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Nucleosome functions in spindle assembly and nuclear envelope formation

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Summary

Chromosomes are not only carriers of the genetic material, but also actively regulate the assembly of complex intracellular architectures. During mitosis, chromosome-induced microtubule polymerisation ensures spindle assembly in cells without centrosomes and plays a supportive role in centrosome-containing cells. Chromosomal signals also mediate post-mitotic nuclear envelope (NE) re-formation. Recent studies using novel approaches to manipulate histones in oocytes, where functions can be analysed in the absence of transcription, have established that nucleosomes, but not DNA alone, mediate the chromosomal regulation of spindle assembly and NE formation. Both processes require the generation of RanGTP by RCC1 recruited to nucleosomes but nucleosomes also acquire cell cycle stage specific regulators, Aurora B in mitosis and ELYS, the initiator of nuclear pore complex assembly, at mitotic exit. Here, we review the mechanisms by which nucleosomes control assembly and functions of the spindle and the NE, and discuss their implications for genome maintenance.

Keywords

chromatin; chromosomes; genome maintenance; mitotic spindle; mitosis; nuclear envelope; nuclear pore complex

Introduction

As cells progress through the cell cycle, complex architectural changes occur. During mitosis, chromosomes condense, the nuclear envelope (NE) disassembles in many eukaryotes such as vertebrates, and the bipolar spindle forms. Following chromosome segregation, the spindle disassembles, chromosomes decondense, and the NE forms anew. The proper execution of all these events is a prerequisite for genome stability and cell viability, and malfunctions can lead to diseases as varied as developmental defects, progeria and cancer [1, 2]. Ultimately, these cellular events depend on gene products encoded in chromosomal DNA. However, chromosomes also regulate spindle assembly as well as NE formation independently of transcription (Fig. 1).

These mechanisms occur through a multitude of chromosomal proteins, which together with DNA form the physical substance of chromosomes, chromatin [3]. The most abundant and

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basal of these are the core histones H2A, H2B, H3 and H4. These organise the majority of nuclear DNA into nucleosomes, in which ~145 bp of DNA wrap around octamers formed by two copies of each histone [4]. Histones are intimately connected to all chromosome functions, but demonstrating their direct in vivo functions is difficult, as histones can modulate processes indirectly through regulating transcription. Recently, two studies determined the effects of the absence of nucleosomes under physiological conditions, revealing important aspects of histone function. Both approaches used vertebrate eggs, where critical chromatin functions proceed in the absence of transcription [5]. In one approach, nucleosome-free chromatin was generated by preventing loading of histones onto mouse sperm (which is largely void of histones) following fertilisation [6]. In the other approach [7], histone loading was prevented in a cell-free system derived from *Xenopus laevis* eggs. In the absence of cellular partitions and transcription, these egg extracts recapitulate complex chromosome functions such as DNA replication, repair, mitosis and NE assembly [8–10]. Histone functions were analysed by the addition of naked DNA or nucleosome arrays generated from purified components in the absence of extract. Together, these studies showed nucleosomes to be key regulators of spindle function and NE assembly. Here, we review the role that chromatin plays in regulating spindle function and NE formation, and the critical contributions nucleosomes make to both processes.

Chromosomes can induce microtubule polymerisation during mitotic spindle assembly

Chromosome segregation during mitosis and meiosis depends on the bipolar spindle (Fig. 1A), whose major constituents are microtubules, polymers of α/β -tubulin dimers [11]. Microtubule organisation varies dramatically during the cell cycle. In interphase animal cells, microtubules form a cytoplasmic network that is mainly nucleated at the centrosome [12]. Upon entry into mitosis, this network disassembles and microtubules reorganise into a dynamic bipolar spindle that directly interacts with chromosomes after NE breakdown. Similar to interphase, centrosomes predominate nucleation of mitotic microtubules in most somatic cells. However, plants and animal oocytes do not contain centrosomes, and yet assemble functional spindles [11, 13]. Much of our insight into the centrosome-independent spindle assembly derives from animal oocytes, where microtubule formation is initiated through signalling events at chromosomes (referred to as chromosome-induced microtubule polymerisation hereafter). Particularly, *Xenopus* egg extracts were instrumental for our understanding of this process. In egg extracts, DNA coupled to beads can induce spindle assembly in a sequence-independent manner [14]. This may be a universal feature of oocytes, as a comparable phenomenon has also been observed with similar beads injected into mouse oocytes [15]. The next section will thus mainly focus on insights gained from egg extracts and other oocyte systems, while the following section will compare these findings with results from somatic systems.

