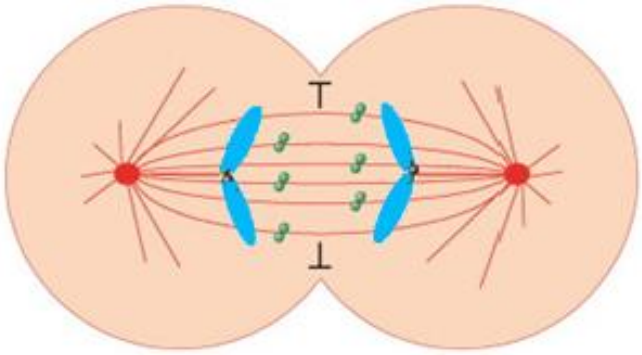
Requirements for CPC localization during anaphase



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Content

1. Introduction

Mitosis

One of the most important processes in dividing cells is mitosis. During mitosis, the chromosomes of a cell are split apart into two identical sets. This is necessary to divide DNA equally over two cells, which later separate during a process called cytokinesis. Mitosis is a complex process that is divided in different stages [1]. The first stage is prophase, in which the DNA is condensed and separate chromosomes become visible. The centrosomes, the microtubule organizing centres, will split apart and localize at opposite sides of the cell. The next stage is prometaphase, in which the nuclear membrane breaks apart and spindle microtubules bind to kinetochores at the chromosomes. Metaphase follows, in which the chromosomes align at the middle of the cell, also called the metaphase plate. When the chromosomes are aligned, anaphase starts. At this phase, the chromosomes start moving towards the centrosomes, while membranes and nucleoporins are recruited at the chromatin [2]. Once the chromosomes are at opposite sides of the cell, telophase starts, in which two daughter nuclei are formed around the pools of chromosomes. Telophase is the last stage of mitosis.

Errors in mitosis can have many consequences, even death in unicellular organisms. In multicellular organisms, this is also possible but via indirect effects of these errors. So it is important that mitosis is tightly coordinated and mistakes are prevented. This is done by several mitotic checkpoints like the checkpoint that is regulated by the cyclin-dependent kinase 1 (CDK1)-cyclin B complex, which is involved in mitotic entry and the Spindle Assembly Checkpoint (SAC) during (pro)metaphase, in which the Anaphase Promoting Complex (APC/C) is involved [1]. CDK-cyclin complexes are global controllers and sufficient to drive the entire cell cycle [3]. Once the division program has been triggered, more specific controllers are needed to regulate mitosis at specific locations and times. One of the most important complexes specifically regulating mitosis is the Chromosomal Passenger Complex (CPC), which consists of Aurora B kinase, INner CENtromere Protein (INCENP), Survivin and Borealin [2]. The CPC is conserved among eukaryotes.

Errors in mitosis can lead to chromosomal instability. For accurate chromosome segregation it is essential that separation of the sister chromatids only occurs if all chromosomes are attached to the mitotic spindle in bi-orientation [2]. The chromosomes need enough time to attach correctly and errors need to be detected. These processes rely on the CPC for an error-

free division, underlining its importance for cell division. Chromosomal instabilities are associated with different disease, for example cancer. Knowing exactly in which processes the CPC is involved and how it functions, can help us understand what is going on in these diseases.

Cytokinesis

During the last phases of mitosis, cytokinesis takes place. This is also under the influence of the CPC. Cytokinesis is a multistep process that starts with the assembly of an actomyosin ring. The structural components of the contractile ring include three interconnected filament systems: actin filaments, bipolar filaments of the motor myosin II, and septin filaments [3]. The septins are a membrane-associated filament system that bind and are recruited to the ring by anillin, a filament cross-linker that also binds directly to actin and to activated myosin II. The next step of cytokinesis is contraction. During this step, myosin filaments are proposed to use their motor activity to move along actin filaments, shortening the ring in a fashion analogous to muscle contraction [4]. However, in contrast to muscle, the ring is progressively disassembled as it constricts.

Contraction is the transition from a broad equatorial band to a compact mature contractile ring. It exists of two steps [3]. In the first step, which is termed "equatorial band formation," contractile ring proteins accumulate on the cell cortex in a broad band that encircles the cell equator (See figure 1). The second step transforms the sheet-like equatorial band into a ribbon-like contractile ring that sits at the furrow tip. During this second step, the equatorial band folds in half to form a furrow that extends into the interior of the cell. Contractile ring proteins become enriched in a ribbon along the furrow tip and are depleted from the region behind the tip (See figure 1). Contraction is now complete, so cells can progress to abscission.

Abscission is the final step in

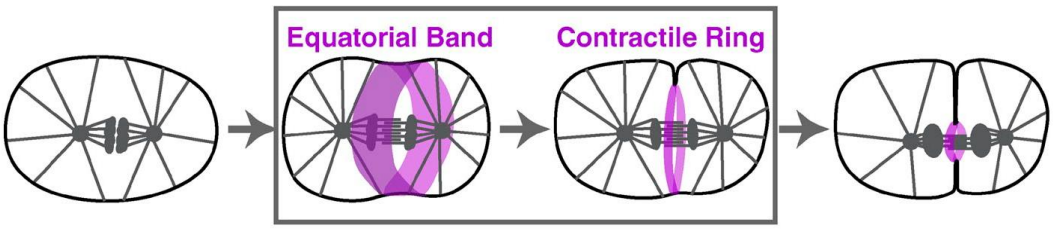


Figure 1. At the start of cytokinesis, an equatorial band is formed by contractile ring proteins that accumulate at the cell equator. This is the first step in formation of the contractile ring. At the second step, the sheet-like equatorial band formed in the first step, is transformed into a ribbon-like contractile ring that is localized at the furrow-tip [3].

cytokinesis. It is a complex process, in which different events succeed each other [61]. The cleavage furrow that is formed at the start of cytokinesis, has to be anchored to the cell cortex. If this does not happen, the furrow will regress. The ensuing event is the disassembly of the microtubules, followed by the splitting of the plasma membrane. If all events happened and the midbody is clear, full abscission can be achieved.

If cytokinesis is not completed, this can lead to tetraploidy and subsequently to aneuploidy. This can cause diseases, such as cancer. The CPC is involved in the proper regulation of cytokinesis and especially abscission by controlling the chromosomes have properly split apart.

Many players are involved in regulating the start of cytokinesis. Genes that are known to be involved, like the microtubule binding protein Anaphase Spindle Elongation (Ase1), are usually located at the spindle midzone [11]. If Ase1 is depleted in cells, it leads to a delay in abscission, because the cleavage furrow forms properly, but it then lingers before abscission happens. Markedly, Ase1p is not the only protein that has an effect on cytokinesis; kinetochore proteins like Ndc10p are also involved. If Ndc10p is depleted, cytokinesis defects are observed [11]. So the midzone and the players that are located there are necessary for proper cytokinesis; in midzone-defective cells, cytokinesis is defective or delayed. Why the midzone is so important, can be for two different reasons: one is that the spindle midzone provides a positive signal that is necessary for cytokinesis. If the midzone is affected, the signal is not provided. An alternative option is that spindle midzone defects are detected by a sensing mechanism which then sends an inhibitory signal which actively inhibits cytokinesis.

Composition of the CPC

Both CPC function and composition is highly conserved among eukaryotes. The CPC consists of one enzymatic unit, Aurora B Kinase, and three non-enzymatic subunits, INCENP, survivin and borealin (See figure 2). Knockdown of any member of the complex leads to delocalization of the others, thereby disrupting mitotic progression [1].

Aurora B kinase is a Ser/Thr kinase that is highly conserved among eukaryotes (See table 1) [1]. Budding yeast has one Aurora kinase, Ipl1, whereas vertebrates have three Aurora kinases, A, B and C. All have distinct functions; Aurora A plays a role in centrosome separation and maturation, Aurora B is the enzymatic component of the CPC and Aurora C, the least studied of the kinases, is mainly restricted to the germ cells [5]. Aurora B has an ever increasing list of substrates, making it a key regulator in mitosis. INCENP is the scaffold protein of the CPC; it interacts with all other members [6]. The Aurora B binding domain of

INCENP is called the IN box. It is involved in both binding and regulating Aurora B. Survivin is a member of the Inhibitor of Apoptosis Protein (IAP) family, because it contains a Baculovirus IAP repeat (BIR) [1]. Interestingly, both Aurora B and INCENP are necessary for CPC targeting to the central spindle [2]. Survivin can homodimerize on itself at the BIR domain [6]. Once bound to INCENP, the Survivin BIR domain is essential for spindle checkpoint functioning and centromere targeting [7]. Survivin can be phosphorylated by Aurora B, but recent work showed that Survivin is also an activator for Aurora B [8]. Borealin was identified most recently as a CPC component in a proteomic screen for components of the mitotic scaffold [9]. Like survivin, Borealin can be phosphorylated by Aurora B. Both Survivin and Borealin do not directly interact with Aurora B but can bind the N-terminal domain of INCENP [6]. Both are required for centromere targeting of the CPC [62].

Localization of the CPC

The CPC has many functions in cell division, in line with its dynamic localization during mitosis and cytokinesis [2]. In early prophase it localizes on chromosomal arms, but during late prophase it is progressively concentrated on inner centromeres. The CPC stays localized there during prometaphase and metaphase, where it ensures accurate segregation of the chromosomes (see figure 3).

At anaphase onset the CPC translocates from the centromeres to the interpolar microtubules of the central spindle and to the equatorial cortex [10]. The region where the CPC is located becomes the midbody during telophase. CPC localization is conserved among eukaryotes [12, 13].

