Aurora B is not required for tension sensing and chromosome bi-orientation, the question remains what the role of centromeric-localized Aurora B is. An important difference between budding yeast chromosomes and fission yeast or mammalian chromosomes is the presence

of multiple microtubule attachment sites per kinetochore in the latter versus only one attachment site per kinetochore in budding yeast. Mammalian cells (and fission yeast cells alike) could simply more heavily depend on high and local centromeric Aurora B activity because these cells have to deal with multiple kinetochore-bound microtubules that need to be destabilized in case of an aberrant attachment. In addition, the centromeric pool might support the active pool of Aurora B at the kinetochore (DeLuca et al. 2011). Alternatively, an explanation why Sli15 Δ NT is effective in suppressing chromosome segregation defects in Bir1 mutant budding yeast might be because syntelic error correction does not require centromere localization of CPC/Aurora B while correction of merotelic attachments, which cannot occur in budding yeast, does (Campbell and Desai 2013). When left unresolved, merotelic attachments are particularly hazardous as these attachments are not sensed by the mitotic checkpoint and are likely to missegregate sister chromatids in anaphase (Cimini et al. 2001). Since fission yeast does have multiple microtubule attachment sites per kinetochore, it would be interesting to generate a deletion mutant of Pic1 (the fission yeast homolog of INCENP and Sli15), which cannot localize to centromeres and study its effect on fidelity of chromosome segregation and cell viability. The reason why merotelic kinetochore–microtubule attachments would specifically require *centromeric* Aurora B for correction could be because the merotelically attached microtubules may be in the vicinity of the centromeres or distort the kinetochore in such a way that the kinetochore attachment site of the incorrectly attached microtubule is positioned towards the centromere (Andrews et al. 2004; Cimini et al. 2006; Gregan et al. 2011; Knowlton et al. 2006). Interestingly, active Aurora B is enriched on merotelic attachment sites (Knowlton et al. 2006). Since microtubules can be co-activators of Aurora B kinase *in vitro*, this could mean that the merotelically attached microtubules might in turn contribute to the activity of the centromeric pool of Aurora B (Rosasco-Nitcher et al. 2008; Tseng et al. 2010).

Alternatively, Aurora B could be required at centromeres to *prevent* merotelic attachments. In fission yeast, Ark1 (Aurora B homolog) mediates clamping of the different microtubule binding sites in one kinetochore via the monopolin and condensin complexes to reduce the chance of acquiring merotelic attachments (Gregan et al. 2007; Tada et al. 2011). Obvious homologs of members of the monopolin complex in mammalian cells have not been found, although the evolutionarily conserved Spc24/25 heterodimer, which is part of the Ndc80/Hec1 complex, shows structural resemblance to the fission yeast Csm1 homodimer, a component of the monopolin complex (Wei et al. 2006). Moreover, in mammalian cells, localization of Condensin I depends on Aurora B activity and depletion of Condensin I gives rise to merotelic attachments (Gerlich et al. 2006; Samoshkin et al. 2009). These data suggest that Aurora B may not only be able to

correct but could also prevent merotelic attachments in mammalian cells, and this latter activity might require centromere localization of the CPC.

Translocation of the CPC from centromeres to the central spindle in anaphase

Even if centromeric localization of the CPC is not required for certain kinetochore regulatory functions of the CPC, the fact that the complex is predominantly found at this site in mitosis and the evolutionary conservation of the centromeric recruitment pathways imply that in most cells the centromere is the preferred spot for CPC clustering and hence activation of Aurora B before anaphase (Kelly et al. 2010; Wang et al. 2010; Yamagishi et al. 2010). As mentioned earlier, perturbations in centromeric localization of the CPC in mammalian cells result in chromosome segregation defects, indicating that the complex is not readily clustered on an alternative spot in the mitotic cell. In anaphase, the CPC leaves the centromeres and is found at the central spindle and equatorial cortex. This change in localization is thought to promote the loss of Aurora B kinase activity from a place where it is no longer needed and gain of activity at a location where its function is now required.

