Nucleosome-Dependent Pathways That Control Mitotic Progression

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The majority of eukaryotic chromosomal DNA exists in the form of nucleosomes, where ~147 bp DNA wraps around histone hetero-octamers, composed of histone H3, H4, H2A, and H2B. Despite their obvious importance in DNA compaction and accessibility, studying their specific roles, such as regulation of mitotic progression, in a physiological environment is associated with critical caveats because of their major contributions in transcriptional control. Through establishing a method to deplete endogenous histones H3 and H4 from frog egg extracts and complementing their functions using recombinant nucleosome arrays, we are now able to analyze their roles in mitotic progression without affecting overall transcriptomic profiles. Here we summarize advancements learned from this system, illustrating that microtubule and nuclear envelope assembly can be regulated by two major nucleosome-bound protein complexes, RCC1–Ran and the chromosomal passenger complex (CPC) containing the mitotic protein kinase Aurora B. We also discuss roles of the CPC on the proteomic composition of mitotic chromatin. The CPC promotes dissociation of a variety of nucleosome remodelers and DNA repair pathway proteins, suggesting its role in suppressing DNA processing activities on mitotic chromosomes. We speculate that this suppression particularly on chromosomes under microtubule tension may be important to preserve genome integrity.

Major characteristics that define eukaryotes are the intracellular membrane system, which forms the nucleus to encapsulate genomic DNA, and cell division through mitosis, where replicated genomic DNA is organized into topologically distinct multiple chromosomal threads, which are distributed equally to two daughter cells. DNA replication and chromosome segregation in eukaryotes rely on formation of microscopic-scale architectures around chromosomes, the nuclear envelope, and spindle microtubules, respectively. Pioneering studies in *Xenopus* eggs and their extracts have shown that exposure of DNA to cytoplasm can trigger formation of the nuclear envelope in interphase and spindle microtubule assembly in M phase, in a DNA sequence-independent manner (Forbes et al. 1983; Karsenti et al. 1984; Heald et al. 1996). Here we will review our current understanding of how these DNA-induced processes are controlled by nucleosomes, the fundamental unit that folds genomic DNAs in eukaryotes.

A SYSTEM TO DIRECTLY MANIPULATE HISTONES H3 AND H4 IN *XENOPUS* EGG EXTRACTS

DNA added to *Xenopus* egg extracts is rapidly chromatinized with the large excess of maternally stored histones in egg cytoplasm. To study the roles of nucleosomes, we therefore established a method to deplete histones H3 and H4 from egg extracts and complement them with recombinant proteins (Fig. 1; Zierhut et al. 2014). We used monoclonal antibodies against histone H4 acetylated at Lys12 (H4K12ac) to deplete the H3–H4 complex, and

depleted extracts were complemented with synthetic DNA preassembled with nucleosomes (Fig. 1A,B; Zierhut et al. 2014). As the great majority of histone H4 in eggs is acetylated at Lys5 and Lys12 and forms a complex with H3 (Nicklay et al. 2009), monoclonal antibodies against H4K12ac were able to deplete >90% of histones H3 and H4. Under this experimental condition, reconstituted nucleosome arrays, coupled to magnetic beads, supported spindle assembly in M phase extracts and nuclear envelope formation with nuclear import activity in interphase extracts (Fig. 1C; Zierhut et al. 2014). Naked DNA beads in interphase Δ H3–H4 extracts were able to recruit membranes but were defective in recruiting the nuclear pore complex (NPC). Naked DNA-beads also failed to induce spindle microtubule assembly in M phase Δ H3–H4 extracts. These experiments directly showed the importance of nucleosomes in spindle formation and NPC. An independent study in mouse oocyte, where de novo formation of nucleosomes on sperm nuclei can be inhibited by depleting H3.3 or HIRA, also showed the importance of nucleosome formation for NPC assembly (Inoue and Zhang 2014).

MASS SPECTROMETRY ANALYSIS OF NUCLEOSOME-DEPENDENT BINDING PROTEINS

Δ H3–H4 egg extracts offered a unique opportunity to compare protein constituents that assemble on nucleosomes and nucleosome-free DNA under physiological conditions using quantitative mass spectrometry (MS)

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Figure 1. *Xenopus* egg extract system to determine functions of the nucleosome and specific histone modifications. (A) End-biotinylated tandem 19-mer arrays of Widom's 601 sequences were coupled to streptavidin-coated magnetic beads, with or without nucleosome assembly by salt dialysis using recombinant core histones. (B) *Xenopus* egg extract conditions. (C) Spindle formation in M phase and nuclear pore complex (NPC) assembly in interphase on DNA/chromatin beads in the absence of histones H3/H4 were visualized by rhodamine-tubulin and antibodies against the NPC component ELYS. Scale bars, 3 μ m. (Modified from Zierhut et al. 2014; Zierhut and Funabiki 2015.)

analysis. In both M phase and interphase extracts, the list of most abundant nucleosome-dependent binders are similar; the linker histone H1M, the FACT complex, Ran-RCC1, DDB1, and the chromosomal passenger complex (CPC) (Zierhut et al. 2014; C Jenness and H Funabiki, unpubl.). Unlike the linker histone and potential nucleosome regulators, FACT and DDB1 (Belotserkovskaya and Reinberg 2004; He et al. 2006; Winkler and Luger 2011), RCC1-Ran and the CPC play critical roles beyond structural organization of mitotic chromatin, as discussed below.