Experiments on the crystal structure of the CPC show that the N-terminal domains of Borealin and Survivin, bound on the N-terminal domain of INCENP, are sufficient to target the CPC to the central spindle and midbody, but targeting to the centromeres requires the C-terminal domain of Borealin in addition [6]. INCENP can bind to the chromatin-associated protein Hp1, leading to targeting at the chromosomal arms [63]. Aurora B and INCENP are important for the CPC localization at the central spindle [62]. INCENP can bind to

Table 1: CPC component orthologs [2]

H. sapiens	*M. musculus*	*X. laevis*	*C. elegans*	*D. melanogaster*	*S. pombe*	*S. cerevisiae*
Aurora B	Aurora B	XAurora-B	AIR-2	ial	Ark1	Ipl1
INCENP	INCENP	XINCENP	ICP-1	Incenp	Pic1	Sli15
Borealin	Borealin	Dasra-A, Dasra-B	CSC-1	Borr [a], Aust [b]	Nbl1	Nbl1
Survivin	Survivin	XSurvivin	BIR-1	Deterin	Bir1/ Cut17	Bir1

[a] Borealin-related. [b] Australin

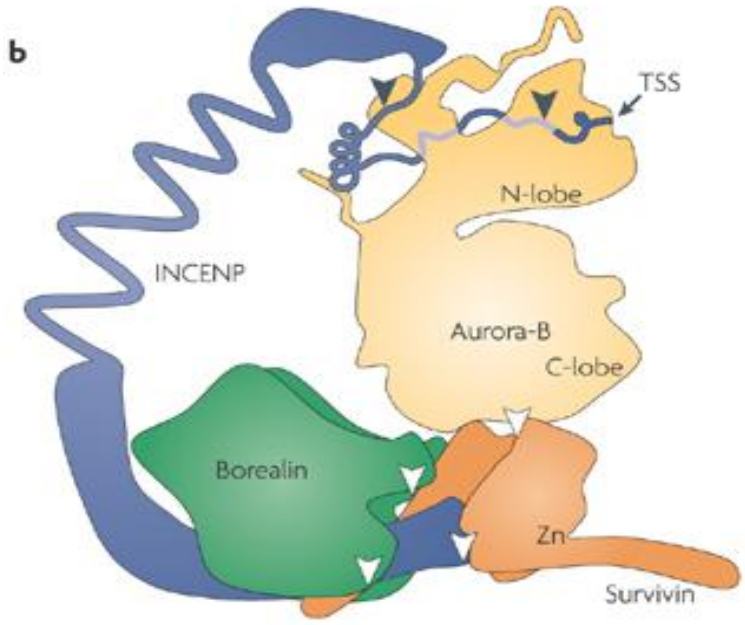


Figure 2: Schematic representation of the CPC. Interactions within the complex are represented by white arrowheads. Survivin and Borealin interact with the N terminus of INCENP, Aurora B interacts with the IN box of INCENP, which is at the C terminus of INCENP. Borealin and Survivin also bind each other [1].

microtubules after Cdk1 inactivation, this requires MKLP2 [13,42].

The importance of central spindle localization of the CPC

The enzymatic subunit of the CPC, Aurora B, needs to reach its substrates at the right time and place during mitosis and cytokinesis. This way, it can carry out its

various functions in the cell. Over 50 substrates of Aurora B have been identified to date [2]. Not surprisingly, tight regulation of localization and timing of Aurora B is important for normal cell division; substrate specificity of Aurora B is mainly determined by localization.

During anaphase, the CPC promotes axial shortening of segregating chromosomes in anaphase. It is also involved in the stabilization of the central spindle. The CPC can only carry out these processes if it is localized at the central spindle. After anaphase, the CPC plays an essential role during cytokinesis by installing an abscission delay for the prevention of chromosome damage and / or tetraploidy [2]. To reach the downstream players for this abscission delay, the CPC needs to be localized at the midbody.

Thus, it is known that localization of the CPC is important for error-free cell

division, but surprisingly, not much is known about how exactly the translocation during anaphase is managed in the cell. Since error-free cell division is necessary for the prevention of many diseases such as cancer, knowing what requirements there are for CPC anaphase localization, can help us to identify therapeutic targets for anticancer therapy. Especially cancer has a linkage to cell division, since it is a disease of overproliferating cells.

This thesis will provide an overview of how CPC localization and functioning in anaphase and cytokinesis is regulated in eukaryotes and the defects found when the CPC is not localized properly.

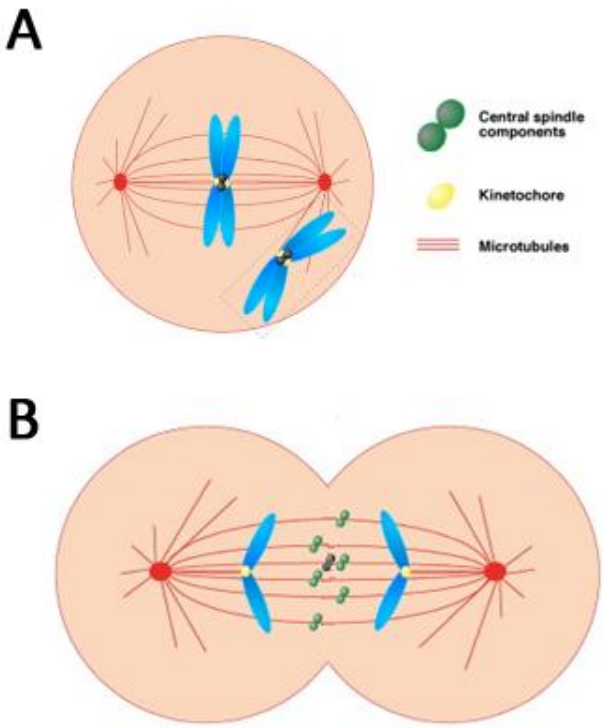


Figure 3: A) During (pro)metaphase, the CPC (green circles) localizes at the centromeres to regulate proper chromosome segregation. B) During anaphase, the CPC is released from the centromeres and localizes to the central spindle. It is retained there to regulate cytokinesis. Adapted from [14].

2. Regulation of CPC localization during anaphase and cytokinesis in unicellular eukaryotes

Yeast as a model organism for studying the CPC

Yeast is a model organism that is much used for studies on cell division, because of its strong genetic possibilities and relatively easy use. Many players involved in regulation of mitosis and cytokinesis are conserved, making it possible to use yeast as a model for how human cell division works.

The yeast CPC is homologous to the mammalian CPC. In the budding yeast *Saccharomyces cerevisiae*, the Aurora kinase homologue is called Increase in PLoidy 1 (Ipl1). Yeast only has one Aurora kinase homologue, which shows similar functions to both Aurora A and Aurora B [15]. The homologue of INCENP is Synthetically Lethal with Ipl1 (Sli15), which was discovered as a protein that associates with Ipl1p in vivo and promotes its function in chromosome segregation [16]. Survivin is called Baculoviral IAP Repeat-containing protein 1 (Bir1) in yeast, because of the two IAP repeat domains (BIR) that it contains. Borealin is called N-terminal-Borealin Like protein 1 (Nbl1) [18]. Only the region of Nbl1p involved in three-helical binding (THB domain) of Sli15p, Bir1p and Nbl1p is present in different *Saccharomyces* species, including *cerevisiae* [18]. This region also shows strong homology to Survivin. Surprisingly, there is evidence that some regions containing consensus residues for Cdk1 phosphorylation, have been switched between Borealin and Survivin homologs. Phosphorylation sites that are found on the Bir1p (Survivin homolog) in yeast, are found in Borealin in humans [19]. The phosphorylation sites are not conserved at the sequence level, but are largely conserved as a cluster in a disordered region. Because of the switch of the domains, mammalian Survivin contains only one BIR domain 1 and is 700 amino acids smaller than the yeast homologue Bir1p [17], while Nbl1p is much shorter than human Borealin, a difference of over 200 amino acids. So interestingly, functionally comparable domains apparantly have moved to different CPC subunits during evolution.

Regulation and function of the CPC in late mitosis and cytokinesis can be divided in two processes, central spindle localization of the CPC during anaphase and the involvement of the CPC in the NoCut checkpoint, while localized at the midbody. All described mechanisms were discovered using *S. cerevisiae,* unless noted differently.

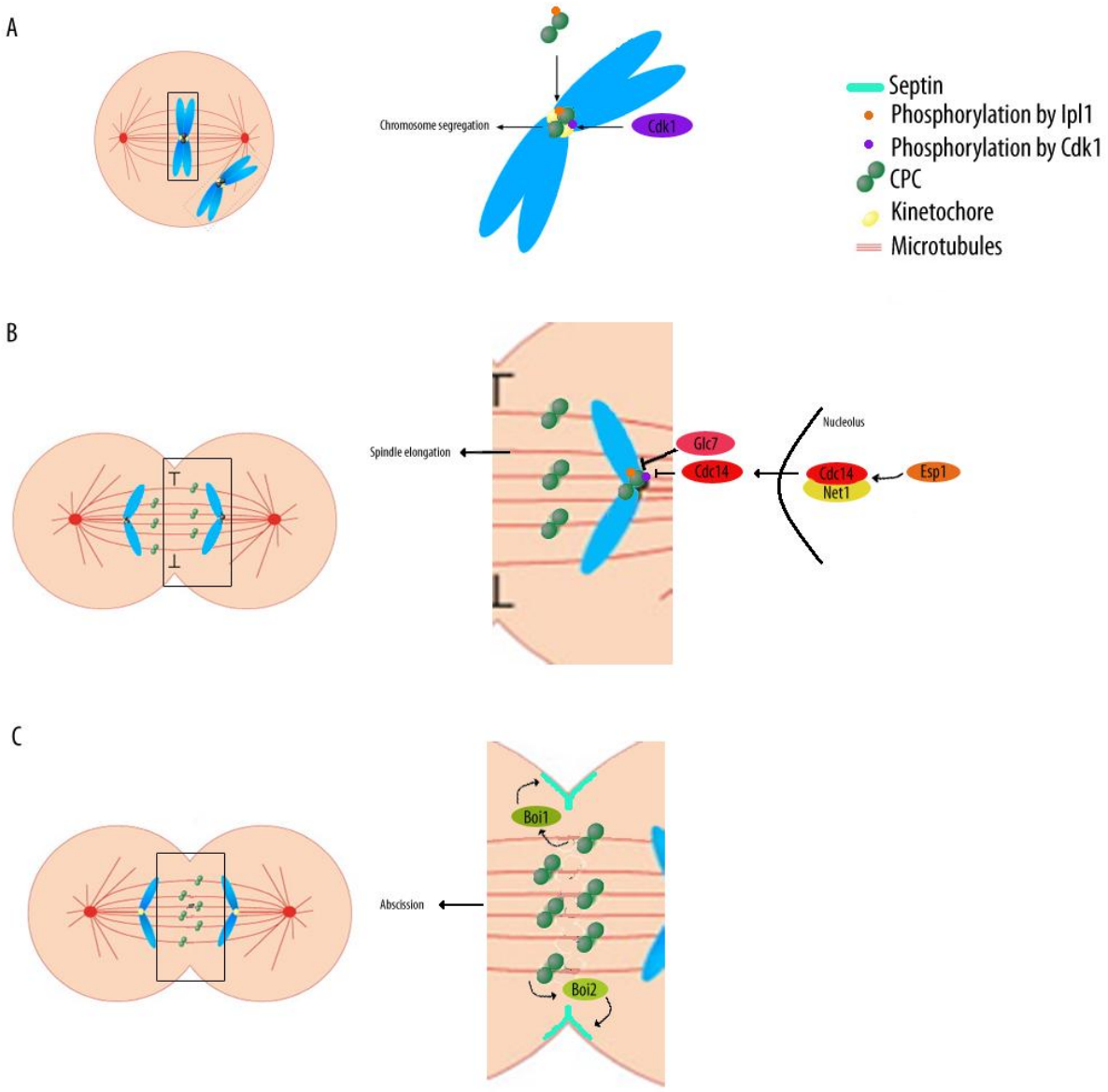


Figure 4. A model for CPC regulation and its effects in *S. cerevisiae.* A) Before anaphase the CPC is located at the centromeres. Sli15p is phosphorylated by Cdk1p and Ipl1p, contributing to this localization. The CPC regulates proper chromosome segregation in this phase. B) At the start of anaphase, Esp1p regulates partial release of Cdc14p, leading to dephosphorylation of Sli15p. C) At late anaphase the CPC is located at the midbody. If no chromatin is present at the midbody, the CPC will localize Boi1p and Boi2p to the nucleus, which leads to a rearrangement of Septins so cytokinesis will proceed. Adapted from [14].