Nucleosomes direct spindle assembly by generating RanGTP and recruiting Aurora B

Chromosomes direct spindle assembly by two signalling pathways (Fig. 2). First, chromosomes generate a local enrichment of the GTP-bound form of the small GTPase Ran (RanGTP). This liberates spindle assembly factors from inhibitory interaction with importin α/β (known also as karyopherin α/β), similar to the function that RanGTP plays in nuclear import (reviewed in [16]). Because RanGTP levels decrease with distance from chromosomes, this mechanism also helps prevent ectopic microtubule polymerisation [16]. However, it has been demonstrated that while generation of RanGTP by chromosomes is critical for microtubule assembly, chromosomes still provide spatial information to locally promote microtubule assembly in the absence of a RanGTP gradient [17]. This is facilitated by the second chromatin-induced pathway, mediated by the chromosomal passenger complex (CPC). Chromosomes activate the kinase subunit of the CPC, Aurora B [18–20], resulting in inhibitory phosphorylation of proteins that destabilise microtubules, such as MCAK and Op18 (stathmin) [11, 20]. As detected by a number of Förster resonance energy transfer based sensors, enrichment for RanGTP and phosphorylated Aurora B substrates extends over roughly the length of the spindle, although high signals are restricted to the immediate vicinity of chromosomes [21–24]. This suggests that while RanGTP and Aurora B substrates may contribute to spindle assembly at a distance, their major activity is restricted to the immediate vicinity of chromatin.

The increased RanGTP concentration in the vicinity of chromosomes results from a combination of three major processes. First, RCC1, the Ran guanine-nucleotide exchange factor (RanGEF), which mediates the exchange of GDP for GTP, is itself a chromosomal protein [16]. Second, the enzyme that stimulates GTP hydrolysis by Ran, RanGAP1, is cytoplasmic, thus ensuring that the GTP-bound form of Ran is short lived once it diffuses away from chromatin [16]. Third, cytoplasmic RanGTP formation is prevented through sequestration of excess RCC1 by RanBP1 [25]. In addition, RanGTP levels can be controlled by RCC1 phosphorylation and methylation [26–28].

How is chromosomal enrichment of RCC1 achieved? RCC1 can bind to the histone H2A–H2B complex, nucleosomes, and naked DNA, with the latter interaction being mediated by RCC1's N-terminal tail [29, 30]. A crystal structure of RCC1 bound to the nucleosome revealed that RCC1 binds to histones H2A and H2B as well as to DNA with loops emanating from its globular domain [31]. Interactions between DNA and the N-terminal RCC1 tail were also observed although the tail was dispensable for nucleosome interaction [31]. Several studies establish the physiological importance of the RCC1-nucleosome interaction. First, nucleosomes and the H2A–H2B complex, but not DNA stimulate the activity of RCC1 [30]. Second, the genomic binding pattern of budding yeast RCC1 is almost identical to that of nucleosomes [32]. Finally, RCC1 can be recruited to nucleosomes, but not to nucleosome-free DNA in *Xenopus* egg extracts and mouse oocytes [6, 7].

Recent findings also illustrated how chromosomes activate Aurora B, and how this activation is restricted to mitosis (Fig. 2A). Central to this mechanism is H3 and a kinase

cascade, resulting in mitotic activation of the kinase Haspin, which phosphorylates Thr 3 of H3 (H3T3ph) [33, 34]. This mark is directly bound by the CPC component Survivin and ensures the chromosomal enrichment of Aurora B [20, 35–37]. This enrichment is key to Aurora B activation, being thought to allow activating trans-autophosphorylation [19]. H3T3ph is the only essential and sufficient Haspin substrate for Aurora B activation, as H3T3A mutant chromatin cannot activate Aurora B in *Xenopus* egg extracts, whereas the phosphomimetic H3T3E mutation allows Aurora B activation in the absence of Haspin [7]. In the *Xenopus* egg cytoplasm, Aurora B autophosphorylation (and activation) is actively suppressed by phosphatases, generating a strong dependency on chromosomes for Aurora B activation (Fig. 2B). Although initially associated along chromosome arms, Aurora B eventually enriches at centromeres, paralleling H3T3ph [20]. At centromeres, Aurora B is important for regulating kinetochore-microtubule attachment and the spindle assembly checkpoint [20], but activated Aurora B may also diffuse away from centromeres, phosphorylating substrates at a distance [38]. For example, proper spindle assembly in egg extracts requires interaction of the CPC subunit INCENP with microtubules [39], which may facilitate substrate recognition by limiting the diffusion dimensionality of the kinase and microtubule-bound substrates [40], and may also play additional kinase-independent roles. However, at present it is unknown where phosphorylation of targets that are critical for spindle assembly occurs.

In addition to H3T3ph, two other histone marks, phosphorylation of Thr 120 of H2A (H2AT120ph), and trimethylation of Lys 9 of H3 (H3K9me3), have also been connected with Aurora B [20]. H2AT120ph may help restrict Aurora B localisation to centromeres [37, 41], and H3K9me3 may contribute to chromosomal localisation of Aurora B through a shared interaction partner, HP1 [20, 42–44]. However, it is unknown whether these two marks contribute to Aurora B activation and chromosome-induced microtubule polymerisation.