Intriguingly, SMC-family protein complexes, condensin and cohesin, show a mild preference for nucleosome-free DNA over nucleosomal DNA (Zierhut et al. 2014; Hirano 2016; C Jenness and H Funabiki, unpubl.). This may reflect the evolutionary conservation of SMC proteins in prokaryotes, which lack nucleosomes. Similarly, the MCM complex, the AAA family ATPase required for DNA replication initiation, effectively binds to nucleosomal and nucleosome-free DNA (Zierhut et al. 2014; C Jenness and H Funabiki, unpubl.), although it is not clear at present if this reflects functional, topological, binding. It seems that these protein complexes evolved to acquire additional modules/factors to interact and deal with nucleosomal DNA, such as FACT (Kinoshita et al. 2015; Shintomi et al. 2015; Hirano 2016; Kurat et al. 2017). In contrast, major critical roles in eukaryote-specific events, nuclear envelope formation, and spindle microtubule as-

sembly are performed by nucleosome-dependent chromatin proteins, RCC1-Ran and the CPC.

THE RCC1-Ran PATHWAY

The GTP-bound form of the small GTPase Ran controls a number of processes by modulating karyopherin family proteins, such as importins and exportins (Cavazza and Vernos 2015). RanGTP disrupts the interaction between importin β and importin α , which recognize a variety of nuclear proteins that contain classical nuclear localization signals (Fig. 2, steps a and b). In the context of nuclear envelope formation, RanGTP liberates components of NPCs from importins (Walther et al. 2003), whereas in M phase, it releases proteins that promote spindle assembly (Cavazza and Vernos 2015). One of many mechanisms involve TPX2, which promotes microtubule nucleation by tethering tubulin dimers and by activating Aurora A and the γ -tubulin ring complex (γ TuRC) (Fig. 2, step c; Groen et al. 2004; Tsai and Zheng 2005; Pinyol et al. 2013; Roostalu et al. 2015; Scrofani et al. 2015; Zhang et al. 2017).

GDP bound to Ran is exchanged with GTP with the help of RCC1 associated with nucleosomes (Fig. 2, step a; Nemerlut et al. 2001; Makde et al. 2010). Although RCC1 can directly interact with DNA, an additional interaction with the acidic patch of H2A-H2B in the nucleosome is critical

Figure 2. Schematics of chromatin-induced microtubule nucleation. In the cytoplasm distal to chromatin, RanGDP forms a trimeric complex with RCC1 and RanBP1 and is targeted to chromatin via RCC1-nucleosome interaction (step a). On chromatin, GDP is replaced with GTP by RCC1, whose activity is stimulated by nucleosome binding. RanGTP releases a number of proteins, such as TPX2 and other proteins promoting microtubule assembly, from Importins (step b). TPX2 binds and stabilizes interfaces between adjacent α/β tubulin dimers to promote microtubule nucleation (step c). In the cytoplasm distal to chromosomes, the chromosomal passenger complex (CPC; composed of Aurora B, INCENP, Borealin, and Survivin) is kept in hypophosphorylated status by PP2A and is bound to NPM2 oligomers. Upon binding to chromatin through Survivin-H3T3ph interaction and the SAH-chromatin interaction, Aurora B and INCENP become phosphorylated, leading to Aurora B activation (step d). Activated Aurora B phosphorylates Op18, which sequesters tubulin dimers. Aurora B-dependent Op18 phosphorylation releases tubulins to promote microtubule assembly (step e). Hypophosphorylated MCAK and Op18 interact with microtubule ends and stimulate depolymerization (step f). The CPC interacts with microtubules through the SAH domain of INCENP. Upon interaction with microtubules, Aurora B is activated (step g). MCAK and Op18 are phosphorylated by Aurora B (step h). Phosphorylated MCAK and Op18 are inactivated, leading to microtubule polymerization (step i). The CPC can directly bind to microtubules and become activated (step j). (Modified from Zierhut and Funabiki 2015; Wheelock et al. 2017.)

(Makde et al. 2010). Ran has been shown to directly interact with histones H3–H4, but this interaction is very weak, and accordingly, our quantitative MS analysis shows that equivalent amount of Ran and RCC1 exist on mitotic chromatin (Bilbao-Cortés et al. 2002; Zierhut et al. 2014; Jenness et al. 2018), even though the concentration of Ran (5 μM) in egg cytoplasm is more than 30-fold higher than that of RCC1 (150 nM) (Wuhr et al. 2015).

A bead coupled directly with RCC1 proteins can promote bipolar spindle assembly, suggesting that local enrichment of RCC1 can act as an effective trigger for microtubule nucleation and subsequent spindle assembly (Halpin et al. 2011). However, spindles are shorter and spindle microtubule density is lower on an RCC1 bead than on a chromatin bead. This may reflect the involvement of RCC1-independent regulation (e.g., through activation of the CPC, see below) or the presence of additional

mechanisms by which chromatin activates RCC1. Indeed, the catalytic activity of RCC1 is stimulated by nucleosome interaction (Nemergut et al. 2001). Furthermore, RanBP1, whose concentration in eggs is 2 μM , forms a trimeric complex with Ran and RCC1 to inhibit the catalytic activity of RCC1 (Zhang et al. 2014). Despite its abundance, no RanBP1 was detected on purified chromatin (Zierhut et al. 2014; Jenness et al. 2018), indicating that binding of RCC1–RanGDP–RanBP1 to chromatin releases RanBP1, and thus licenses RanGDP for conversion to RanGTP. It would be interesting to know if RanBP1 is defective in binding to RCC1 that is coupled to beads.