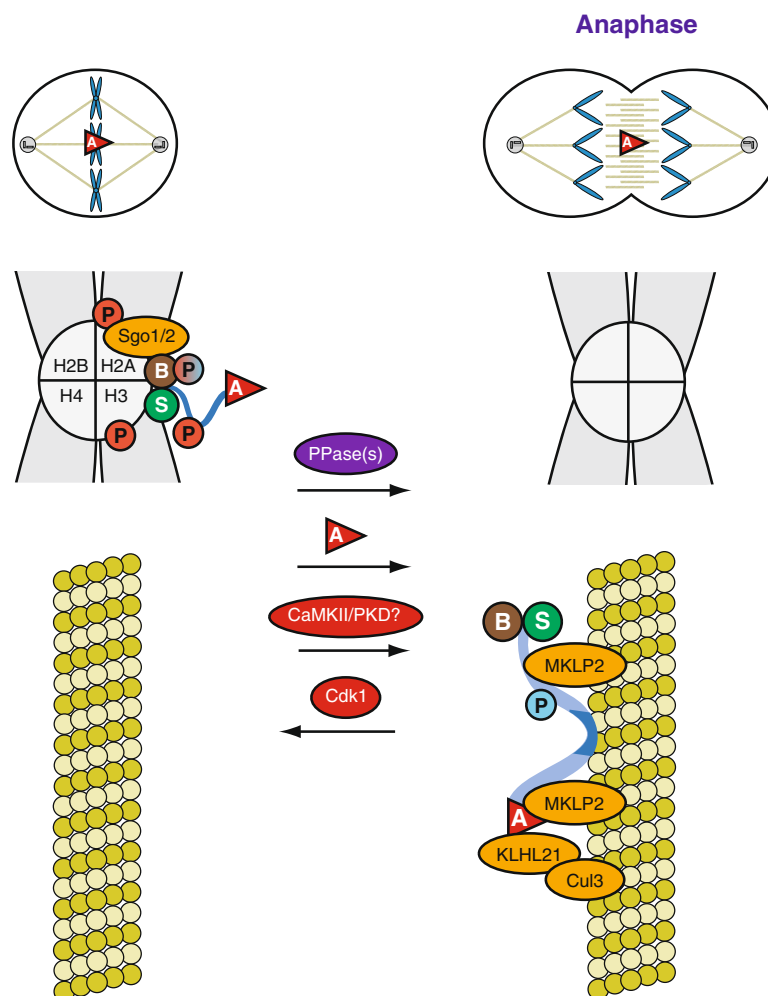
Although the pool of Aurora B found at the equatorial cortex in anaphase may not be necessarily derived from centromeres, the pool localizing to the spindle midzone is (Murata-Hori and Wang 2002). The translocation from centromeres to the central spindle changes the CPC from a Borealin/Survivin-directed, histone-bound state into an INCENP/Aurora B-directed, microtubule-bound state and the drop in Cdk1 activity due to the degradation of Cyclin B, is a major contributing factor in yeast and mammalian cells (Fig. 4) (Murata-Hori and Wang 2002; Pereira and Schiebel 2003). In *Saccharomyces cerevisiae*, it depends on the Cdc14 phosphatase, which dephosphorylates multiple Cdc28 (Cdk1 homolog in budding yeast) and Ipl1 sites that reside mainly in the microtubule-binding domain of Sli15. Phosphorylation of these sites prevents microtubule-binding of Sli15 before mitotic exit (Mirchenko and Uhlmann 2010; Nakajima et al. 2011; Pereira and Schiebel 2003). In addition, Cdc28-dependent phosphorylation of Ipl1 represses its interaction with the microtubule plus-end tracking protein Bim1 (Zimniak et al. 2012). Notably, the putative microtubule-binding domain in mammalian INCENP (the coiled-coil domain, Fig. 1) does not contain any Cdk1 or Aurora B consensus sites suggesting that this mode of regulation may not be applicable to mammalian INCENP. However, mammalian INCENP is heavily phosphorylated in mitosis on Cdk1 consensus sites residing in the unstructured region of the protein. Except for Threonine-59 (T59; see below), the function of these phosphorylation events and their contribution to CPC translocation

in anaphase is unknown (Dephoure et al. 2008; Hegemann et al. 2011; Malik et al. 2009; Nousiainen et al. 2006; Olsen et al. 2010; Hummer and Mayer 2009).

