HHS Public Access

Author manuscript

Curr Opin Cell Biol. Author manuscript; available in PMC 2021 June 07.

Published in final edited form as:

Curr Opin Cell Biol. 2005 February ; 17(1): 35–46. doi:10.1016/j.ceb.2004.12.009.

Kinetochore–spindle microtubule interactions during mitosis

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Abstract

The kinetochore is a proteinaceous structure that assembles onto centromeric DNA and mediates chromosome attachment to microtubules during mitosis. This description is deceivingly simple: recent proteomic studies suggest that the diminutive kinetochores of *Saccharomyces cerevisiae* are comprised of at least 60 proteins organized into as many as 14 different subcomplexes. Many of these proteins, such as the centromeric histone variant CENP-A, and entire subcomplexes, such as the Ndc80^{Hec1} complex, are conserved from yeast to humans despite the diverse nature of the DNA sequences on which they assemble. There have recently been advances in our understanding of the molecular basis of how kinetochores establish dynamic attachments to spindle microtubules, and how these attachments are correctly oriented to ensure segregation of sister chromatids to daughter cells.

Introduction

The many tasks of the mitotic kinetochore include attaching chromosomes to the mitotic spindle, coupling force production by microtubule polymer dynamics and/or motor proteins to chromosome movement, and inhibiting the anaphase segregation of chromatids until all chromosomes are attached and properly aligned [1,2]. Here, we discuss recent work on the binding of kinetochores to spindle microtubules, on the regulation of the dynamics of kinetochore-attached microtubules, and on the mechanisms that ensure proper orientation of attached chromosomes on the spindle. We will not discuss the spindle checkpoint [2,3] or the specification of kinetochore assembly [4], which have been reviewed recently elsewhere.

Forming and maintaining stable microtubule attachments

The kinetochore forms on centromeric chromatin to generate a microtubule-binding interface that links chromosomes to the mitotic spindle. Numerous proteins and protein subcomplexes have been implicated in proper kinetochore assembly and microtubule attachment [2,5–7], although the precise functions of the majority of these proteins remain unclear. New ideas about how the kinetochore attaches chromosomes to spindle microtubules have emerged from studies of the Ndc80 complex (Ndc80^{Hec1}, Nuf2, Spc24, and Spc25), which is conserved from fungi to humans [8–17,18•,19,20••,21••,22•,23•] (Table 1). The variation in chromosomal architecture and kinetochore-microtubule binding capacity within the animal kingdom [24] makes the conservation of this complex an exciting

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finding. Immunofluorescence and immuno-electron microscopic analyses of vertebrate cells reveal that Ndc80 complex proteins localize to the outer kinetochore plate [14,15,19,25••], where microtubule plus ends terminate [26]. Ndc80^{Hec-1} and Nuf2 are stably bound to kinetochores, as assessed by fluorescence recovery after photobleaching [19], and their levels at the kinetochore remain unchanged during the formation of kinetochore-microtubule attachments [15,19,20••,22•, 25••,27]. These attributes make components of the Ndc80 complex good candidates for playing a direct role in mediating interactions between kinetochores and microtubules.

Defects in the Ndc80 complex disrupt kinetochore-microtubule attachments, chromosome congression and chromosome segregation in all systems that have been analyzed, although the underlying cause is controversial (please see Table 1 and all references therein). In addition to technical variations between studies, particularly with respect to mammalian cell RNAi, difficulties in distinguishing between a role in outer kinetochore assembly versus a direct role in kinetochore-microtubule interactions probably underlie many of the apparently contradictory conclusions. Recent findings in tissue culture cells, budding yeast and *Caenorhabditis elegans* embryos indicate that targeting of most outer kinetochore proteins is largely preserved upon disruption of Ndc80 complex proteins, although some spindle checkpoint protein levels are decreased [12,15,18•,21••,22•,23•,27, 28,29•,30••,31••,32] (Table 1). In contrast, chromosomes assembled in *Xenopus* egg extracts immunodepleted of Ndc80 and Nuf2 fail to localize multiple outer kinetochore components [20••]. However, as these assembly defects have not yet been rescued using purified proteins, the possibility that they result from the depletion of additional interacting proteins cannot be excluded [20••] (Table 1). Recent work in metazoans has identified a larger network of conserved interacting proteins that includes the Ndc80 complex, making this a likely possibility [21••]. In budding yeast, mutational inactivation of Ndc80 complex subunits results in an inability of microtubule-binding proteins, such as the Dam1 complex and Stu2, to localize to kinetochores as judged by chromatin immunoprecipitations [28,29•,32,33]; however, neither the Dam1 complex nor Stu2 can associate with centromeric DNA in the absence of microtubules [34,35•] (Table 1). Thus, the apparent assembly defects following inhibition of Ndc80 complex function are more likely to be a consequence of defects in forming stable microtubule attachments.

Several lines of evidence indicate that microtubule attachments can form when Ndc80 complex proteins are depleted, but these attachments are unstable and cannot support chromosome congression and segregation. In vertebrate cells depleted of Nuf2 or Ndc80^{Hec1}, stable fibers of kinetochore microtubules fail to form, but kinetochore checkpoint proteins, such as Mad1 and Mad2, are still significantly depleted from kinetochores [15,19, 20••,22•,23•,25••,27,30••] (Table 1). Interestingly, drug-induced depolymerization of microtubules restores Mad1 and Mad2 targeting to normal levels, indicating that kinetochores assembled in Nuf2- or Ndc80^{Hec1}-depleted cells are fully capable of binding checkpoint proteins [22•,30••]. However, a recent study claims that with more penetrant depletions of Nuf2 and Ndc80^{Hec1}, checkpoint proteins fail to target even after microtubule depolymerization [36•] (Table 1). Although the current data exclude a stoichiometric role for the Ndc80 complex in the targeting of checkpoint proteins, the discrepancy between the different mammalian cell RNAi studies remains to be resolved.