Adding a Ran mutant defective in GTP hydrolysis to egg extracts is sufficient to drive microtubule nucleation and assembly, highlighting the importance of local enrichment of RanGTP (Cavazza and Vernos 2015). However, the dominant negative RanT24N mutant, which inhibits

RCC1's nucleotide exchange activity, inhibits spindle formation in commonly used *Xenopus laevis* egg extracts, but not in egg extracts of *Xenopus tropicalis* (Helmke and Heald 2014). This was attributed to the higher concentration of TPX2 in *X. tropicalis* eggs than in *X. laevis* eggs, which was itself thought to cause *X. tropicalis* spindles to be shorter. Therefore, there must be an additional mechanism that restricts microtubule assembly on chromatin. As described below, our laboratory has shown that the CPC, which directly interacts with nucleosomes (Kelly et al. 2010), also contributes to the mechanism by which chromatin locally restricts microtubule assembly.

At the transition from M phase to interphase, RanGTP acquires another function and drives NPC formation in the reassembling nuclear envelope (Fig. 3). RanGTP promotes NPC assembly through liberating components of the NPC, such as those of the NUP107 complex, and excess RanGTP promotes NPC formation in chromatin-free membrane structures, annulate lamellae (Walther et al. 2003). However, RCC1 does not appear to be the sole essential protein that needs to be targeted to chromatin. We showed that ELYS, which links to the NUP107 complex, is recruited to chromatin through directly interacting with histone H2A and H2B and supports nucleosome-dependent NPC formation (Zierhut et al. 2014). Thus, for both spindle assembly and NPC assembly, RCC1 and additional nucleosome-binding proteins are required.

THE CPC IN SPINDLE ASSEMBLY

Before the realization of RanGTP's importance in spindle assembly, Karsenti and colleagues showed that phosphorylation of Op18 (also known as Stathmin), a regulator of microtubule dynamics, is induced by chromatin in M phase *Xenopus* egg extracts (Andersen et al. 1997). Op18 promotes microtubule destabilization by two distinct mechanisms: sequestration of tubulin dimers to reduce the effective concentration of tubulins and binding to curved microtubule protofilaments (Fig. 2, steps e and f; Cassimeris 2002; Gupta et al. 2013). This Op18-mediated destabilization is suppressed by phosphorylation (Cassimeris 2002). Although two of the mitotic phosphorylation sites are Cdk1 targets, Aurora B mediates chromatin-induced phosphorylation at a third site (Ser16 in *Xenopus*) (Gadea and Ruderman 2006; Kelly et al. 2007).

Aurora B is the kinase subunit of the CPC, which also contains INCENP, Borealin (also known as Dasra, CDCA8), and Survivin (Fig. 2; Carmena et al. 2012). CPC depletion from *Xenopus* egg extracts or addition of the Aurora B inhibitor ZM447439 inhibits spindle formation (Sampath et al. 2004; Gadea and Ruderman 2005). In addition to Op18, Aurora B also phosphorylates the major microtubule depolymerizing enzyme, MCAK (also known as XKCM1, KIF2C), and suppresses its microtubule depolymerizing activity and chromosome arm local-

Figure 3. Mechanism of nucleosome-dependent nuclear envelope formation and interference by microtubules. (Left) In M phase, the RanGTP pathway and the chromosomal passenger complex (CPC) pathways suppress microtubule depolymerization, whereas DNA-binding protein DPPA2 promotes it. (Right) At the transition into interphase, H3T3 is dephosphorylated, resulting in suppression of Aurora B on chromatin. Microtubules inhibit functional nuclear envelope assembly, but DPPA2-mediated microtubule disassembly facilitates nuclear envelope formation. Nuclear membrane can associate nucleosome-free DNA, whereas NPC formation is supported by at least two nucleosome-dependent mechanisms. First, RanGTP liberates building blocks of nuclear pore complex (NPC) microtubule assembly, such as subunits of the NPC107 complex. Second, specifically during interphase, ELYS, which interacts with the NPC107 complex, is recruited to nucleosomes. (Modified from Zierhut and Funabiki 2015; Wheelock et al. 2017.)

ization (Andrews et al. 2004; Lan et al. 2004; Ohi et al. 2004; Zhang et al. 2007) (Fig. 2, step i; also see Table 2).

The kinase activity of Aurora B is regulated in multiple steps. By itself, Aurora B is a poor protein kinase, but binding of the carboxy-terminal IN-Box module of INCENP allosterically stimulates Aurora B (Bishop and Schumacher 2002; Honda et al. 2003; Sessa et al. 2005). However, binding of INCENP to Aurora B is insufficient to promote effective phosphorylation in egg extracts, where the majority of Aurora B exists in the complex with INCENP along with Dasra A (egg form of Borealin family protein) and Survivin (Bolton et al. 2002; Sampath et al. 2004; Kelly et al. 2007). For full activation of Aurora B, phosphorylation at its catalytic loop (T-loop) and IN-Box must be phosphorylated by Aurora B, but those sites are generally not phosphorylated in egg extracts because of active type 2A (and also likely type 1) phosphatases (Kelly et al. 2007). Nucleoplasmin/nucleophosmin (NPM2) proteins also interact with unphosphorylated, cytoplasmic CPC, although its functional significance remains to be tested (Hanley et al. 2017). However, Aurora B autophosphorylation (a hallmark of kinase activation) can be induced by chromatin or taxol (a microtubule stabilizing drug) in M phase egg extracts (Fig. 2, steps d and j; Kelly et al. 2010; Tseng et al. 2010). Because adding antibodies that cluster the CPC can also promote Aurora B activation, we have proposed that local enrichment of the CPC can activate Aurora B (Kelly et al. 2007). Thus, Aurora B activity can be coupled to intracellular localization.