CPC localization during anaphase

At the start of mitosis, the Cdk1-Cyclin B complex associates, so Cdk1p becomes active [1]. Cdk1p and Ipl1p can phosphorylate Sli15p, which leads to CPC localization at the centromeres [15] (see figure 4A). Importantly, most Cdk1p phosphorylation sites are located

in the microtubule binding domain of Sli15p, leading to the inability of Sli15 to bind to microtubules. [18]. At anaphase onset, Cdk1p activity decreases and activity of the Sli15-specific protein phosphatase Cell Division Cycle 14 (Cdc14) increases [1]. Cdc14p shows a strong binding to Sli15p [12], and regulates its dephosphorylation. After Cdk1p activity decreases, Ipl1p remains active and phosphorylates Sli15p at various serine / threonine phosphorylation sites [15]. Interestingly, most Ipl1p phosphorylation sites are also found on the microtubule binding domain of Sli15p, preventing the CPC to localize at the microtubules of the central spindle (see figure 4B). These Ipl1p-phosphorylation sites on Sli15p are dephosphorylated by GLyCogen 7 (Glc7), also called PP1 [15].

During preanaphase, Cdc14p is trapped in the nucleolus because it binds to the inhibitor Nucleolar silencing Establishing factor and Telophase regulator 1 (Net1) [20]. Net1p is part of a complex that is called RENT. At the metaphase – anaphase transition, the separase Extra Spindle Pole bodies 1 (Esp1), which is part of the FEAR pathway, is activated. Esp1 activates the kinase Cdc5p, which then phosphorylates Cdc14p-Net1p, leading to the destabilization of the complex [21]. This stimulates a transient and partial release of Cdc14p into the nucleus [22]. Subsequently, the Mitotic Exit Network (MEN) pathway, a GTP-ase driven signaling cascade, initiates the cleavage of the remaining Cdc14p-Net1p complexes during anaphase and maintains Cdc14p in its free state by phosphorylation of the NLS-residue of Cdc14p [23]. Now, higher levels of Cdc14p are reached to mediate mitotic exit and cytokinesis onset [22]. Ipl1p-activity then decreases. At that time, Glc7p and Cdc14p will provide complete dephosphorylation of Sli15p [15]. Non-phosphorylated Sli15p can bind to microtubules, thereby allowing the CPC to translocate from the centromeres to the bundled microtubules at the central spindle (see figure 4C).

Importantly, besides the effect of the MEN pathway on the release of Cdc14p, it is also directly involved in CPC localization via the protein kinases complex Mob1p/Dbf2p [64]. Mob1p is required for the dissociation of Ipl1p from the kinetochores. Binding of Mob1p to Dbf2p is necessary for kinase activation by Cdc15p [65]. This is required for CPC localization on the central spindle. This makes the MEN pathway an important pathway for CPC localization in *S. cerevisiae.*

The Fission yeast *Schizosaccharomyces pombe* has a cdc14-like protein, Cdc14-like phosphatase 1 (Clp1). Its sequence is well conserved between *S. pombe* and *S. cerevisiae* and as in *S. cerevisiae,* Clp1p is the main antagonizer for Cdk1p. However, it functions differently during cell-cycle control [24]. It is not important for mitotic exit, but regulates mitotic entry and coordinates

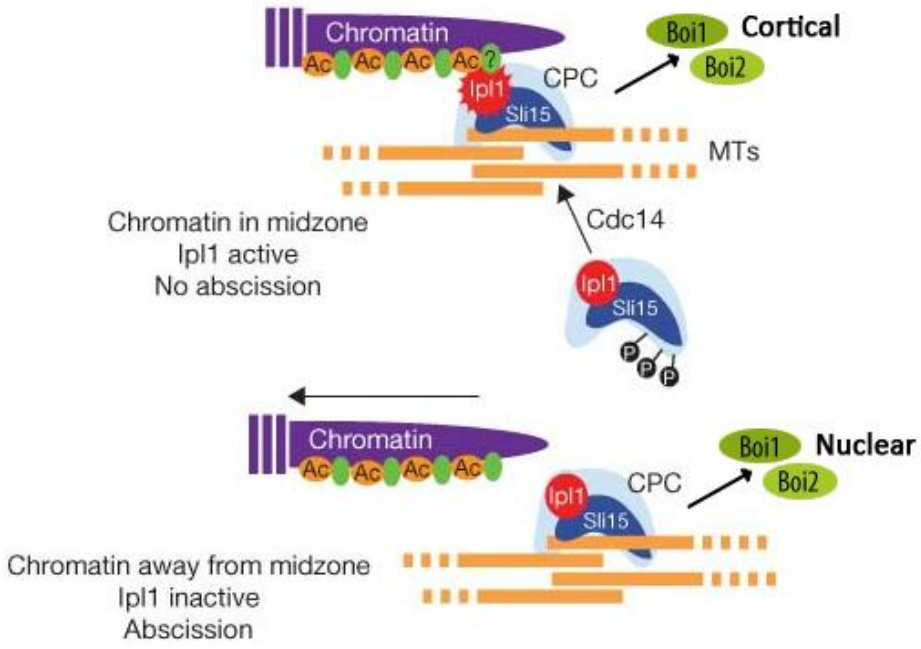


Figure 5: A model for the interaction of the CPC with chromatin during anaphase. Ipl1p and Sli15p subunits are depicted in red and blue, respectively. Green are chromatin-associated factors, chromatin is depicted in purple. Microtubules in orange. In the upper panel, the CPC is targeted to the spindle midzone microtubules in early anaphase. When chromatin is present in the midzone, Ahc1p contributes to the interaction of the CPC to the chromatin, leading to Ipl1p activation, which leads to translocation of Boi1p and Boi2p to the cortex, where they inhibit abscission by inhibition of septin filaments. Lower panel: if chromosome segregation happens correctly, the CPC is no longer bound to chromatin and Ipl1p is not activated. Boi1p and Boi2p will remain nuclear, so abscission is not inhibited. Adjusted from [30].

cytokinesis, with subsequently entry into the next cell cycle. It functions before anaphase, by interacting with Ipl1p and stimulating its function in regulation of chromosome bi-orientation.

There are hints that sumoylation may play a role, since Bir1p and Sli15p can be sumoylated, and it has recently been found that sumoylation apparently plays a role in CPC targeting in mammals [25,26].

The role of the CPC for cytokinesis

To find whether the midzone provides a positive and / or negative signal for cytokinesis, Ipl1p-mutant yeast was used [27]. *Ipl-321* mutants are temperature-sensitive cells that have reduced Ipl1p kinase activity. These mutants do not show a cytokinesis defect [11]. However, *Ipl-321* can rescue

midzone-defective cells by restoring abscission. This is the same for *Sli15-1* mutants. Thus, midzone defects cause Ipl1 and Sli15-dependent inhibition of abscission [11]. So the midzone gives an inhibitory signal. This does not exclude the possibility that it is also giving a positive signal for abscission.

Not only Ipl1 and Sli15 are important for cytokinesis, Bir1 is also essential. Bir1 mutants show disorganized septin rings that do not disassemble properly, even when cells start to form new buds [28]. Sli15 mutants show the same phenotype. Since the CPC complex is not localized at the bud neck itself (See figure 4C), other players are expected to be involved. These players are the anillin proteins Bem1 Interacting Protein 1 and 2 (Boi1 and Boi2) [11]. Anillins are a family of proteins that can shuttle between the cell cortex and the nucleus and have a pleckstrin-homology (PH) domain at their C-terminus. Ipl1-activity is necessary for proper localization of Boi1p and Boi2p (see figure 5). Co-expression of *Boi1* with *GFP* showed that Boi1p localizes to the bud neck of anaphase cells. During the separation phase of cytokinesis, no Boi1p could be detected. The same result was obtained for Boi2p, using co-expression of *Boi2-GFP*. In midzone-defective cells, Boi1p and Boi2p are localized at the bud neck, supporting their inhibitory role. But in *Ipl-321* mutants, Boi1p and Boi2p are localized at the nucleus [11]. To test the effect of Boi1 and Boi2 on abscission, abscission timing was compared between *Ase1* mutants that have defects in the spindle midzone, in the absence of Boi1p and Boi2p. *Ase1* mutants take approximately 24 minutes for completion of abscission after start of contraction, a delay of around 9 minutes compared to wildtype, while *Ase1 / Boi1 / Boi2* mutants show no delay [11]. So *Boi1 / Boi2* double mutants have accelerated abscission compared to wildtype, meaning that Boi1 and Boi2 are involved in abscission timing [11]. Since Bir1 is involved in septin organization, the CPC probably does this via regulation of Boi1 and Boi2 localization, leading to the delay in abscission.