Chromosome-induced microtubule polymerisation during oocyte maturation

While the RanGTP-pathway and the CPC pathway are both essential for chromatin-induced spindle assembly in *X. laevis* egg extracts, their importance varies among systems (Tables 1 and 2). Even between *Xenopus* egg extract systems, a striking difference exists; in egg extracts of *X. tropicalis*, RanGTP is dispensable for spindle assembly [45]. This largely appears to be due to the different abundance of the spindle assembly factor TPX2 and importin α in *X. tropicalis* eggs [45, 46]. In *X. tropicalis* eggs, spindle assembly may thus primarily depend on the CPC to locally promote microtubules, although this has not been tested yet. In *X. laevis* extracts, the RanGTP and the CPC pathway act complementary to provide spatial information. When RanGTP concentration is made uniformly high, spindle formation is inhibited on DNA beads but not on sperm chromosomes [17]. However, spindle formation on DNA beads can be rescued by artificial enrichment of the CPC, indicating that Aurora B can indeed drive spindle assembly in the absence of spatial information provided by RanGTP [17]. Similarly, under certain conditions, RCC1-coated beads can promote spindle assembly in *X. laevis* egg extracts in the absence of CPC enrichment [47]. Thus, the

contributions by spatial information of RanGTP and Aurora B can be modulated to fit each specific system's requirements to ensure correct spindle function.

In intact oocytes, RanGTP and Aurora B are also used in a developmentally regulated and species-specific manner (Tables 1 and 2). In *X. laevis* oocytes, knockdown of RCC1 interferes with spindle assembly at the second meiotic division [48]. However, the same treatment does not cause obvious spindle defects during the first meiotic division [48]. In contrast, in *Drosophila* oocytes, neither interference with the formation of RanGTP by overexpression of a dominant negative version of Ran, RanT24E (which cannot bind guanine nucleotides), nor flattening the RanGTP gradient by overexpression of RanQ69L (which cannot hydrolyse GTP) prevents spindle assembly [49]. However, CPC perturbation interferes with spindle assembly [50, 51], suggesting that *Drosophila* oocytes rely on the CPC more than on RanGTP. RanGTP still regulates microtubule aspects in this system, as the microtubule-mediated fusion of the male and female pronucleus is defective after RanGTP perturbation [49].

Similar to the situation in *Xenopus*, mouse oocytes require RanGTP more during meiosis II than during meiosis I [48], and the microinjection of RanT24E or RanQ69L causes severe spindle defects during meiosis II. Unlike *Xenopus*, both Ran perturbations also perturb meiosis I, severely delaying spindle assembly [48, 52]. The spindle that eventually forms requires other chromatin factors, as it was not observed in oocytes from which the nucleus was removed [52]. While it is possible that one of these factors is Aurora B, this has so far not been tested. By itself, Aurora B inhibition (using kinase inhibitors), or INCENP knockdown causes chromosome misalignment but only mild, if any, spindle assembly defects [53–55]. The involvement of RanGTP in oocyte meiosis I spindle assembly may be a general principle of mammals, as recent analysis of human oocytes revealed an absence of spindles in response to microinjection of RanT24N [56]. This analysis also revealed that human oocyte meiosis I spindle assembly is rather slow and error prone. The mechanistic basis of this phenomenon is at present unclear.

Does chromosome-induced microtubule polymerisation contribute to somatic spindle assembly?

In most animal cells, centrosomes act as the major microtubule organising centre. There even is a *Drosophila* mutant in which spindles can form in the absence of chromosomes [57]. However, in all cells, microtubules eventually enrich around chromosomes, indicating a contribution to microtubule polymerisation, stabilisation or attraction. In evidence for this, there are animal clades that do not contain centrosomes [58], and in *Drosophila*, centrosome-free animals can be generated, in which mitosis is relatively normal [59]. Similarly, spindles can form in mammalian cells after removal of centrosomes [60, 61]. These results suggest that somatic cells maintain intact chromosome-induced microtubule polymerisation pathways.

While the chromosome-induced spindle assembly in *Xenopus* egg extracts does not depend on specific chromosomal regions, during centrosome-mediated spindle assembly, centromeres and the kinetochores that are assembled thereon play a pivotal role in attracting

microtubules and can also be shown to nucleate microtubules themselves. For example, kinetochore-induced microtubule polymerisation can be detected after disassembly of spindle microtubules with nocodazole and subsequent drug removal [62, 63]. Similarly, if monopolar spindles are assembled by monastrol treatment, kinetochores facing away from the half-spindle can induce microtubule polymerisation [64]. Microtubule nucleation from the kinetochore is also observed in unperturbed budding yeast [65]. In contrast, chromosome arms, although contributing to chromosome congression through binding chromokinesins [66], appear dispensable for spindle assembly in somatic cells. This is most obvious when mitosis is prematurely induced in the presence of under-replicated chromosomes [67]. Under these conditions, small centromeric fragments containing kinetochores align at the spindle while chromosome arms are excluded. Even in this extreme situation, and despite the capacity of chromosome arms to attract microtubules – uncovered by ablation of kinetochores – does microtubule nucleation remain restricted to kinetochores [68]. Together, these results suggest that chromosome arms are not a major inducer of spindle formation in somatic cells.