How can the CPC be recruited to chromatin? We and others showed that Survivin, a CPC subunit important for chromatin targeting (Carvalho et al. 2003; Lens et al. 2003; Yue et al. 2008), directly interacts with the H3 tail when phosphorylated at threonine 3 (H3T3ph) (Fig. 2, step d; Kelly et al. 2010; Wang et al. 2010; Yamagishi et al. 2010). The H3 amino-terminal tail binds to a cleft of the BIR domain of Survivin, in a coordination similar to how the BIR domain of XIAP interacts with SMAC (DIABLO) and caspase-9 (Kelly et al. 2010; Jeyaprakash et al. 2011; Du et al. 2012; Niedzialkowska et al. 2012). In egg extracts, we showed that H3T3ph is critical for chromatin-induced activation of Aurora B, as nucleosomes with a phosphorylation-defective H3T3A mutant fail to activate Aurora B, whereas nucleosomes with a phosphomimetic H3T3E mutant bypass the requirement for the H3T3 kinase Haspin (Kelly et al. 2010; Zierhut et al. 2014). Surprisingly, although Aurora B, INCENP, and Dasra A bind to interphase chromatin at a level comparable to mitotic chromatin (Jenness et al. 2018), activated Aurora B can be seen only on mitotic chromatin and not on interphase chromatin (C Jenness and H Funabiki, unpubl.). This interphase chromatin association may be supported by the single α helix (SAH) domain of INCENP, which also contributes to chromatin binding (Wheelock et al. 2017), but this is not sufficient to activate Aurora B. Thus, beyond the chromatin enrichment, the M phase-specific H3T3ph-Survivin interaction activates Aurora B by an additional mechanism, perhaps involving structural reorganization of the CPC on chromatin related to dimerization capacity of Borealin (Bourhis et al. 2009; Bekier et al. 2015).

The requirement for chromatin-induced mechanisms to activate Aurora B for spindle assembly in *Xenopus* egg extracts can be bypassed by artificial activation of Aurora B through antibody-mediated clustering (Kelly et al. 2007). Adding the CPC clustering anti-INCENP antibody also facilitates assembly of microtubules that are not attached to chromatin. However, Aurora B activation is only sufficient to promote spindle assembly if INCENP interacts with microtubules through the SAH domain (Fig. 2, steps g and j; Tseng et al. 2010). Therefore, the SAH-microtubule interaction may be required for optimal substrate phosphorylation, whereby the microtubule-binding capacity of the CPC facilitates phosphorylation of substrates on microtubules (Noujaim et al. 2014). It may be worth noting that Aurora B-dependent phosphorylation often weakens microtubule-binding activity of substrates (Cheeseman et al. 2006; Wang et al. 2007; Gestaut et al. 2008; Alushin et al. 2010), and this is related to the fact that Aurora B is a basophilic kinase (Alexander et al. 2011), whereas basic amino acids are often used to recognize negatively charged E hooks of tubulins. Thus, it is possible that the Aurora B substrate-microtubule interaction may limit the substrate accessibility by Aurora B, necessitating the CPC-microtubule interaction for effective Aurora B substrate recognition.

A microtubule-targeted fluorescence resonance energy transfer (FRET)-based sensor revealed that Aurora B-dependent phosphorylation on microtubule-bound substrates can be broadly observed across the mitotic spindle in human tissue culture cells (Tseng et al. 2010; Tan and Kapoor 2011). Before the metaphase to anaphase transition, higher phosphorylation levels occur near bulk chromosomes than at regions close to poles. However, on metaphase spindles, the gradient of Aurora B-dependent phosphorylation is not obvious unless Aurora B activity is partially inhibited (Tan and Kapoor 2011; Wang et al. 2011). In contrast, during anaphase, a clear gradient of Aurora B-dependent phosphorylation was seen, centering on the spindle midzone, where the CPC is relocated from chromosomes at the metaphase to anaphase transition (Fuller et al. 2008; Tan and Kapoor 2011). This difference likely reflects the more stable Aurora B enrichment at the anaphase spindle midzone than in preanaphase mitotic stages when the CPC preferentially enriches on the inner centromere over microtubules.

The weakly tuned microtubule-binding property of INCENP during preanaphase is functionally important. Robust microtubule binding by the INCENP SAH domain requires the adjacent phospho-regulatory domain (PRD) (Wheelock et al. 2017). CDK-dependent phosphorylation of the PRD suppresses, but not completely inhibits, microtubule binding. Strikingly, although deleting the SAH domain prevents the CPC from supporting spindle assembly, replacing the SAH domain with alternative microtubule-binding domain from PRC1 or Tau promotes spontaneous microtubule assembly in the absence of chromatin (Tseng et al. 2010). We therefore speculate that feedback activation of Aurora B by assembled microtubules causes chromatin-independent microtubule assem-

bly, explaining why the SAH-microtubule interaction must be tuned by Cdk1-dependent phosphorylation.