Translocation of the mammalian CPC seems to depend on a reduction in the centromere binding affinity and an increase in the microtubule binding affinity of the CPC. In line with this idea, upon Cdk1 inhibition, the H3-T3 and H2A-T120 phosphorylation marks are lost, most likely due to the activity of PP1 γ phosphatase (van der Horst and Lens, unpublished data) (Qian et al. 2011; Vagnarelli et al. 2011). In addition, a Borealin-7A mutant (in which seven putative Cdk1 sites were mutated into alanine) no longer localizes to centromeres due to reduced affinity for Shugoshin suggesting that Borealin dephosphorylation at anaphase onset could also play a role (Tsukahara et al. 2010). The increase in microtubule binding affinity is in part mediated by dephosphorylation of the T59 Cdk1 site in INCENP. Dephosphorylation of T59 in anaphase is required for INCENP to interact with the kinesin MKLP2, to bind microtubules and translocate to the central spindle midzone (Goto et al. 2006; Gruneberg et al. 2004; Hummer and Mayer 2009). Like most kinesins, MKLP2 can multimerize and this multimerization could be responsible for the characteristic localization of MKLP2 and the CPC at the site where the anti-parallel microtubules of the central spindle overlap (Lee et al. 2010; McDonald et al. 1979). Knockdown of MKLP2 or expression of an INCENP mutant in which T59 is changed into a phospho-mimicking glutamic acid precludes the CPC from translocating to the central spindle resulting in cytokinesis failure (Gruneberg et al. 2004; Hummer and Mayer 2009). While the phosphatase that dephosphorylates INCENP in anaphase in yeast is known (Cdc14), in mammalian cells the T59 phosphatase remains to be identified. In addition to INCENP's interaction with MKLP2, the coiled-coil domain of INCENP itself is required for central spindle localization, potentially by increasing the microtubule affinity of the CPC-MKLP2 complex (van der Horst et al., manuscript in preparation) (Vader et al. 2007). Apart from INCENP, also Aurora B can directly interact with MKLP2 as well as with the Cul3-KLHL21 complex that localizes to the central spindle in anaphase (Gruneberg et al. 2004; Maerki et al. 2009). Interestingly, in KLHL21-depleted cells, MKLP2 localization to the central spindle is reduced, which probably reflects the reduced localization of the CPC to this region. Whether localization of KLHL21 also depends on the presence of MKLP2 or the CPC, similar to the mutual dependency of MKLP2 and INCENP, is currently unknown.

Finally, in mammalian cells Aurora B kinase activity is required for translocation of the CPC to the central spindle as the CPC remains on chromosomes in anaphase when Aurora B activity is inhibited (Xu et al. 2009). While in budding yeast Aurora B-dependent phosphorylation sites in the microtubule-binding domain of INCENP need to be dephosphorylated for efficient translocation of the CPC to the central spindle

Fig. 4 Scheme illustrating the regulation of translocation of the CPC from centromeres (in *metaphase*) to the central spindle (in *anaphase*). The mechanism behind the requirement for Aurora B activity is unknown. The phosphatase(s) (PPase) required for dephosphorylation of Threonine-59, important for binding of MKLP2 to INCENP, has not been identified yet (Hummer and Mayer 2009). Phosphorylation of Serine-197 has been suggested to be involved in central spindle/midbody localization (Yang et al. 2007). The motif surrounding this serine fits the consensus motifs for Calmodulin-Dependent Protein Kinase II (*CaMKII*) and protein kinase D (*PKD*). The part in sapphire that binds to microtubules is INCENP's coiled-coil domain



(Mirchenko and Uhlmann 2010; Nakajima et al. 2011), in mammalian cells the Aurora B substrates that need to be phosphorylated and the ones that potentially need to be dephosphorylated to support CPC translocation remain to be determined.