During centrosomal mitosis, kinetochore-induced microtubule polymerisation may serve two purposes. First, separation of duplicated centrosomes sometimes occurs belatedly, and chromosome-induced microtubule formation may compensate for the transient lack of a second spindle pole [69]. Second, kinetochore-induced microtubule polymerisation may allow timely attachment of kinetochores to the spindle. Initially, it was thought that kinetochore attachment is achieved by “search and capture” of microtubules originating from centrosomes. However, later analyses indicated such a process to be inefficient and slow [70]. Chromosome-induced microtubule polymerisation may thus ensure the presence of microtubules around kinetochores, and facilitate spindle attachment. These notions are supported by observations that microtubules formed at kinetochores can be captured by the spindle [64, 65].

Evidence for the importance of kinetochore-induced microtubule polymerisation in somatic cells also comes from RanGTP- and CPC perturbations (Tables 1 and 2). In most systems, the contribution of the CPC is largely unknown, although in *Drosophila* S2 cells engineered to lack centrosomes, knockdown of CPC subunits causes small and/or multipolar spindles [71], and kinetochore microtubule polymerisation after nocodazole washout is defective after knockdown of Survivin in HeLa cells [62]. More is known about RanGTP, which affects centrosomal spindles in all species tested (Table 1). In human model systems, the involvement of RanGTP in spindle assembly has most extensively been analysed in HeLa cells. Here, uniformly high RanGTP levels result in ectopic microtubule polymerisation [23], as well as spindle assembly and chromosome alignment delays [72]. Similarly, inhibition of RanGTP results in spindle defects, aberrant chromosome alignment, spindle positioning defects, and defects in kinetochore microtubule formation following nocodazole washout [23, 62, 73–75]. In agreement with these results, a steep mitotic RanGTP gradient can be detected in many cell lines such as HeLa cells, although other cell lines, and particularly untransformed ones, often do not show clear gradients [72]. The reasons behind this phenomenon appear to be complex, but one major factor may be the cellular DNA content, as fusion of cells of a gradient-free cell line resulted in cells with a gradient [72].

However, this also resulted in an increase in cell volume, which itself may be important for formation of a RanGTP gradient. The majority of tumour cells have unstable karyotypes, often showing partial or complete polyploidy [2], raising the possibility of steeper RanGTP gradients in many tumour cells. One aspect correlating with such gradients is faster spindle assembly [72], which itself may cause chromosome attachment errors. Therefore, by negatively affecting chromosome segregation, extremely steep RanGTP gradients may contribute to tumour karyotype alterations.

At present, neither the mechanism nor the functional significance of the differential contributions of chromosome arms and kinetochores to spindle assembly in the different model systems is known. Restricting chromosome-induced microtubule polymerisation to kinetochores may be particularly important in smaller cells where free soluble tubulin is limited. In contrast, in oocytes, which contain large excess stockpiles of materials, microtubule assembly must be strongly suppressed in the cytoplasm. These suppressive activities may locally be overcome by utilising chromosome arms.

Chromosome-induced processes orchestrate nuclear envelope reformation

Following mitotic exit, chromatin becomes encapsulated by the nuclear envelope (NE, Fig. 3), formed by two sheets of lipid bilayers, the inner nuclear membrane (INM) and the outer nuclear membrane (ONM) [76]. Specialised channels, nuclear pore complexes (NPCs), connect these and allow regulated protein trafficking between the nucleoplasm and the cytoplasm [76]. A protein scaffold interior to the INM, the nuclear lamina, helps the nucleus to maintain its shape and to resist external forces [76]. Many NE components are connected to each other and to chromatin: INM proteins often contain nucleoplasmic domains that bind lamins or chromatin [76], various NPC components interact with chromatin [6, 7, 76], and lamins may also directly interact with chromatin [77]. However, during mitosis in vertebrates and many other eukaryotes, these structures are deconstructed: the lamina depolymerises, the INM and ONM retract into the endoplasmic reticulum (ER), and NPCs disassemble [76].

Post-mitotic NE reassembly is largely driven by reversal of mitotic phosphorylation events [76]. In addition, disassembly of spindle microtubules may also be a prerequisite for proper NE formation [78]. In *Xenopus* egg extracts, this mechanism involves another protein whose activity depends on chromosomes (Fig. 2A). This protein, Dppa2, can disassemble microtubules in vitro and also interacts with chromosomes, presumably by directly binding DNA [7, 78]. In the absence of Dppa2, or in the presence of mutants that are unable to bind DNA or to disassemble microtubules, NE formation is defective. Artificial microtubule depolymerisation with nocodazole can rescue NE formation in the absence of Dppa2, indicating that microtubule depolymerisation is indeed critical for nuclear envelope reassembly. Prior to NE assembly, Dppa2 may also participate in balancing RanGTP and Aurora B as microtubule growth is increased in Dppa2 extracts [78]. At present, it is unclear whether Dppa2 or equivalent mechanisms operate in other systems, although the presence of microtubules around NE-free chromatin regions following mitotic exit of human cells suggests microtubule depolymerisation to generally be important for NE reformation (reviewed in [79]).