In HeLa cells, where spindle assembly does not require Aurora B activity, this tuned microtubule-binding capacity of INCENP is critical for activation and suppression of the spindle assembly checkpoint (SAC) (Wheelock et al. 2017). We have shown that SAH-microtubule binding is important to support SAC activation in HeLa cells upon taxol treatment, likely through promoting phosphorylation of kinetochore proteins, such as Hec1, and through destabilizing kinetochore-microtubule interactions. When the microtubule-SAH interaction is enhanced by mutating CDK-dependent phosphorylation sites in the PRD to alanines, cells show a difficulty in silencing the SAC even after metaphase plate formation in otherwise untreated cells. Similarly, the microtubule-binding capacity of INCENP, which is under the negative control of CDK1-dependent phosphorylation but not centromere targeting of the CPC, is critical for essential functions of the CPC in budding yeast (Campbell and Desai 2013; Fink et al. 2017). In addition, it was proposed that the interaction between the microtubule plus-end tracking protein EB1 and Aurora B mediates microtubule-dependent Aurora B activation and CPC recruitment to the centromere (Banerjee et al. 2014), and this interaction may also contribute to the function of Aurora B in the SAC.

Despite the capacity of chromatin to stimulate RanGTP production and Aurora B activation, which are both thought to stabilize microtubules, no spatial difference in microtubule stability (and instability) can be found across the metaphase spindle in *Xenopus* egg extracts (Brugués et al. 2012). Based on a series of experiments and mathematical simulations, Needleman and colleagues have proposed that the spindle size and microtubule length distribution can be explained by spatial regulation of microtubule nucleation but not by that of microtubule stabilization. Incorporation of new microtubules is preferred at the center of the spindle, and microtubules are shorter at the poles. This can be explained by enhanced microtubule nucleation at the spindle equator and the transport of the microtubules by motor proteins (Brugués et al. 2012). A chromatin-centered gradient of microtubule nucleation activity can be generated by RanGTP-mediated activation of spindle assembly factors (SAFs) that interact with microtubules (Carazo-Salas et al. 2001; Groen et al. 2004; Cavazza and Vernos 2015; Oh et al. 2016). The Aurora B pathway may also contribute to local microtubule nucleation around chromatin by inhibiting Op18 and MCAK, which can prevent the formation of elongation-competent microtubule plus ends (Wieczorek et al. 2015). Consistent with this idea, it was reported that the Op18-tubulin interaction is suppressed near mitotic chromatin (Niethammer et al. 2004), and that depletion of Haspin, required for chromatin-induced Aurora B activity, decreases spindle size (Kelly et al. 2010). However, unhydrolyzable RanGTP can promote microtubule nucleation in CPC-depleted extracts (Sampath et al. 2004), suggesting that the CPC is not required in the presence of excess RanGTP. It is likely that RanGTP and Aurora B both act in a cooperative manner to recognize chromatin and promote microtubule

assembly, although their importance in providing spatial information of chromatin can be redundant depending on the system. This may explain why depletion of the CPC subunits or Aurora B inactivation in somatic cells and mammalian oocytes may cause chromosome misalignment and spindle morphology defect but usually does not inhibit spindle assembly (Zierhut and Funabiki 2015), whereas the CPC supports kinetochore-induced microtubule nucleation in somatic cells (Tulu et al. 2006) and is absolutely essential for spindle assembly in *Xenopus* egg extracts (Sampath et al. 2004).

FUNCTIONAL COORDINATION OF MICROTUBULE DYNAMICS AND NUCLEAR ENVELOPE FORMATION BY CHROMATIN FACTORS

Although the RCC1-Ran pathway promotes both spindle assembly and nuclear envelope formation (Cavazza and Vernos 2015), the CPC acts to promote spindle assembly but inhibits nuclear envelope formation (Fig. 3). The CPC, which is enriched on centromeres during the preanaphase stages of mitosis, is relocalized to the spindle midzone in anaphase. Preventing this process by either inhibition of the p97-Cdc48 pathway or excessive activity of Haspin, which retains H3T3ph and thus Aurora B activity on chromosomes, delays chromosome decompaction and nuclear envelope formation (Ramadan et al. 2007; Kelly et al. 2010). One of the mechanisms by which Aurora B prevents proper nuclear envelope formation is through microtubules. In *Xenopus* egg extracts, we have shown that a DNA-bound microtubule-destabilizing protein, Dppa2, is required for formation of a sperm pronucleus of proper size and shape (Xue et al. 2013). This function is opposed by the CPC, suggesting that suppression of microtubule-chromosome interaction by chromatin recruitment of Dppa2, as well as concomitant CPC inhibition, is important for proper nuclear assembly. Although artificial microtubule stabilization with taxol recapitulated this effect, suppression of microtubule assembly by nocodazole also delayed kinetics of nuclear expansion, indicating that microtubules play both positive and negative roles in reformation of the nucleus after mitosis, highlighting the importance of spatiotemporal control of microtubule assembly (Xue et al. 2013). The carboxy-terminal microtubule-destabilizing domain of Dppa2 is not conserved in mammalian orthologs, and Dppa2 family proteins are not found in fish and birds, indicating that the role of xDppa2 in microtubule destabilization may not be evolutionarily conserved. However, the inhibitory function of microtubules in nuclear formation appears to be universal for eukaryotes undergoing open mitosis (Lu et al. 2011; Xue and Funabiki 2014).