The NoCut pathway

Importantly, the CPC is not only involved in starting cytokinesis, but also in completion of the process. This is done through regulation of the NoCut pathway, a signaling pathway that can inhibit cytokinesis in the presence of spindle-midzone defects by installation of an abscission delay [11]. The pathway is called NoCut since it prevents the 'cut' phenotype, which has been clearly described in *S. pombe* [29]. The cut phenotype is seen when cytokinesis takes place in the absence of normal nuclear division, when the nucleus is still in the cleavage plane and no proper chromosome segregation has taken place, leading to chromosome breakage.

The trigger to activate NoCut is the presence of chromatin at the spindle midzone

[30], as was found out by use of a Topoisomerase II (Top2) mutant Top2-4, in which the chromatin stays in the spindle midzone after anaphase and by use of the Tet-repressor to fuse Ipl1p to chromatin containing Tet operator repeats. If Ipl1p is forced onto chromatin using the Tet-repressor, NoCut is even activated in the absence of defects of the spindle midzone. Ipl1p can interact with chromatin via the Ada Histone Acetyltransferase 1 (Ahc1) element and maybe via other yet unknown chromatin-associated factors [30] (see figure 5). Exactly how NoCut activation by the presence of chromatin is reached is not known; clustering of CPC complexes can lead to auto-activation [31], but there could also be other players involved. What is known, is that the CPC has to be located at the central spindle during anaphase to trigger the NoCut response, indicating that the signal is generated at this location and time [30].

3. Regulation of CPC localization during anaphase and cytokinesis in multicellular eukaryotes

Yeast is a relatively easy model for studying mitosis and other cellular processes. But it is a unicellular organism, so not all information that is abstracted from yeast research, can be directly extrapolated to more complex, multicellular eukaryotes, such as humans. Therefore, a lot of research on cell division is also done on different multicellular eukaryotes, which gives more insight in CPC function and localization. Much used multicellular organisms include *Caenorhabditis elegans, Drosophila melanogaster* and *Xenopus laevis.* A lot of early work on the CPC was done on chicken (*Gallus gallus*). For mammalian research, mouse model systems and human cell lines are much used. All of this research is done to determine how the CPC localizes and functions, which will hopefully lead to therapeutic purposes.

CPC anaphase localization in *C. elegans*

The nematode *C. elegans* is a lower eukaryote for which every single somatic cell in the adult has been mapped, so it is completely known which cells divide and give rise to certain offspring. Studying cell division in a mapped model organism makes it easier than studies in non-mapped, more complex models as higher eukaryotes, to detect defects in mitosis. In *C. elegans*, the CPC-components have different names. For nomenclature, see table 1.

The *C. elegans* CPC has the same localization pattern as in yeast: at the start of anaphase, it is localized to the centromeres and it then relocalizes to the central spindle during anaphase. In yeast, this relocalization is dependent on the phosphatases cdc-14 and glc-7. Importantly, an RNAi-screenin *C. elegans* showed that also in this multicellular organism, cdc-14 and the glc-7-homolog gsp-2 (yeast Glc Seven-like Phosphatase 2) are also required for relocalization of the CPC and for central spindle assembly [32] (See figure 6). If cdc-14 is depleted by RNAi, air-2 does not localize to the midbody but is restricted to small microtubule bundles. This leads to a defective central spindle. Another protein that is mislocalized in cdc-14-depleted embryos is the mitotic kinesin-like protein Zygotic epidermal ENclosure defective (zen-4, also known as CeMKLP1). Normally, cdc-14 and zen-4 show the same localization pattern and both are separately mislocalized if one of them is depleted, thus they depend on each other for localization [32]. So if zen-4 is depleted, cdc-14 shows mislocalization and this leads to mislocalization of air-2. But zen-4 can also directly bind to air-2, as was shown by yeast two hybrid analysis [33]. Like in yeast,

in *C. elegans,* cdc-14 regulates CPC localization at the midbody.

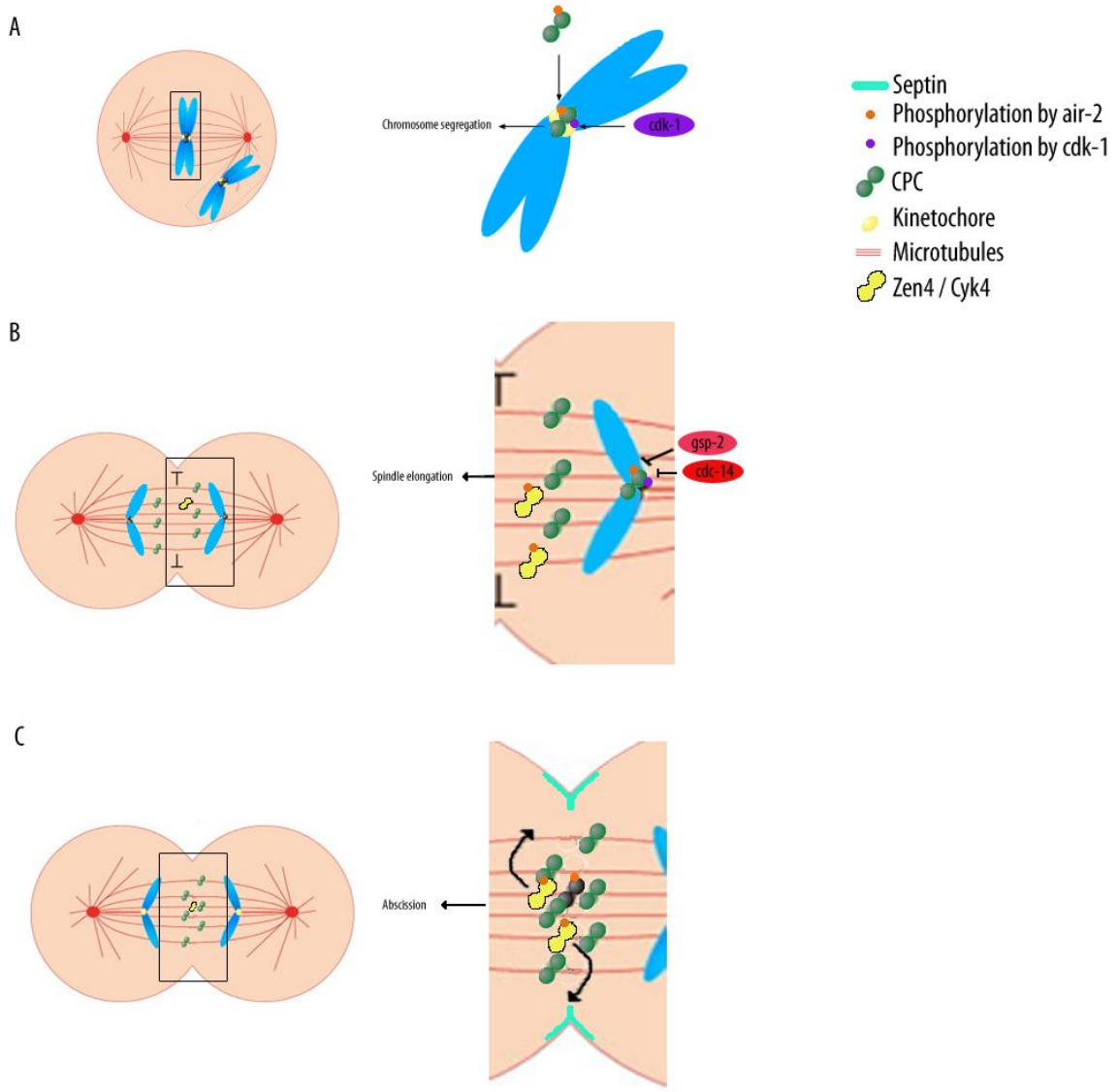


Figure 6. A model for CPC regulation and its effects in *C. elegans.* A) During (pro)metaphase, the CPC is located at the centromeres, because icp-1 is phosphorylated by cdk-1 and air-2. The CPC regulates proper chromosome segregation in this phase. B) At the start of anaphase, cdc-14 dephosphorylates icp-1. Zen-4 / cyk-4 localization is both under the influence of cdc-14 and the CPC. It is localized to the central spindle, where it is phosphorylated by air-2 to promote microtubule bundling for midzone stability. C) At late anaphase, the central spindle turns into the midbody, where the CPC is localized. If no chromatin is present at the midbody, the CPC will rearrange the septins via the septin unc-59, so abscission can proceed. The zen-4 / cyk-4 complex is also involved in abscission, but via another pathway, including ced-10. Adapted from [14] .

CPC involvement in cytokinesis in *C. elegans*

As in yeast, the central spindle that is formed during anaphase is important for cytokinesis [34]. Depletion of ZEN-4 in *C. elegans* embryos causes a defect in the formation of the central spindle because interpolar microtubules do not become bundled [35]. Furrow ingression starts, but fails before completion. RNAi of a Rho Family Gap protein, CYtoKinesis defect 4 (cyk-4), gives the same phenotype [36]. Zen-4 and cyk-4 co-localize on the central spindle and form a complex *in vivo* [34]. This complex is sufficient to bind microtubules *in vitro*. If air-2 is knocked down or does not localize properly, cyk-4 and zen-4 do not localize properly [37]. This is because air-2 phosphorylates the complex, as determined by immunofluorescence using antibodies recognizing phosphorylated zen-4 or phosphorylated cyk-4 [37] (See figure 6B). Phosphorylation of the complex leads to bundling of the microtubules to form a stable midbody, which is needed for the cleavage furrow to form and ingress properly.

During the first embryonic division of a *C. elegans* embryo, depletion of AIR-2 or depletion of ZEN-4 / CYK-4 shows the same effect: a two- to threefold reduction in constriction rate of the contractile ring, compared to wildtype [3]. This fitted in the linear pathway that is seen for midzone stability for the complex and air-2. But if both AIR-2 and ZEN-4 are depleted using temperature-sensitive mutants, an additive constriction effect is seen, of less than half the constriction rate as in a single mutant. This suggests that air-2 and zen-4 both make an independent contribution to contractile ring constriction (see figure 6C), via other players.

One of these players is the small GTPase CEll Death abnormality 10 (CED-10). If only CED-10 is depleted, this leads to a slight delay in cytokinesis [3,38]. If CED-10 is depleted together with ZEN-4, this reverses the *ZEN-4* phenotype [3]. Another player involved is the septin Uncoordinated 59 (UNC-59). Depletion of UNC-59 can rescue the *AIR-2* phenotype but not the *ZEN-4* phenotype, thus it plays a role in the air-2 pathway for constriction.