INM proteins target membranes to DNA

NE membranes are provided by the ER [80], being targeted there by INM proteins such as the lamin B receptor (LBR) and LEM domain proteins such as emerin, MAN1 and Lap2 β [76, 81]. Membrane recruitment involves the nucleoplasmic domains of many of these INM proteins, which bind multiple chromatin-associated targets in vitro (and which are cytoplasmic during mitosis). For LBR, these potential interactors include DNA [82, 83], histones [84, 85] and HP1 [86]. LEM domain proteins can interact with DNA [83] but their DNA binding activity is not sufficient for chromatin association, which requires an adapter protein, BAF [87]. BAF itself can bind to DNA and histones in vitro [88, 89]. Consistent with the idea of redundancy between INM proteins, single knockdowns do not cause strong NE formation phenotypes, and co-depletions have additive effects [81]. In vitro, INM proteins can recruit purified membranes to DNA in the absence of other co-factors [83, 90]. However, because many of these proteins also bind histones or histone interactors, it was not clear whether the interaction with DNA is sufficient under physiological conditions. The recent data from nucleosome-free DNA in egg extracts [7] and mouse oocytes [6] demonstrated that, under physiological conditions, nucleosomes are indeed not required for membrane recruitment. Furthermore, despite its potential association with histones, BAF does not require nucleosomes for chromatin association [7]. Therefore, NE membranes appear to primarily be targeted directly to DNA (Fig. 4A).

Once bound to chromatin, membrane fusion in egg extract was suggested to require GTPase activity, thought to be due to Ran [91]. Furthermore, Ran-coated micron-scale beads can assemble nuclear import-compatible NEs in the absence of chromatin in *Xenopus* egg extracts [92]. In *C. elegans* embryos, interfering with Ran itself or the balance between RanGDP and RanGTP caused defects in NE formation [93]. The mechanism by which Ran promotes NE assembly remains largely unclear, but at least one INM protein, LBR, interacts with importin β [94], suggesting that Ran may regulate the interaction of some INM proteins with chromatin by releasing them from importin β . However, more recent studies indicate fusion of membranes to require RanGTP only when NE assembly is reconstituted with vesicular precursors in the absence of a functional ER network, a situation occurring in an egg extract based system that is sometimes used to study NE assembly [90]. Strikingly, nucleosome-free pronuclei in mouse oocytes can form closed NEs without enrichment of RCC1, and thus, of RanGTP [6]. Thus, similar to the situation in spindle assembly, the requirement for RanGTP in NE formation may be context dependent.

Nuclear pore complex assembly is initiated by nucleosomes

Two major mechanisms ensure that NPCs and the lamina are formed at chromosome-associated membranes, and not generally at the ER, the Golgi or the plasma membrane. First, similarly to mitotic spindle assembly, NPC formation requires RanGTP [16, 91, 92, 95, 96]. Second, lamina assembly only occurs once nuclear import has been established [6, 7, 97]. Analysing the consequences of the absence of nucleosomes also revealed that nucleosomes are required for NPC assembly [6, 7]. The NPC defect observed in the absence of nucleosomes could only slightly be improved by forcing RCC1 to DNA [7], suggesting the existence of a second NPC assembly step, independently of the generation of RanGTP,

that is regulated by nucleosomes. Indeed, it was found that this factor is ELYS, the initiator of NPC assembly [6, 7, 76] (Fig. 4B), and that NPC formation on nucleosome-free DNA could be rescued by expressing both RCC1 and ELYS fused to DNA binding domains [7]. ELYS can bind the H2A–H2B dimer, suggesting an interaction with these histones is important in recruiting ELYS to nucleosomes [7]. ELYS also contains a variant type of AT-hook DNA binding domain [98], and it had previously been thought that NPC formation is initiated by recruitment of ELYS to DNA. However, biochemical experiments showed that the AT-hook is insufficient for binding to either naked DNA or nucleosomes, although it does contribute to nucleosome interaction [7]. Altogether, although remaining to be clarified by structural analyses, this suggests that interaction with both H2A–H2B and DNA within the context of a nucleosome is required for chromatin recruitment of ELYS.