Interestingly, INCENP may have to be phosphorylated on Serine-197 to localize to the midbody in telophase as mutation of this residue to alanine prevents midbody localization (Yang et al. 2007). The amino acid sequence surrounding Serine-197 (LPRTL_{S197}PT) does not conform to the optimal Aurora B consensus motif ([R/K]_x[S/T] Φ), but does fit the Cdk1, protein kinase D (LxRxx[S/T]), and CaMKII consensus motifs (Φ x[K/R]_{xx}[S/T] Φ [D/E]) (Doppler et al. 2005; Ubersax and Ferrell 2007). A role for Cdk1 is unlikely as its activity drops significantly during mitotic exit, although low or localized residual Cdk1 activity at this stage of the cell cycle cannot be formally excluded. It remains to be explored whether protein kinase D or CaMKII can function as the INCENP-Serine-197 kinase and how CPC localization depends on phosphorylation of Serine-197.

Is CPC (re-)localization required for anaphase progression and cytokinesis?

As mentioned earlier, relocalization of the CPC and thus Aurora B activity in anaphase most likely serves a dual purpose: elimination of activity from a site where it is no longer needed or wanted and increase in activity at a site where its function is now required. In both *Xenopus* egg extracts containing sperm chromatin and *Caenorhabditis elegans* embryos, nuclear envelope reformation is impaired when Aurora B cannot be extracted from chromatin upon knockdown of Cdc48/p97, suggesting that the removal of the CPC from centromeres or chromatin in anaphase might be important for reformation of the nucleus in telophase (Ramadan et al. 2007). In addition, it might prevent microtubule destabilization and reactivation of the mitotic checkpoint during anaphase due to relieve of inter-kinetochore tension after cohesin cleavage. Indeed, a mutant of the mitotic exit phosphatase Cdc14 in budding yeast caused Bub1 kinetochore localization and Mad1 phosphorylation in anaphase indicative of mitotic checkpoint reactivation (Mirchenko and

Uhlmann 2010). This phenotype was rescued by expression of a mutant of Sli15 that could no longer be phosphorylated by Cdk1 and allowed the CPC to leave centromeres in the absence of Cdc14. Also in mammalian cells, preventing CPC translocation by depleting the mitotic kinesin MKLP2 resulted in the recruitment of the mitotic checkpoint proteins BubR1 and Bub1 to anaphase kinetochores (Vazquez-Novelle and Petronczki 2010). However, sister chromatid separation and Cyclin B degradation still took place indicating that the anaphase-promoting complex was active and the mitotic checkpoint had been silenced. Moreover, chromosome segregation in MKLP2-depleted cells was not impaired indicating that retention of Aurora B at anaphase centromeres does not affect the stability of kinetochore–microtubule attachments (Vazquez-Novelle and Petronczki 2010). Recent data provided an explanation for these findings in MKLP2-depleted cells, since Aurora B activity towards the kinetochore protein Dsn1 in anaphase is counteracted by PP1 activity and the two PP1-targeting subunits Sds22 and Repo-Man are responsible for this effect (Wurzenberger et al. 2012). Taken together, removal of Aurora B from centromeres may only be essential for some aspects of mitotic exit, such as nuclear envelope reformation (Ramadan et al. 2007).

CPC translocation from centromeres to the central spindle might be more important for gain of function since the presence of the complex at the central spindle appears to be required for proper execution of cytokinesis. Alternatively, the central spindle may merely be a site where clustering and thus activation of the CPC occurs in anaphase, and precise localization in anaphase may not be required for CPC function during mitotic exit. However, the following pieces of evidence do to some extent suggest central spindle localization of the CPC to be important. Chromatid arm shortening that occurs in early anaphase to prevent chromatin from being trapped in the cleavage furrow, is most evident in chromosomal arms that are close to the spindle midzone, and appears to depend on the pool of Aurora B present on the midzone microtubules (Mora-Bermudez et al. 2007; Neurohr et al. 2011). Moreover, suppression of Aurora B activity in anaphase or telophase or inhibition of translocation of the CPC from centromeres to the central spindle by expression of an INCENP T59 phosphomimicking mutant causes binucleation possibly due to a failure to generate and/or maintain a stable midbody. Midbody instability may in turn be a consequence of misregulation of the kinesins Kif2a and Kif4a, both of which are substrates of Aurora B and regulate central spindle size (Gruneberg et al. 2004; Guse et al. 2005; Hummer and Mayer 2009; Nunes Bastos et al. 2013; Steigemann et al. 2009; Uehara et al. 2013). Further details on how the CPC controls mitotic exit and cytokinesis have recently been reviewed (Carmena et al. 2012b).