Contraction occurs in two steps. The first step is called equatorial band formation, the second step transforms the equatorial band into a contractile ring [3] . Using a cortical imaging method, Lewellyn *et. al* found that the first step of contraction will be initiated in embryos lacking air-2 or zen-4, but the second step fails. Thus, the zen-4 / cyk-4 complex and the CPC are not required for equatorial band formation but are required to form the contractile ring. Both complexes do this independently from each other, zen-4 / cyk-4 via ced-10 activity and the CPC is associated with the septin unc-59 [3]. It is likely that the CPC is involved in the rearrangements that the septins make during cytokinesis,

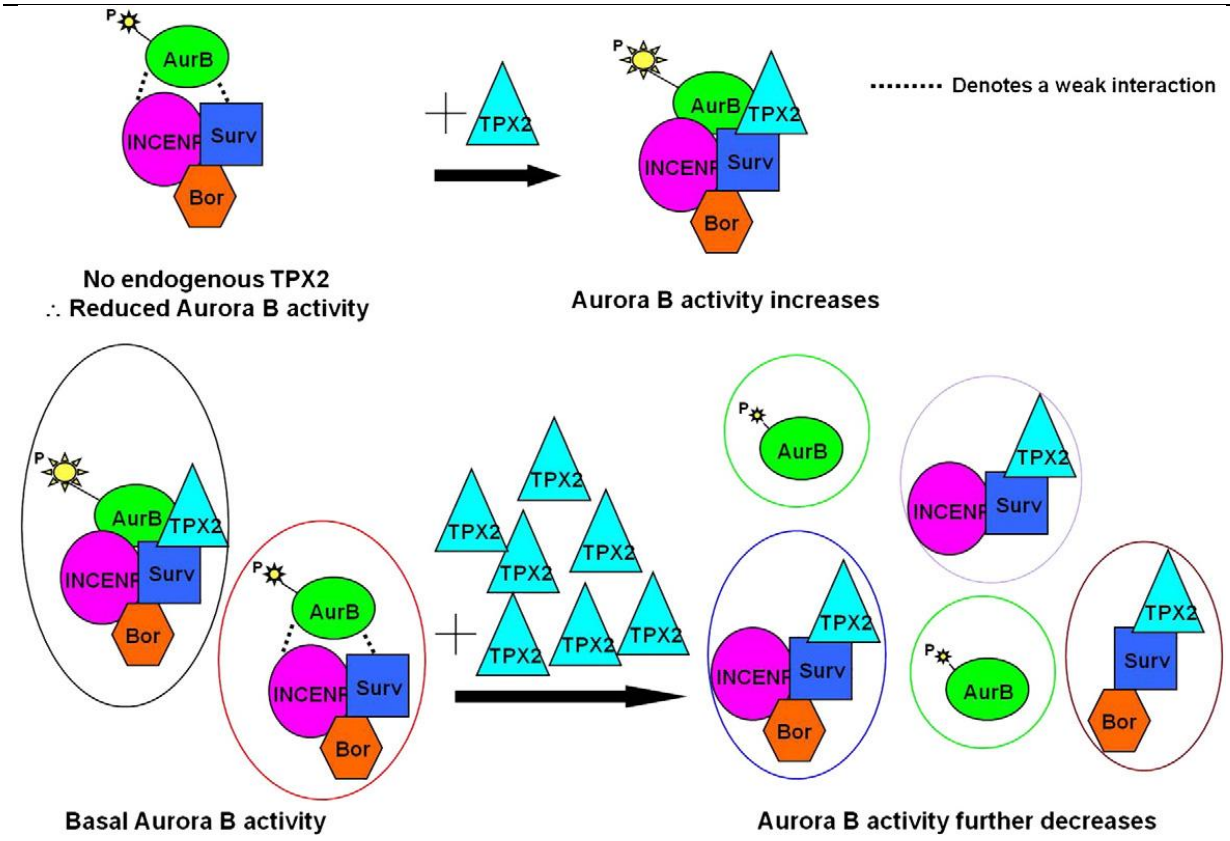


Figure 7. Model for TPX2 activation of Aurora B, as a scaffold for the CPC. If TPX2 is depleted, Aurora B shows a weak binding to the other CPC components. Addition of TPX2 can rescue this. However, high levels of TPX2 inhibit binding of the CPC components to Aurora B. The size of the phosphorylation mark on Aurora B represents the amount of Aurora B phosphorylation. Different colored circles indicate different protein complexes. AurB = Aurora B, Surv = Survivin, Bor = Borealin. Adapted from [5].

cytokinesis, since that has also been found in yeast (see chapter 2).

CPC regulation in *Xenopus laevis*

Xenopus laevis is a model organism often used to do research on higher eukaryotes, especially because the embryos are easy to manipulate. Nevertheless, not much research on the CPC has been done using the *Xenopus* system. Research that has been done is on the turnover rates of the CPC at the centromers [66]. High turnover rates are observed, but if they are perturbed, this leads to mitotic exit without cytokinesis. So also in *Xenopus,* the CPC is involved in cytokinesis entering.

Recently, it has been found that the Aurora A activator protein TPX2 can act as a novel scaffold and co-activator protein of the CPC [5]. TPX2 roughly shows the same localization pattern as the CPC and can bind to

it. If TPX2 is depleted, less Aurora B activity is registered. High levels of TPX2 however, sequester the other CPC components away from Aurora B, also leading to decreased activation (See figure 7). This model is quite new, so it is not included in the general model (See figure 8).

CPC regulation in chicken

A lot of the early research on the CPC was done in chicken, by Earnshaw. Chicken is not used much as a model organism, since it is definitely not the easiest model for genetic analysis. However, the embryos are easily accessible, so it is most used embryonic research. The functional domains of INCENP where discovered by mapping experiments on chicken [63]. Borealin also has first been discovered in chicken [67]. More recently, chicken cell lines are being used to produce conditional knockouts of the different CPC components. Experiments on these cell lines are used to confirm previous experiments on the complex [68].

CPC anaphase localization in mammals

The mammalian system for CPC regulation is studied by the use of model organisms such as mouse and rat, but also by use of cell culture systems for human, mouse or rat cells. The use of cells makes it easy to image processes in the cell, like mitotic progression and protein translocation.

Since cells are so useful for protein location analysis, rat kidney epithelial cells were transfected with different constructs containing GFP linked to CPC components [39]. This shows the dynamics of the CPC, including the translocation from centromeres to midbody during anaphase, showing its conservation from lower to higher eukaryotes. It also shows that Cdk1-Cyclin B activity is required to maintain the CPC complex at the centromeres, another conserved mechanism. The human Cdc14A is also required for proper cytokinesis, although it has not been proven that this is via the CPC [40].

In *C. elegans,* CPC localization is not only under the control of Cdc14, but also under the control of a mitotic kinesin, zen-4. Mammals not only have a zen-4 homologue, they have two mitotic kinesins, Mitotic Kinesin-Like Protein 1 and 2 (MKLP1 and MKLP2). [34]. Using antibody stainings, it was determined that they both localize to the central spindle during anaphase, at similar but not identical locations [13]. After anaphase, MKLP1 becomes restricted to the central spindle and MKLP2 localizes to two bands on either side of the central spindle [41]. The localization of the latter overlaps with Aurora B. The CPC and MKLP2 depend on each other for proper localization at the midbody: if INCENP is depleted by RNAi, MKLP2 does not localize properly and

vice-versa [42]. This localization dependance suggests a similar role for MKLP2 in mammals as zen-4 has in *C. elegans.*

MKLP2 is the most likely candidate to function as zen-4 in *C. elegans* from both mammalian mitotic kinesins. MKLP2 can bind