Once on chromatin, ELYS induces NPC formation by recruiting the Nup107 complex, an important structural NPC component [76, 99]. The RCC1-dependent and ELYS-dependent NPC assembly branches intersect, and chromatin association of the Nup107 complex requires RanGTP, at least partially explaining the RanGTP requirement in NPC formation (Fig. 4B) [95]. Similarly, addition of RanQ69L to *Xenopus* egg extracts can increase the chromatin association of ELYS [98, 100]. However, RanGTP may not be absolutely required, as preventing the formation of RanGTP does not interfere with the association of ELYS with chromatin [100]. At present, it is not clear how ELYS association with chromatin can occur independently of RanGTP [100] even though chromatin association of the Nup107 complex requires RanGTP [95]. Fractionation experiments showed ELYS and the Nup107 complex to be associated with each other in the absence of RanGTP and in the presence of importin β [100, 101], suggesting RanGTP not to regulate the interaction between ELYS and the Nup107 complex. Future studies will be needed to resolve this matter. Species-specific differences may also exist, as interference with the Ran-system impedes chromatin association of ELYS in *C. elegans* [102]. Finally, RanGTP is also involved in later steps in NPC assembly (Fig. 4B) [91, 95, 98, 103].

Whatever the exact function of RanGTP in NPC formation is, it can be bypassed under certain conditions. Fusing ELYS with the INM protein emerlin allows NPC formation on nucleosome-free oocyte pronuclei that do not contain RCC1 [6]. At first glance, this mechanism may appear to be related to a second pathway for NPC assembly, which mediates insertion of NPCs into intact membranes [104–106]. However, this pathway normally occurs independently of ELYS and requires RanGTP [105, 106]. Therefore, the mechanistic basis of this phenomenon is currently not understood.

Altogether, by recruiting membrane components and NPC components of the NE, DNA and nucleosomes together specify the assembly of a functional NE. NPCs can assemble both at mitotic exit, concomitantly to NE formation, as well as into an intact envelope. NPC assembly at mitotic exit is much faster, and rapidly dividing cells may thus be more dependent on this pathway.

Nuclear pore complex proteins contribute to spindle function

Several nucleoporins have secondary functions that connect NPCs with mitotic spindles. Both ELYS and the Nup107 complex can be detected in an interdependent manner at kinetochores and spindle poles [107–110], and seem to regulate both kinetochore and spindle function in a species-specific manner. During chromosome-induced spindle assembly in *X. laevis* egg extracts, immunodepletion of either the Nup107 complex or ELYS causes severely defective spindle assembly [110–112]. This may be due to faulty microtubule polymerisation as the Nup107 complex and ELYS are thought to be important for recruiting the microtubule-nucleating γ -TuRC complex to spindles [110, 112]. The C terminus of ELYS directly binds to microtubules in a manner dependent on the region containing the AT-hook and a nuclear localization sequence and this binding is inhibited by importin α/β [110]. Because this inhibition is relieved by RanGTP [110], ELYS-mediated microtubule nucleation appears to act downstream of the Ran pathway.

Similarly, in human cells, ELYS and the Nup107 complex contribute to kinetochore microtubule assembly through recruiting the γ -TuRC complex to kinetochores [112]. In addition, ELYS and the Nup107 complex may support the centromeric localization of the CPC [113, 114]. It is also noteworthy that in plant prophase cells, the outer nuclear envelope is capable of nucleating microtubules and coordinating bipolar spindle assembly before nuclear envelope breakdown [13]. Thus, in plants, NPCs may also help assemble spindle microtubules in the vicinity of chromosomes. The functional interaction between this nucleoporin pathway and the Ran/CPC pathway would provide further spatial coordination.

General principles and functional implications

The observation that nucleosomes are the central platform for spindle and NPC assembly highlights an important regulatory principle by which local and temporal regulation coincide to specify intracellular architecture. Both processes require nucleosomes to recruit RCC1 to chromosomes, thus generating a high local concentration of RanGTP. Depending on the cell cycle stage, however, nucleosomes also acquire specific regulators: ELYS recruitment in interphase contributes to NPC formation, and Aurora B activation by H3T3ph in mitosis supports spindle microtubule polymerisation. Aurora B activation, in turn, needs to be undone for proper NE formation [35, 115].

Why are these processes coupled to nucleosomes, rather than directly to DNA? We envisage two non-mutually exclusive possibilities. First, the coupling of NE formation to nucleosomes may be crucial to provide timing in early embryonic development, as male pronucleus formation requires the loading of histones [6]. Second, coupling NE assembly to nucleosomes, rather than naked DNA, may be a quality-control mechanism against NE formation on aberrant DNA in the cytoplasm [7]. For example, many pathogens such as viruses and intracellular bacteria can give rise to cytoplasmic DNA. Certain cell types such as phagocytes are furthermore expected to carry a heavy burden of exogenous DNA, resultant from the phagocytosis of extracellular pathogens, or apoptotic and necrotic cells [116]. Cytoplasmic DNA may also arise from endogenous sources such as retroelements or excessive amounts of DNA damage [116]. Lastly, nucleosomes can be removed from

chromatin on anaphase bridges, which can be caused by multiple defects in mitosis [117, 118]. In this respect, it is of interest that chromatin trapped on anaphase bridges as well as lagging chromosomes frequently give rise to micronuclei [119, 120], on which NEs form only very poorly [119–122]. It is believed that, as a result of NE aberrations, replication is defective in these micronuclei, leading to massive amounts of DNA damage [119–122]. In healthy cells, the resulting cell cycle arrest and apoptosis may protect against the propagation of cells with nuclear abnormalities, as perhaps suggested by a p53-dependent growth arrest following chromosome missegregation [123]. However, this mechanism may be a double-edged sword, as it may lead to genome rearrangements that may enable tumour adaptation and progression through the process of chromothripsis [120, 124].