ROLE OF THE CPC ON THE PROTEOMIC COMPOSITION OF CHROMATIN

Major chromatin substrates of Aurora B are the Ser10 and Ser28 residues of histone H3 (Hsu et al. 2000; Goto

et al. 2002), but their molecular functions are largely mysterious. In budding yeast, it was shown that H3S10ph promotes mitotic chromosome compaction through recruiting the histone H4 deacetylase Hst2 (Wilkins et al. 2014), but it is unclear if this process is conserved in vertebrates. In human cells, it was suggested that the serine/arginine-rich splicing factors (SRSFs), SRp20 (SFRS3), and ASF/SF2 (SFRS1), dissociate from mitotic chromosomes by Aurora B-dependent H310 phosphorylation (Loomis et al. 2009). At heterochromatin, H3S10ph, inhibits bind-

ing of chromodomain of HP1 to its adjacent heterochromatin associated modification, trimethylated Lys9 (H3K9me3) (Fischle et al. 2005; Hirota et al. 2005).

To verify these reported phenomena and to seek novel factors whose binding is influenced by Aurora B-dependent phosphorylation, we examined the impact of CPC depletion on the proteomic profile of chromatin-binding proteins in the presence or absence of H3K9me3 (Fig. 4; Tables 1–3; Jenness et al. 2018). Consistent with our previous findings, we found that HP1 exclusively interacts

Figure 4. Effect of the chromosomal passenger complex (CPC) depletion, H3K9me3, and cell cycle on the proteomic profile of chromatin. Chromatin beads with or without H3K9me3 were incubated with four different egg extract conditions (control Δmock extracts or ΔCPC extracts in M phase or in interphase) and isolated and then identity and quantity of bound proteins were determined by liquid chromatography–tandem mass chromatography (LC-MS/MS). (A) Based on the similarity of relative abundance across the varying conditions, proteins were clustered using hierarchical clustering algorithm. Relative abundance was expressed as a heatmap (low/black to high/yellow). (B) Clades of proteins exclusively enriched on chromatin in M phase, including a few showing CPC dependency. (C) Clades of proteins showing exclusive binding to H3K9me3-nucleosomes in ΔCPC extracts. (D) Clade of proteins showing reduced binding to M phase chromatin in a manner dependent on the CPC. (Modified from Jenness et al. 2018.)

Table 1. Most abundant M phase chromatin-associated proteins in a manner dependent on the chromosomal passenger complex (CPC)

Gene	Alt name	Complex	Δ CPC chromatin abundance (A.U.)	Δ mock chromatin abundance (A.U.)	Δ mock/ Δ CPC
CCNB2	Cyclin B2		0	1.8×10^8	—
CDCA8	Dasra A, Borealin	CPC	0	1.7×10^8	—
AURKB	Aurora B	CPC	0	1.3×10^8	—
ZFP161	ZBTB14, ZNF478		0	1.1×10^8	—
TPX2		HURP	3.0×10^7	1.1×10^8	3.8
ZMYM4	ZNF262		0	1.1×10^8	—
DLGAP5	HURP	HURP	1.7×10^7	1.0×10^8	—
PSMB8		Proteasome	0	8.7×10^7	—
INCENP		CPC	0	8.3×10^7	—
EML4	EMAP-4		0	6.2×10^7	—
EVC			0	6.1×10^7	—
TRIM2			0	6.0×10^7	—
RDBP	NELFE		0	5.9×10^7	—
FAM98B			0	5.7×10^7	—
KIFC1	HSET		0	4.6×10^7	—
NME2	NDP-kinase 2		0	3.8×10^7	—
BIRC5	Survivin	CPC	0	3.8×10^7	—
RPSA	Laminin Receptor 1		1.0×10^7	3.7×10^7	3.5
CCNB1	Cyclin B1		0	3.1×10^7	—
MAP4			0	3.0×10^7	—
EEF2	EF2		0	3.0×10^7	—
HMMR	RHAMM	RHAMM- γ TuRC-TPX2	0	3.0×10^7	—

Data are generated from Supplemental Table S1 in Jenness et al. 2018.

Abundance (arbitrary units) of proteins that associate with chromatin beads in M phase *Xenopus* egg extracts in a manner dependent on the CPC are shown. The top 22 most abundant proteins (except for tubulins, actins, mitochondrial proteins, and highly abundant glycolytic enzymes) showing at least threefold enrichment on chromatin in control Δ mock egg extracts over Δ CPC extracts are listed. Subunits of the CPC and the HURP complex are colored in red and blue, respectively. Other known microtubule-binding proteins are shown in purple.

with H3K9me3 particular in CPC-depleted (Δ CPC) extracts (Fig. 4C). Other known H3K9me3-binding proteins containing a chromodomain, Suv39h1, Suv39h2, and Suv420h2, also showed this pattern. In addition, Haspin

(GSG2), whose fission yeast homolog is known to interact with HP1 homolog Swi6 (Yamagishi et al. 2010), showed similar behavior, indicating the evolutionary conservation of HP1-Haspin interaction. In contrast, despite their high

Table 2. Proteins that dissociate from M phase chromatin in a manner dependent on the chromosomal passenger complex (CPC) (I)