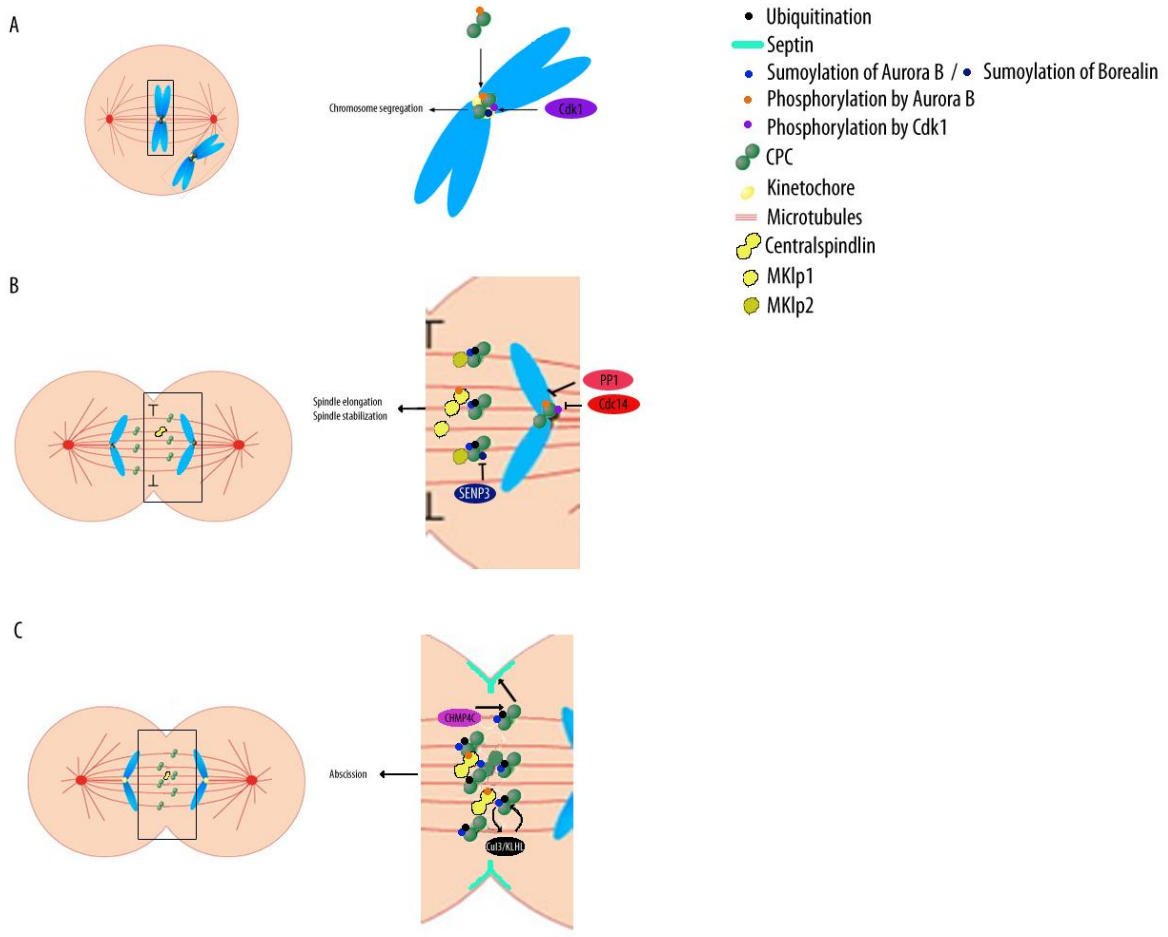


Figure 8. A model for CPC regulation and its effects in mammals. A) During (pro)metaphase, the CPC is located at the centromeres, because INCENP is phosphorylated by Cdk1 and Aurora B. Borealin is sumoylated at the centromeres. The CPC regulates proper chromosome segregation in this phase. B) At the start of anaphase, Cdc14 dephosphorylates INCENP. MKLP1 and MKLP2 localization is under the influence of the CPC. Both are localized to the central spindle, where they promote microtubule bundling for midzone stability. SENP3 desumoylates Borealin at the midbody, but Aurora B is sumoylated. This facilitates the formation of Cul3/Klhl complexes for CPC ubiquitination. C) At late anaphase, Cul3/Klhl complexes ubiquitinate the CPC to retain it there. Centralspindlin is phosphorylated by Aurora B, leading to recruitment of abscission factors to the midzone. Abscission only takes place if there is no chromatin present at the midbody, this goes via CHMP4C binding to chromatin. Figure adapted from [14].

to Aurora B, MKLP1 cannot [13]. Recently, it has been found that the β-tubulin binding domain of INCENP interacts with MKLP2, which is required for localization of the CPC at the central spindle microtubules [42]. So MKLP2 functions in CPC localization during anaphase, not MKLP1.

Furthermore, MKLP2 is required for proper Cdc14A targeting at the central spindle, whereas Cdc14A can dephosphorylate INCENP. This means that MKLP2 can act on the CPC in two ways: by directly affecting Aurora B through binding to it, and by targeting Cdc14A to the central spindle to dephosphorylate INCENP [13]. The localization of MKLP2 at the stable microtubule ends of the midzone requires INCENP [42]. However, this association is negatively regulated by Cdk1: if Cdk1 is present, MKLP2 and the CPC do not localize at the midbody but are refined at the centromeres. So anaphase onset, that starts when Cdk1 levels decrease, triggers the association of the CPC and MKLP2 and their localization at the central spindle (See figure 8B).

Ubiquitination of the CPC

An important process for CPC localization during mitosis is CPC ubiquitination [43] (See figure 8C). Different CPC components are targeted by ubiquitination, like Survivin [43]. Ubiquitination of Survivin promotes its association with centromeres during early mitosis but also triggers its dissociation from these structures at later stages. Next to Survivin, Aurora B can be ubiquitinated by the a complex of the Cullin 3-based E3 ligase (Cul3) with its substrate-specific adaptors Kelch-like 9, 13 and 21 (KLHL9, KLHL13 and KLHL21). These complexes are required for correct chromosome alignment, proper midzone and midbody formation and also for completion of cytokinesis [14]. The substrate-recognition domains of the three KLHL adaptors can directly bind Aurora B, which has been found to be ubiquitinated in a Cul3-dependent manner. Interestingly, the complexes mainly seem to not only polyubiquitinate the CPC, but also monoubiquitinate it. Polyubiquitinated proteins are usually targeted for degradation [44]. There could be players involved that are still to be discovered.

During anaphase, ubiquitination of the CPC by the complexes containing KLHL9 or KLHL13 mainly happens in the cytoplasm of the cell, while Cul3/KLHL21 complexes are located at the spindle midzone [44]. This leads to the assumption that Cul3/KLHL9 and Cul3/KLHL13 monoubiquitinate Aurora B in the cytoplasm, leading to its localization at the spindle midzone. At the spindle midzone itself, Cul3/KLHL21 can also monoubiquitinate Aurora B to contain it on the spindle midzone. This eventually leads to a shift in equilibrium

of Aurora B binding to chromosomes outside the spindle midzone, to a stable binding of Aurora B to the microtubules in the spindle midzone during anaphase [45] (See figure 9). This hypothesis includes an unknown Ubiquitin Binding Protein (UBP) to be localized at the spindle midzone, to retain the CPC at the spindle midzone.

Another ubiquitin ligase that influences the CPC, is the Anaphase Promoting Complex or Cyclosome (APC/C) ubiquitin ligase. This ligase depends on it coactivator Cdh1. APC/C^{Cdh1} becomes active at anaphase onset, because it is inhibited by Cdk1. APC/C^{Cdh1} leads to stabilization of Aurora B. The complex is therefore indirectly necessary to localize the CPC correctly to the spindle midzone [46]. If APC/C^{Cdh1} is depleted in cells, the CPC prematurely localizes to the equatorial cortex, resulting in a weak anaphase spindle.

CPC involvement in cytokinesis in mammals

The Centralspindlin complex is essential for the formation of microtubule bundle structures and the equatorial recruitment of factors critical for cytokinesis [47]. It is a tetrameric complex that consists of a dimer of the kinesin 6 motor protein MKLP1, bound to a dimer of the Rho family GTPase-activating protein (GAP) CyK4, also called MgcRacGAP. Markedly, Centralspindlin is homologous to the previously described zen-4 / cyk-4 complex in *C. elegans.* Centralspindlin localizes to the centre of the central spindle (See figure 8), where it promotes central spindle microtubule bundling and recruits regulators of abscission, by forming clusters at the central spindle [48]. As in *C. elegans,* both Centralspindlin subunits depend on each other for localization. Notably, Centralspindlin can be phosphorylated by Aurora B, which is necessary for proper Centralspindlin localization. Two phosphorylation sites of MKLP1 are important, S708 and S710, because these sites affect the binding of the phosphoserine- / phosphothreonine binding protein 14-3-3 [49]. S710 seems to be phosphorylated by a constitutively active kinase. If phosphorylated, 14-3-3 can bind to MKLP1, preventing it from clustering. S708 is phosphorylated by Aurora B at the central spindle. This phosphorylation site lies within the MKLP1 motif to bind 14-3-3, inhibiting its binding by decreasing the S710 monophosphorylated form of MKLP1. So CPC localization at the central spindle is required for the stable clustering of Centralspindlin in that area, to properly recruit abscission regulators.

The NoCut pathway has been described for yeast in chapter 2. This pathway is conserved in eukaryotes. In yeast, the CPC can interact with chromatin to activate NoCut during cytokinesis [30]. However, it was unknown how this interaction is established

and what players are involved. But recently, work in mammals showed that charged multivesicular body (MVB) protein 4C (CHMP4C) is involved in chromatin binding at the midbody [50]. CHMP4C is a subunit of the endosomal sorting complex required for transport III (ESCRT-III), which is known to play a conserved role in abscission. Interestingly, if CHMP4C is depleted in cells, midbodies are resolved quicker than in a wildtype situation, but if it is overexpressed cytokinesis is inhibited. Using a yeast two hybrid screening, it was discovered that CHMP4C can interact with Borealin [50]. While bound to the CPC, CHMP4C is phosphorylated by Aurora B on S210 that is placed in a specific insertion (INS) that contains multiple serine and threonine residues at residues 201 - 217. S210 is only conserved in mammals. Interestingly, phosphorylation of the INS is required for midbody localization of CHMP4C. Since CHMP4 proteins were first discovered as chromatin-modifiers that associate with chromatin, CHMP4C could be the key for CPC activation of an abscission delay in respons to the presence of chromatin at the midbody. Importantly, this is likely because if S210 is mutated in cells, so that it cannot be phosphorylated anymore, abscission is not delayed, even if chromatin bridges are present at the midbody. Accumulation of phosphorylated histone H2AX is detected in these cells, which reveals accumulation of genetic damage. So CHMP4C probably is the key to the activation of the CPC in cells containing chromatin bridges at the midbody, this is a process specific for mammals.

Sumoylation of the yeast-homologues of Survivin and INCENP is important for stable CPC localization at the central spindle (see chapter 2). But remarkably, in mammals, sumoylation is found for Borealin and Aurora B [26, 51]. Borealin is sumoylated by SUMO2/3, that are found at the centromeres and chromatin during prometaphase and metaphase [52]. Borealin is sumoylated at the centromere before anaphase and at the start of anaphase, loss of sumoylation is observed [51]. This is the same as the sumoylation pattern for Bir1 (Survivin) in yeast. If Borealin cannot be sumoylated, segregation defects are found. This is probably via an unknown player that can bind to the sumo-attachment of Borealin. Surprisingly, Borealin localization at the centromere is not dependent on sumoylation, also the same for Bir1 in yeast. This could mean that the lysine residues that can be sumoylated, are switched between Survivin and Borealin, like the Cdk1 phosphorylation residues described in chapter 2 and thus the function.

Aurora B can only be sumoylated if it is bound to INCENP [26]. Sumoylation of Aurora B leads is necessary for proper centromere localization of the CPC. Markedly, sumoylation has often been linked to ubiquitination. There are hints that sumoylation of Aurora B

facilitates the formation of the Cul3/Klhl9 and Cul3/Klhl13 complexes. Interestingly, each adaptor contains three SUMO-interacting motifs (SIM) and Cul3 has one SIM. This leads to a theory that sumoylation of Aurora B is necessary for promoting ubiquitination of the CPC, which has also been found to be involved in both centromere targeting and the release of the CPC from the centromere [43] (See figure 8).