Notably, inhibition of Aurora B activity before the onset of furrow ingression prevents ingression resulting in binucleation

(Ahonen et al. 2009). However, if one inhibits Aurora B when ingression has just started, ingression continues for some time before the furrow regresses (Ahonen et al. 2009; Guse et al. 2005). In contrast, inhibition of Aurora B at the stage that a cell is ready for abscission forces fission of the intercellular bridge, indicating that Aurora B activity has to drop below a certain threshold to allow abscission (Steigemann et al. 2009). These results also provide a rationale as to why completion of cytokinesis is such a slow process because it seems to depend on a gradual decrease in Aurora B activity in the midbody, which may in part be degradation-mediated and in part phosphatase-regulated (Floyd et al. 2008; Mallampalli et al. 2013; Steigemann et al. 2009). In line with this, defective nuclear pore assembly or a chromosome bridge traversing the midbody delays abscission and this is associated with prolonged Aurora B activity in the midbody (Mackay et al. 2010; Steigemann et al. 2009). The induction of an abscission delay by potentially hazardous situations has been dubbed abscission checkpoint and appears to act via Aurora B-mediated phosphorylation of the abscission inhibiting protein CHMP4C on Serine-210 (Capalbo et al. 2012; Carlton et al. 2012; Steigemann et al. 2009). This serine sits in a region that is not present in the other CHMP proteins that are required for abscission and is involved in CHMP4C's recruitment to the site of abscission (Carlton et al. 2012; Guizetti et al. 2011). One important question that remains is how seemingly divergent defects that can cause an abscission delay are communicated to Aurora B. Finally, recent findings suggest that the assembly of CHMP proteins into filaments that support abscission can only take place when daughter cells have attached to a substrate (Lafaurie-Janvore et al. 2013). This adhesion would release tension in the intercellular bridge and stimulate filament assembly. Whether this type of tension release is somehow related to the very recently described role for Aurora B in the regulation of cell spreading via the formin FHOD1 is presently unclear (Floyd et al. 2013).

Concluding remarks

One of the most striking features of the CPC is its dynamic localization in the dividing cell. Tight regulation of its localization has for long been thought essential for execution of its various functions ranging from kinetochore–microtubule error correction, mitotic checkpoint control, and cytokinesis. Especially its localization at the inner centromere is key to a spatial restriction model explaining how Aurora B can discriminate between correctly and incorrectly attached kinetochore microtubules (leaving the former intact, but destabilizing the latter). Recent data in budding yeast challenge this model and they suggest that mere clustering of the CPC near kinetochores may be sufficient to promote proper chromosome segregation. Future research will have to tell whether this also applies to

higher organisms in which one kinetochore binds more than one microtubule. However, if this turns out to be the case then we have to re-evaluate how the CPC discriminates between amphitelic, syntelic, and merotelic attachments. In addition, if centromeric localization is not essential for error correction, why is its centromeric localization under such tight control throughout evolution? Could it be that certain specific functions of the CPC, such as for instance prevention of merotelic or activation of the mitotic checkpoint, do require its specific recruitment to this site? Alternatively, centromere recruitment of the CPC might guarantee that in anaphase the central spindle pool is strictly dependent on the centromere-localized pool, which may prevent premature execution of anaphase events. Finally, in early anaphase, the CPC is not only present on the central spindle but also on the equatorial cortex. The exact contribution of these two CPC pools to the early and late events of cytokinesis is a topic for future research.

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