Gene	Alt name	Complex	Δ CPC chromatin abundance (A.U.)	Δ mock chromatin abundance (A.U.)	Δ CPC/ Δ mock
CDCA7		CHIRRC	4.6×10^8	0	—
HELLS	LSH, SMARCA6	CHIRRC	4.6×10^8	2.0×10^7	22
CHD1			1.9×10^8	0	—
SMARCA5	SNF2H, ISWI	ASF/WICH/CHRAC/ RSF	1.9×10^8	5.5×10^7	3.3
EIF2B3			1.6×10^8	0	—
BAZ1B	WSTF	WICH	1.5×10^8	4.5×10^7	3.3
CCDC39			1.4×10^8	0	—
UBE3C			1.4×10^8	0	—
CUL9			1.3×10^8	0	—
PLCH2			1.1×10^8	0	—
CDH23	Cadherin-23		9.5×10^7	0	—
BAZ1A	ACF1	ASF/CHRAC	7.4×10^7	0	—
TRIP12			6.5×10^7	1.8×10^7	3.6
MDC1			6.3×10^7	1.8×10^7	3.5
ATAD2B			6.3×10^7	0	—
DAZAP2			6.0×10^7	0	—
TTN			5.6×10^7	0	—
AIM1	CRYBG1		5.3×10^7	0	—
PSMD8	RPN12	26S proteasome	5.2×10^7	1.1×10^7	4.8
KIF2C	MCAK, XKCM1		5.0×10^7	8.4×10^6	5.9
RCC2	TD-60		5.0×10^7	1.4×10^7	3.4
IGF2BP3			4.8×10^7	4.2×10^6	11
ATAD2			4.1×10^7	0	—

Data are generated from Supplemental Table S1 in Jenness et al. 2018.

Abundance (arbitrary units) of proteins that associate with chromatin-beads in M phase *Xenopus* egg extracts preferentially in Δ CPC extracts are shown. The top 23 most abundant proteins showing at least threefold enrichment on chromatin in Δ CPC egg extracts over control Δ mock extracts are listed. Subunits of the CHIRRC are colored in red. Proteins forming a complex with SMARCA5 are in blue.

Table 3. Proteins that dissociate from M phase chromatin in a manner dependent on the chromosomal passenger complex (CPC) (II)

Alt name	Complex	Δ CPC chromatin abundance (A.U.)	Δ mock chromatin abundance (A.U.)
RAD1	9-1-1	3.2×10^7	0
SGOL1	Sgo1	3.1×10^7	0
POLE3	CHRAC17	2.9×10^7	0
HUS1	9-1-1	2.9×10^7	0
FEN1		2.1×10^7	0
PRPF19	PRP19	2.0×10^7	0
KPNA2	Importin α	2.0×10^7	0
MCM7	MCM	2.0×10^7	0
MMS22L	TONSL-MMS22L	1.9×10^7	0
APEX1	SET	1.9×10^7	0
CSNK2A1	Casein kinase 2	1.9×10^7	0
RAD9A	9-1-1	1.8×10^7	0
HIST1H1D	Histone H1.3	1.8×10^7	0
THOC2	THO	1.8×10^7	0
MCM2	MCM	1.8×10^7	0
UHRF1		1.6×10^7	0
TONSL	TONSL-MMS22L	1.6×10^7	0
RDM1	RAD52B	1.6×10^7	0
THOC1	THO	1.5×10^7	4.5×10^6
PPP2R5D	PP2A B56Delta	1.5×10^7	0
RSF1	RSF	1.4×10^7	0
MCM3	MCM	1.4×10^7	0
PMS2	MutL α	1.1×10^7	0
KPNB1	Importin β	1.0×10^7	0

Data are generated from Supplemental Table S1 in Jenness et al. 2018.

Abundance (arbitrary units) of notable proteins that associate with chromatin-beads in M phase *Xenopus* egg extracts preferentially in Δ CPC extracts are shown. Proteins forming a complex with SMARCA5 are in blue.

abundance in egg extracts (100–1000 nM) (Wuhr et al. 2015), none of the SRSF proteins could be copurified with chromatin at any conditions, even in Δ CPC extracts (Jenness et al. 2018). SIRT2 is one of the most abundant known histone deacetylases in *Xenopus* eggs (178 nM) (Wuhr et al. 2015), but SIRT2 could also not be detected on purified chromatin at any condition including M phase (Jenness et al. 2018). The only histone deacetylase that could be detected on chromatin was HDAC1, but its chromatin association was restricted to interphase (Jenness et al. 2018). Thus, the proposed interaction between Hst2 and H3S10ph may be too dynamic to detect by our method, limited to anaphase, not conserved in *Xenopus* egg extracts, or require additional conditions that may not be met on the DNA beads that we used.

Several proteins showed CPC-dependent binding to or dissociation from chromatin beads (Tables 1–3). Many of the CDC-dependent binders were microtubule-binding proteins, likely reflecting the role of the CPC in microtubule assembly (Table 1; Jenness et al. 2018). It remains to be clarified if these interactions depend on microtubules that survived our bead-washing procedures. Although it has been suggested that Aurora B contributes to condensin association with mitotic chromosomes (Tada et al. 2011), CPC depletion did not show any impact on chromatin enrichment of condensin and DNA topoisomerase II in *Xenopus* egg extracts (Fig. 4B; MacCallum et al. 2002; Jenness et al. 2018). Proteins whose chromatin association is negatively regulated by the CPC include several nucleosome-remodeling complexes such as CHIRRC (HELLS-CDCA7), WICH (ISWI-WSTF), CHRAC (ISWI-ACF1-POLE3), and RSF (ISWI-RSF1) (Fig. 4D; Tables 2,3; MacCallum et al. 2002; Jenness et al. 2018), but neither

H3S10ph nor H3S28ph contributed to dissociation of HELLS and ISWI from chromatin in M phase (Jenness et al. 2018), indicating that these effects are not mediated through phosphorylation of H3S10 or H3S28. In addition to nucleosome remodelers, the CPC also dissociates a variety of proteins involved in DNA repair and replication (Tables 2 and 3). Although future investigations are required to establish the role of H3S10ph and H3S28ph outside the context of H3K9me3, these data suggest that the CPC (and perhaps H3S10ph/H3S28ph) contribute to suppressing DNA processing during mitosis.