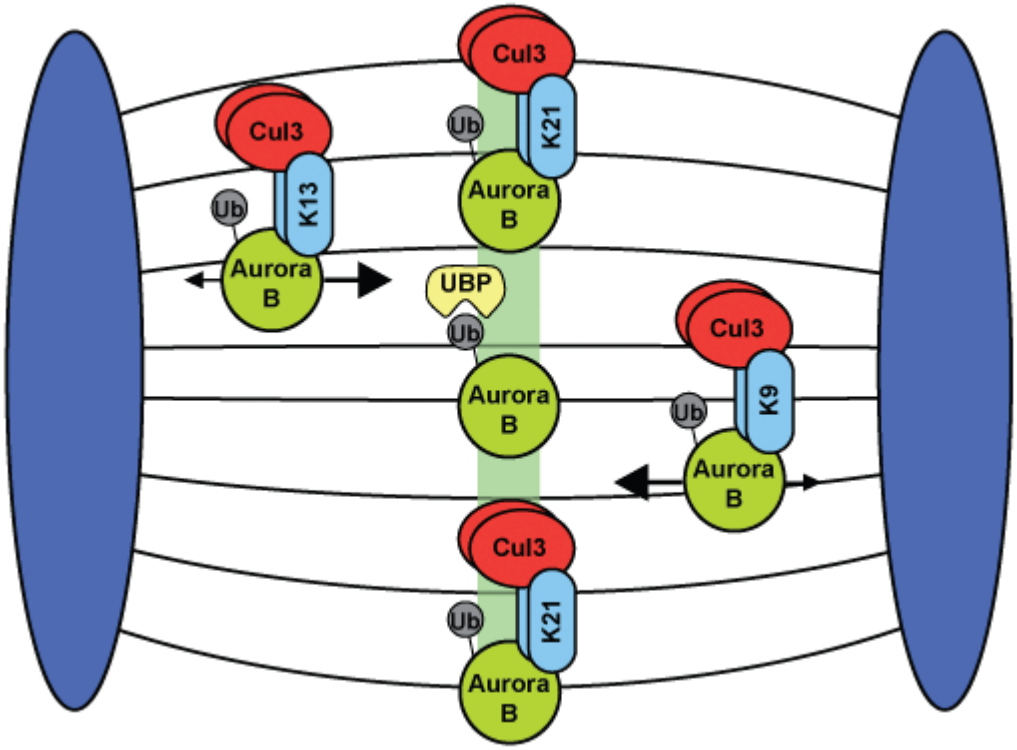


Figure 9. Model for how Aurora B midzone recruitment might be regulated by Cul3 complexes. Cul3/KLHL21 complexes localize to the spindle midzone (green zone), where they ubiquitinate Aurora B. Cul3/KLHL9 and Cul3/KLHL13 complexes are located in the cytoplasm, where they monoubiquitinate Aurora B. This leads to CPC localization at the midzone. At the midzone, a ubiquitin binding protein (UBP) that is unknown leads to CPC retention at the midzone. K21, KLHL21; K9, KLHL9; K13, KLHL13; Ub, ubiquitin. Adapted from [45].

4. CPC defects

After reading chapter 2 and 3, it is clear that the CPC is an important player in many processes during mitosis and cytokinesis. This is also why it is linked to many diseases including cancer. One of the most important events during mitosis is the translocation of the CPC during anaphase from the centromeres to the central spindle. If the CPC does not localize properly, this influences many players in the cell.

Mitotic checkpoint inactivation

The mitotic checkpoint delays anaphase onset until all sister kinetochores are attached to opposite poles [53]. This is done by inhibition of the APC/C. When all kinetochores are attached, tension is created at the kinetochores and this leads to progression into anaphase. The CPC is located at the centromeres and is able to detect this tension [69]. If the kinetochores are not properly attached, a lack of tension will lead to the activation of the mitotic checkpoint, together with Cdk1 activity, which is necessary for full mitotic checkpoint activation in both lower and higher eukaryotes [53]. If the CPC is not translocated to the central spindle during anaphase, this will partly activate the mitotic checkpoint because the tension at the kinetochores is lost [53]. That is why the translocation of the CPC at the start of anaphase is needed to completely inhibit mitotic checkpoint activation.

Spindle stability and elongation

During anaphase, the CPC is required for central spindle stability [12]. If the CPC is not translocated there, proteins that are involved in its stabilization and in abscission will not localize properly, like Slk19 and Ase1 in yeast [72], zen-4 in *C. elegans* [32] or MKLP1 in mammals [49]. So, this is conserved among eukaryotes. If the midzone is not stabilized by the CPC, this will lead to breakage of elongating spindle microtubules. Without spindle elongation, the chromosomes will not get separated far enough for a cleavage furrow to form, as found in the higher eukaryote *X. laevis* [66]. Notably, this is also found in chicken, where a conditional knock-out of INCENP leads to shorter spindles [74].

Axial shortening of chromosomes

As mentioned in the introduction, the CPC also has a role on axial shortening of segregating chromosomes [70]. This axial shortening happens in anaphase, after sister chromatid segregation. If Aurora B is not at the central spindle during anaphase or cannot be activated, this will influence the process of axial shortening in mammals. Axial shortening is important for the packing of all chromosomes in the daughter cell in one

nucleus with a smooth surface. If axial shortening did not happen, cells will enter G1 with severely lobed daughter nuclei [70]. This can lead to many effects on gene regulation because of the greater contact surface in the nuclei [71], contributing to many diseases.

Cytokinesis

Cytokinesis is the final step in cell division and mistakes in cytokinesis can lead to problems such as tetraploidy [54]. The process of cytokinesis is controlled by the CPC (see chapter 2 and 3), which regulates furrow ingression [55]. Interestingly, if there is something wrong with CPC localization or Aurora B activation in mammals, furrow regression will occur when there is a chromosome bridge at the midbody. The mammalian abscission machinery cannot cut the chromatin in the bridge, but Aurora B cannot delay the abscission. This will lead to tetraploid cells.

Chromosome bridges arise at the midbody as the result of segregation defects [75]. The presence of these chromosome bridges will lead to activation of Aurora B and this stabilizes MKLP1 at the midbody via phosphorylation. This shall suppress furrow ingression because the phosphorylated MKLP1 stabilizes the canal at the cleavage plane, providing time for the chromosome bridge to be resolved [55]. When there is no chromatin present at the midbody anymore, Aurora B will be inactivated, leading to abscission (See figure 10).

Tetraploidy has been shown to be involved in cancer formation [54]. If cytokinesis

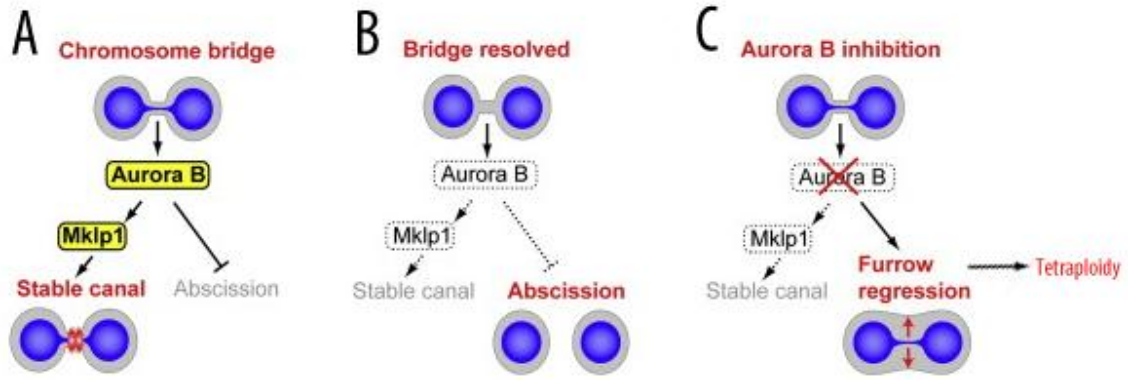


Figure 10. Model for the prevention of tetraploidy by Aurora B. Yellow indicates a phosphorylated an thus active state. A) The presence of a chromosome bridge leads to Aurora B activity at the (post)telophase stage. Aurora B phosphorylates MKLP1, leading to stabilization of the intercellular canal and a delay in abscission. B) If no chromosome bridge is present, there is no chromatin at the cleavage site so Aurora B is not phosphorylated, so abscission can happen. C) If Aurora B is inhibited, the presence of a chromosome bridge will lead to furrow regression. This will lead to tetraploid cells. Adapted from [55].

is blocked in p53 $^{-/-}$ mouse mammary epithelial cells, this will lead to tetraploidy. If these cells are then transplanted in nude mice, they will give rise to malignant mammary epithelial cancers, in which the frequency of chromosomal alterations is greatly enhanced. Tetraploid cells are likely to be a first onset before aneuploidy is detected. But since this experiment was done on p53 $^{-/-}$ cells, this could have had consequences on the formation of cancer. P53 is known to arrest cells in G1 when they are under stress, like the occurrence of tetraploidy [76]. Tetraploid cells show errors in spindle formation, because they contain the double amount of centrosomes. This can lead to the presence of chromosome bridges at the midbody. Without CPC, there will not be an abscission delay to resolve this and cells will develop aneuploidy, which can lead to cancer [55].

Nuclear pore complexes

Next to chromosome bridges, the presence of improperly formed Nuclear Pore Complexes (NPC's) are also found to lead to activation of Aurora B [56]. Within minutes after anaphase onset, membranes and nucleoporins (Nups) are recruited to the surface of the decondensing chromatin to enclose the DNA with a membrane. This

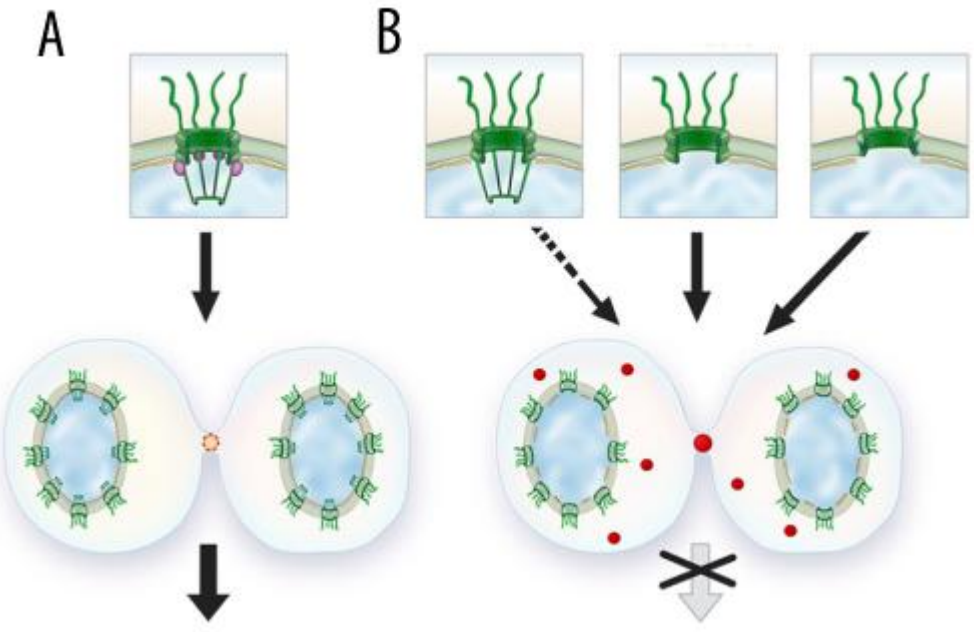


Figure 11. Model for the involvement of nuclear pore assembly in the abscission checkpoint. A) In normal conditions, NPC assembly happens fast after anaphase. Aurora B is inactivated (red circle) and abscission happens. B) If the assembly of the nuclear basket is disrupted by small or large depletions of nuclear basket components, Aurora B is phosphorylated throughout the cytoplasm and at the midbody (solid red circles). This leads to an abscission delay. Adapted from [56].

membrane contains NPC's. These are best known for their role as key regulator for molecular traffic between the nucleus and the cytoplasm, but they are also involved in many more processes around the nuclear envelope, like organization of the nuclear architecture [58]. Importantly, this can have great influences on gene expression [71]. They are also involved in the delivery of the right genetic information to the protein synthesis machinery. Interestingly, NPC's that are not functioning properly, can cause several diseases.