The finding that NE formation is coupled to nucleosomes rather than to DNA itself may also have implications for efforts in gene therapy using non-viral delivery methods [125]. Besides DNA unpacking, a major limiting step in this approach is the translocation of transfected DNA into the recipient nucleus, which is required for expression, but the mechanism whereof is poorly understood. In many cases, it appears that transfected DNA can only be incorporated into the nucleus following mitotic NE disassembly (see [126] for an example, reviewed in [125]), which, however, cannot occur in post-mitotic target tissues. Perhaps the reconstitution of transfected DNA with nucleosomes would allow it to acquire its own NE, mediating gene expression independently of incorporation into the main nucleus.

Conclusions and future outlook

Altogether, the recent years have seen a surge in our understanding of the mechanistic basis of spindle assembly and nuclear envelope formation. Nucleosomes have emerged as central for both processes, combining local and temporal regulation to ensure the implementation of the right architectural program at the right place and time. Future studies will provide insight into poorly understood questions, such as how NE membranes fuse, why NPC formation is coupled to nucleosomes, and how these processes are adapted by cancer cells and in a tissue-specific manner. These insights may provide vital clues for the development of novel strategies for cancer diagnostics and therapy, as well as for the treatment of congenital diseases.

Abbreviations

CPC	chromosomal passenger complex
INM	inner nuclear membrane
NE	nuclear envelope
NPC	nuclear pore complex
ONM	outer nuclear membrane

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Figure 1.

Nucleosome functions in nuclear envelope formation and spindle assembly. **A:** During spindle assembly, nucleosomes (top) generate signals (yellow) that induce polymerisation of microtubules (red). In cells without centrosomes (left), these signals are responsible for spindle assembly. In cells with centrosomes (right), these signals support spindle assembly through microtubule formation at kinetochores. **B:** After mitosis, nucleosomes (top) generate signals (green gradient in middle picture) around decondensing chromosomes (blue) to specify nuclear envelope formation (bottom).

Figure 2.

Chromosomal pathways regulating spindle assembly. **A:** Activation of microtubule polymerisation by nucleosomes. Left side: Nucleosomes mediate the chromosomal enrichment of RCC1 and allow the preferential generation of RanGTP in the vicinity of chromosomes. RanGTP induces microtubule polymerisation by liberating spindle assembly factors (SAFs) from inhibitory interaction with importin α/β . Right side: Phosphorylation of Thr3 of H3 by Haspin allows the chromosomal enrichment and activation of Aurora B. Aurora B phosphorylates and inhibits microtubule destabilising factors such as Op18 and MCAK. For the sake of simplicity, phosphorylation on the CPC is here only indicated on Aurora B. Please note that other sites, particularly on INCENP are also important for Aurora B activity. Middle part, bottom: In *Xenopus* egg extracts, a DNA binding protein, Dppa2 is important for timely microtubule disassembly around chromosomes following mitosis, and may also be important for balancing the microtubule polymerising activities of RanGTP and the CPC. At present, it is not clear if similar mechanisms are at work in other systems. **B:** Cytoplasmic inactivation of RanGTP and Aurora B. RanGTP is inactivated by soluble RanGAP1. Aurora B is inactivated by dephosphorylation, most likely mediated by PP2A.

Figure 3. Overview of the main components of the nuclear envelope. Lamins, the NPC and INM proteins – either directly or via the adaptor protein BAF – are all able to interact with chromatin. See text for further explanations.

Figure 4.

The coincidence of DNA- and nucleosome-specific interactors orchestrates post-mitotic nuclear envelope formation. **A:** Nuclear envelope membranes are recruited by interaction of endoplasmic reticulum (ER)-bound inner nuclear membrane proteins with DNA. At present, it is not clear whether vesicles also are a source of membranes in NE assembly. Note that although this interaction is shown to be in the absence of nucleosomes (in agreement with the preferential binding of ER membranes to naked DNA [90]), it cannot presently be ruled out that in vivo this interaction may also occur with nucleosomal DNA. **B:** Nuclear pore complex (NPC) formation. Nucleosome-bound RCC1 causes enrichment of RanGTP in the vicinity of chromosomes, which contributes to NPC formation at multiple points. RanGTP can stimulate ELYS and Nup107 association with chromatin. However, note that RanGTP may not absolutely be required for the chromatin association of ELYS. Regardless of the exact involvement of RanGTP, ELYS eventually is recruited directly to nucleosomes. Recruitment of downstream nucleoporins is again mediated by RanGTP. Although not described here, there is also another, although slower, pathway for NPC formation, which works on intact membranes. Nup107 complex and NPC shape assembled using data from [99].