An intriguing possibility is that local suppression of the CPC pathway enables targeting some of these factors at specific chromosomes or loci (Fig. 5). For example, chromosome arm binding of Sgo1 and MCAK are suppressed by the CPC (Tables 2,3; Zhang et al. 2007; Rivera et al. 2012), but their centromeric enrichment can be positively regulated by Aurora B (Tanno et al. 2010). A similar mechanism may be relevant when chromosomes undergo missegregation and are lagged behind during anaphase. Aurora B-dependent phosphorylation on chromosome substrates (e.g., H3S10) is maintained on lagging chromosomes even when segregated chromosomes are dephosphorylated (Su et al. 1999; Fuller et al. 2008). As proposed for regulation of nuclear envelope reassembly (Afonso et al. 2014), preventing loading of nucleosome remodelers and other DNA processing proteins to lagging chromosomes may be important to preserve genome integrity, particularly, when these chromosomes are interacting with microtubules (Fig. 5). The pushing and pulling forces generated by a single microtubule fiber are estimated to be ~ 50 pN (Nicklas 1983; Jannink et al. 1996; Grishchuk et al. 2005; Bloom 2008). This is comparable to the

Figure 5. Hypothetical role of Aurora B-dependent removal of mitotic chromatin proteins. During preanaphase stages of mitosis (*left*), Aurora B activated by chromosomes and/or microtubules promote dissociation of the microtubule-destabilizing protein MCAK and chromatin-remodeling complexes, CHIRRC, ACF, CHRAC, and WICH. In an anaphase/telophase cell with a lagging chromosome associated with spindle midzone (*right*), Aurora B-dependent phosphorylation is restricted to regions adjacent to the midzone, where reloading of the chromatin remodeling complexes is inhibited.

measured force (20 pN) required to evict nucleosomes from DNA *in vitro* (Cui and Bustamante 2000; Bloom 2008). In *Xenopus* interphase egg extracts, as little as 4 pN is sufficient to unwrap nucleosomes in the absence of ATP, and the presence of ATP further destabilizes nucleosomes, making them vulnerable to a force as low as 1 pN (Yan et al. 2007). It would be interesting to explore the possibility that displacement of chromatin remodeling or processing proteins is important to suppress microtubule-dependent alteration of chromatin structure and the formation of DNA damage in lagging chromosomes.

CONCLUSION

Nucleosomes are critical for microtubule formation during M phase and NPC assembly in interphase. RCC1–Ran and the CPC are key nucleosome-binding components that regulate both of these processes. Although RCC1–Ran acts positively for both events, the CPC promotes spindle assembly but suppresses nuclear formation. Many nucleosome-binding factors, such as nucleosome-remodeling proteins and histone chaperones, are used to control DNA accessibility. Unlike these regulators, RCC1–RanGTP and the CPC help to form eukaryote-specific macrostructure assembly through regulating proteins that are not necessarily directly interacting with chromatin. In addition, it has been suggested that RanGTP converts the chromatin-remodeling factor ISWI into a microtubule-binding protein to control anaphase spindle stability (Yokoyama et al. 2009). Therefore, it is plausible that

other nucleosome regulators may also have other distinct functions.

Our efforts to understand nucleosome-dependent and -independent processes raise the question of why nuclear envelope formation is broken into steps that can be mediated by nucleosome-free DNA, which can recruit membranes, and steps that require nucleosomes, which are necessary for NPC assembly. We speculate that this mechanism may be actively used to avoid spontaneous formation of functional nuclei from exogenous nucleosome-free DNA, such as that of viruses and other pathogens. An exception is the DNA provided by sperm, where sperm-specific protamines tightly pack DNA in a manner that prevents DNA replication and transcription. Thus, fertilization is a unique developmental event where external DNAs that penetrate into the egg cytoplasm rapidly assemble into nucleosomes. This is made possible by pre-stored soluble histone pools and histone chaperones that support *de novo* nucleosome assembly. In contrast, invasion of foreign DNA into somatic cells, whose cytoplasm harbors little soluble histones, may trigger innate immune response (Chen et al. 2016). We hypothesized that nucleosome-dependency of spindle assembly and NPC formation allows eukaryotic cells to distinguish between foreign nucleosome-free DNAs, such as those from virus and bacteria, and genomic nucleosomal DNAs, so that foreign DNAs cannot easily hijack the replication and segregation system (Zierhut et al. 2014; Zierhut and Funabiki 2015). We are currently exploring the possibility that nucleosomes could suppress a response by a cytoplasmic DNA sensor involved in innate immune response (Zierhut and Funabiki 2017). It is tempting to speculate that suppres-

sion of nucleosome remodeling and unwrapping on lagging chromosomes inhibits such sensors that recognize pathogenic or foreign DNA. In this sense, nucleosome loss in mitosis can be sensed as an aberration, and if difficult to be repaired, it may trigger a mechanism to purge these problematic cells.

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