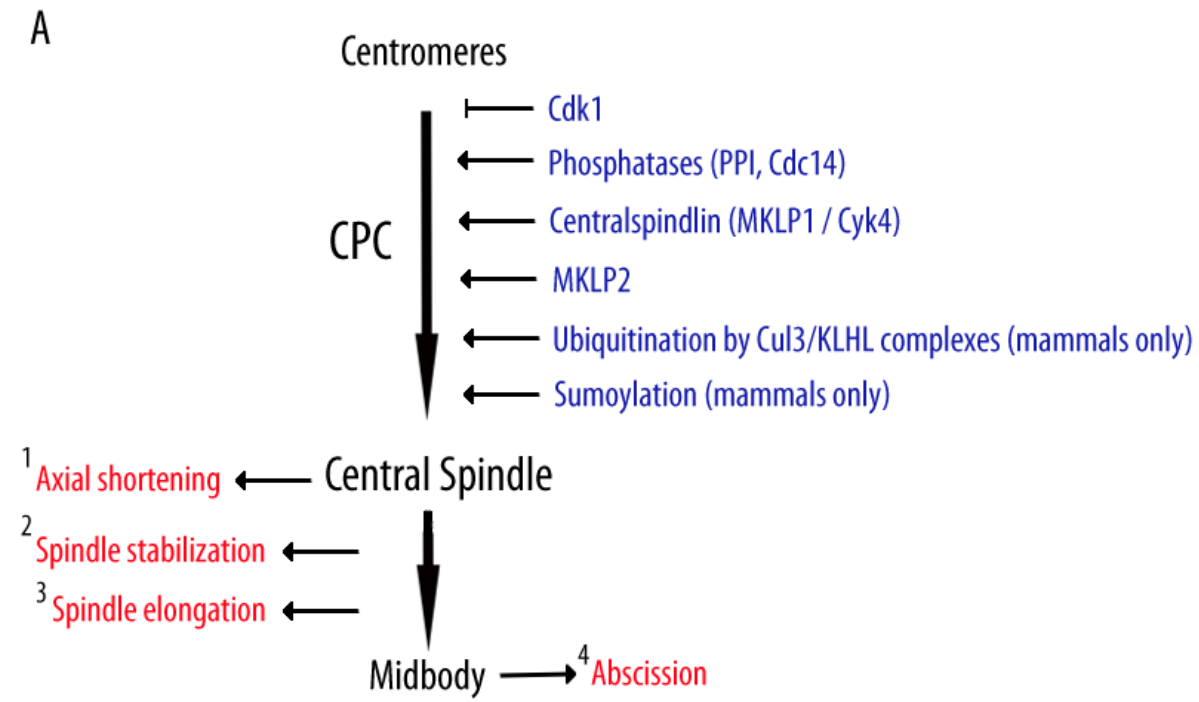
Nup153, a component of the NPC basket, seems to be required for timely cytokinesis, is because it is linked to the Aurora B mediated abscission checkpoint [56,57]. If Nup153 is depleted in HeLa cells, this will lead to activation of Aurora B at foci in the cytoplasm and at the midbody (See figure 11). The foci do not contain INCENP, which means that the CPC is not complete in this situation. The activation of Aurora B will lead to an abscission delay [56]. Depletion of another NPC component, Nup50, similarly triggers the abscission checkpoint. Noticeably, this shows that the CPC does not only control whether the chromosomes are segregated properly before progressing into cytokinesis, but it also seems to check whether the DNA is packaged in functional nuclei.

The CPC mainly controls proper chromosome segregation, preventing tetraploidy and the onset of cancer. But the CPC is also controlling more processes, like the proper formation of NPC's. Further research will maybe point to more processes that regulate activity of the CPC during mitosis or cytokinesis.

5. Conclusion and discussion

Regulation of the CPC is conserved

The CPC is an important player that is necessary for mitosis and cytokinesis (see figure 12). The complex is highly conserved among eukaryotes. One of the most important events during mitosis is the translocation of the CPC from the centromeres at metaphase, to the central spindle at anaphase. This translocation is important to inactivate the mitotic checkpoint and prepare the cell for cytokinesis. The basis of the regulation of this translocation is conserved in eukaryotes, but gets more complex in higher eukaryotes (see figures 4, 6



B

1) Severe lobbed daughter nuclei.
2) Breakage of spindle microtubules and abscission defects.
3) Abscission through chromosomes.
4) Abscission through chromosomes (lower eukaryotes) / Furrow regression (mammals).

Figure 12. Model for CPC localization and functioning in eukaryotes. A) During anaphase, the CPC is translocated from the centromeres to the central spindle. Factors involved are depicted in blue. The localization is required for 4 processes, depicted in red. CPC localization at the central spindle is important for axial shortening of chromosomes and for spindle stabilization and elongation. After anaphase, the area where the CPC is localized is called the midbody. The CPC regulates furrow ingression, leading to abscission. If the CPC is mislocalized or cannot be activated, problems arise with mitosis and cytokinesis. These problems are described in B).

and 8). This is probably because of the importance of CPC localization for the cell, leading to the appearance of different control systems during evolution.

An example of a basically conserved process during cytokinesis is the NoCut checkpoint / Aurora B mediated abscission delay. The sensing mechanism for chromatin at the midbody is present in both lower and higher eukaryotes and will lead to a delay in abscission, to provide time for the cell to clear chromatin from the midbody. This prevents the cell from abscission through the DNA, leading to chromosomal breakage. From yeast to human, this process is conserved. But the difference is noticed when the CPC is knocked down or chemically made inactive; in yeast, the NoCut mechanism will not be active anymore. This will lead to abscission, even when there is chromatin at the midbody. But in mammals, abscission does not go through chromatin. The presence of chromatin at the midbody, without a functional CPC, leads to regression of the cleavage furrow, resulting in tetraploid cells. In both cases, the origin differs, but both can lead to the death of unicellular eukaryotes or cancer formation in multicellular eukaryotes.

Unknown elements for CPC regulation

Many aspects of the regulation of CPC localization and functioning are known. But the picture is not complete yet; precise mechanisms of many downstream factors are unknown and it could be possible that even more regulators can be found.

A new development is the discovery that sumoylation plays a role in CPC localization and functioning (see chapter 3), but the exact mechanism and its role remains unknown. There are hints that sumoylation of Aurora B has an effect on the formation of ubiquitination complexes that are involved in the ubiquitination of Aurora B, contributing to its midzone localization. More research is needed to confirm or disprove the role of sumoylation for proper localization of the CPC, but it is an example of a mechanism who's contribution was unknown until recently, but knowledge of it can greatly contribute to find out where things go wrong in CPC functioning or localization, leading to disease.

The importance of the CPC

A very important player involved in CPC localization and function in lower eukaryotes is cdc14. One of the processes it is involved in, is the localization of the CPC at the central spindle during anaphase. Lower eukaryotes have only one cdc14 homologue, but higher eukaryotes have two or even three, as found in many mammals [24]. If Cdc14 is knocked down in yeast or *C. elegans*, the CPC remains on the centromeres. But surprisingly, knocking down any of the Cdc14

homologues in human cell lines does not show such a strong effect. This is probably because the different Cdc14 proteins can act redundantly. This is seen for more proteins that are involved in proper CPC localization: multiple proteins allow the cell to achieve fine control over the CPC. This shows how important the complex is for cell division.

Another example where multiple proteins contribute to the same process, is the role of the kinases Cdk1 and Aurora B during mitosis. Both can phosphorylated CPC components [15]. The reason why more than one kinase regulates the same substrate is again to fine-tune the spatiotemporal control over the CPC. Both kinases are regulated differently, which is consistent with this idea. So the CPC provides a surveillance mechanism to prevent complete mitotic failure if one component is defect.

Cells that have a malfunctioning CPC, can show many defects. If the CPC does not localize properly at the central spindle during anaphase, this can lead to partial activation of the mitotic checkpoint [53]. Since Cdk1 activity is required for the checkpoint to fully work, this will have minor effects on the cell. The real problem arises later, during cytokinesis. The CPC is required for proper abscission by regulating the abscission checkpoint. If the chromosomes have not separated correctly or no proper NPC's are formed during anaphase, abscission will be delayed to provide time to correct errors [55, 56]. But if the CPC is not correctly localized or it cannot be activated, this will lead to tetraploidy or cells entering G1 with incomplete NPC's. Tetraploidy is known to be involved in cancer onset [54], while malfunctioning of NPC's can effect gene expression or protein synthesis [58]. This can lead to many diseases. Tetraploidy can be a big threat, since estimates are that in around 1% of somatic dividing cells, segregation defects occur that lead to the presence of chromosome bridges at the midbody [59]. If this 1% is not corrected due to defects in the CPC, these cells will become tetraploid and therefore possible cancer-precursors. Luckily, CPC functioning is rarely affected, as for example by mutations of Aurora B [78]. So far, research on this has been done in $p53^{-/-}$ cells, but the chance that a defective CPC will lead to cancer is much smaller in the presence of p53, since it can arrest the cell cycle if the cells are under high stress [76].

It could be possible that the CPC controls many more aspects of cell division. This will even make it a more important factor for the cell. Further research will shed light on this.

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