Table 1

Spindle-assembly phenotypes of RanGTP system perturbations in various species. N/A, not analysed; Y, yes; RanT24N, nucleotide-free Ran mutant; RanQ69L, constitutively GTP-bound Ran mutant.

Species	Cell type	Ran gradient	Perturbation	Phenotype
<i>Xenopus laevis</i>	Oocyte	N/A	Meiosis I: Partial knockdown of RCC1.	No apparent spindle defects (did not test if RanGTP gradient was perturbed) [48].
		N/A	Meiosis II: Partial knockdown of RCC1.	Monopolar spindles, spindles not assembled [48].
	Egg extract	Y [21]	Addition of RanQ69L	Ectopic microtubule polymerisation [127–129]; lack of RanGTP gradient [21].
			Addition of RanT24N	Spindles not assembled [127, 129], lack of RanGTP gradient [21].
Mouse	Oocyte	Y [48]	Meiosis I: Microinjection of RanQ69L	Ectopic microtubule polymerisation, longer spindles, exclusion of longer polar bodies [48].
			Meiosis I: Microinjection of RanT24N	Severe delays in spindle formation [48, 52].
		Y [48]	Meiosis II: Microinjection of RanQ69L	Disorganised spindles, spindles not assembled [48].
			Meiosis II: Microinjection of RanT24N	Disorganised spindles, spindles not assembled [48].
	3T3 cells	N/A	Overexpression of RCC1 inhibitor RanBP1	Monopolar, multipolar, apolar spindles [130].
Chinese hamster	tsBN2 cells	Y [26]	Temperature-sensitive RCC1 mutation	Spindle assembly and chromosome alignment delays [72], spindle positioning defects [74], no microtubules polymerised at kinetochores following nocodazole washout [63].
Human	Oocyte	N/A	Microinjection of RanT24N	Spindles not assembled [56].
	HeLa cells	Y [23]	Microinjection of RanQ69L	Ectopic microtubule polymerisation [23].
			Microinjection of importin β	Multipolar spindles [23], prometaphase delays [23], no microtubule formation at kinetochores following nocodazole washout [62].
			Mutation of RanGTP- sensitive importin β binding region in HURP	Delay in anaphase entry, chromosome alignment defects [131].
			Treatment with importazole, an inhibitor of the release of importin β cargos	Flattening of RanGTP gradient, spindle defects (no analysis to further detail provided), chromosome alignment defects, spindle positioning defects [73, 75].
			Transfection of RanT24N	Spindle position defects [74].
			RanGAP1 RNAi	Spindle formation delays and chromosome alignment defects [72].

Species	Cell type	Ran gradient	Perturbation	Phenotype
	HEK293 cells	N/A	Expression of an RCC1 mutant that does not interact with Ran	Multipolar spindles, disorganised spindles, spindle positioning defects [132].
<i>Caenorhabditis elegans</i>	embryo	N/A	RNAi of Ran or RanGAP	Spindle not assembled, chromosome alignment defective [93, 133].
			RNAi of RCC1	Normal spindle assembly, defects in pronuclear migration [93].
<i>Drosophila melanogaster</i>	Oocyte	N/A	Meiosis I & II: Expression of RanT24N or RanQ69L	No obvious spindle defects (not clear if overexpression is to sufficient levels to interfere with Ran function) [49].
			Fertilised egg: Expression of RanT24N or RanQ69L	Defects in formation of the microtubule apparatus that mediates fusion of male and female pronuclei [49].
	S2 cells	Y [71]	Artificially induced acentrosomal spindle assembly, RCC1 RNAi	No obvious phenotype, but RanGTP gradient can still be detected after RNAi [71].

Table 2

Spindle-assembly phenotypes of Aurora B perturbations in various species.

Species	Cell type	Perturbation	Phenotype
Zebrafish	Oocyte and early embryo	Hypomorphic survivin mutation	Assembly of bent spindles [134].
<i>Xenopus laevis</i>	Egg extract	CPC depletion	No spindles formed [18].
Mouse	Oocyte	Knockdown of INCENP	Spindles form normally, chromosome alignment defects [135].
		Chemical inhibition of Aurora B	Chromosome alignment defects [135]; chromosome alignment defects and spindle morphology defects [53].
	Early embryonic cells	Knockout of survivin	Spindle morphology defects [136].
		Knockout of INCENP	Multipolar spindles and other spindle morphology defects [137].
Human	HeLa cells	Knockdown of Survivin	Reduction in kinetochores that induce microtubule polymerisation following nocodazole washout [62].
Drosophila	Oocyte	Hypomorphic INCENP mutant	Meiosis I: Spindle formation delayed, multipolar spindles [50].
		RNAi of Aurora B or INCENP	No spindles formed [51].
	S2 cells	Artificially induced acentrosomal spindle assembly, RNAi of CPC subunits	Multipolar or small spindles [71].