The microtubule-dependent reduction of checkpoint proteins at kinetochores depleted of Ndc80 complex proteins suggests that these kinetochores can form transient, albeit unstable, microtubule attachments (Figure 1). In support of this idea, an electron microscopic analysis of Nuf2-depleted cells indicates that the outer kinetochore morphology is severely perturbed and the number of embedded microtubule plus ends dramatically decreased in these cells [25••]. Furthermore, analyses of *C. elegans* embryos have demonstrated that partial chromosome alignment and segregation still occur in embryos depleted of Ndc80, Nuf2^{HIM-10} or Spc25^{KBP-3}, although attachments between chromosomes and the spindle are mechanically unstable [18•,21••] (Table 1). In contrast, a complete failure of chromosome segregation occurs in embryos depleted of the more chromatin-proximal kinetochore components, CENP-A^{HCP-3} and CENP-C^{HCP-4} [18•,37].

Cumulatively, these results suggest that the Ndc80 complex does not act as a targeting scaffold for kinetochore proteins, but rather plays a crucial role in stabilizing microtubule attachments by maintaining the structural integrity of binding sites for microtubule plus ends at the outer kinetochore [25••]. Determining how the Ndc80 complex interacts with other components at the kinetochore should provide a more detailed understanding of how mechanically stable microtubule attachments are formed and maintained. For example, recent studies in *C. elegans* have identified two novel kinetochore components, KNL-1 and KNL-3, which are required to target the Ndc80 complex to kinetochores [18•,21••]. In addition, both proteins biochemically associate with components of the Ndc80 complex, and this interaction is conserved in human cells [18•,21••]. Coupled *in vivo* and *in vitro* analyses of the Ndc80 complex and the larger KNL-1/3 interacting protein network should help decipher how stable kinetochore-microtubule attachments are formed.

Growth of kinetochore-attached microtubules

Growth of kinetochore microtubules by the addition of tubulin at the kinetochore is important for chromosome movements prior to anaphase. An example of this type of growth is seen during chromosome congression to the metaphase plate, when microtubule depolymerization at the leading kinetochore is coupled to microtubule polymerization at the lagging kinetochore [1,38]. Kinetochores contain multiple proteins that can promote microtubule growth (for example, see [39–48]), although the physiological contributions of these proteins to kinetochore-microtubule dynamics have not been defined. Recent data have identified the CLASP family of microtubule-associated proteins (MAPs), which includes CLASP1 in humans [49,50•] and MAST/Orbit in *Drosophila* [51,52], as potential regulators of kinetochore-microtubule dynamics. CLASPs localize near microtubule plus ends and stabilize microtubules when overexpressed as GFP-fusion proteins in interphase cells [49,50•]. CLASPs also localize to kinetochores in a microtubule-independent manner and are the outermost kinetochore proteins identified to date [50•,52]. Inhibition of CLASP activity by antibody microinjection or RNAi does not prevent the formation of kinetochore-microtubule attachments, but rather leads to shortened kinetochore microtubules and suppressed chromosome oscillations [50•,52]. Although exactly how the dynamics of kinetochore fibers are perturbed in these cells remains unclear, current data suggest that CLASPs stimulate the growth of kinetochore-attached microtubules (Figure 2a). This idea is

supported by the ability of microtubule-growth-promoting drugs to rescue the short kinetochore microtubule defect in CLASP-inhibited cells [50•,52].

Dissecting how kinetochore-bound CLASP regulates microtubule dynamics may provide molecular clues about the mechanism of chromosome alignment, and perhaps about other occurrences of microtubule growth at kinetochores, such as during microtubule flux [53••]. Addition of tubulin at kinetochore-attached microtubule ends may also prove to be an integral part of mitotic spindle assembly. This idea has arisen from recent observations of outward growth of kinetochore microtubules on unattached kinetochores facing away from the spindle. Kinetochore fibers formed in this manner are translocated poleward, connecting the chromosome to the spindle pole [54••]. This phenomenon occurs both in somatic cells recovering from treatment with a drug that prevents separation of spindle poles and in untreated cells, suggesting that cells possess a kinetochore-based pathway for the formation of kinetochore microtubules that is independent of the capture of centrosome-nucleated microtubules [54••]. In addition, laser cutting experiments performed in grasshopper spermatocytes indicate that kinetochore fibers can grow outward from the kinetochore, and must reach the spindle pole before chromosome segregation can occur [55••]. Overall, these studies suggest that kinetochore-mediated growth of microtubules is an intrinsic activity of kinetochores in multiple systems, where it may play a role in attaching chromosomes to the spindle and/or in chromosome movement on the spindle.

Depolymerization of kinetochore-attached microtubules

Depolymerization of microtubules at the kinetochore is thought to drive poleward movements during the alignment and segregation of chromosomes [1,38]. The most logical candidates for this activity are the kinesin-13 family members (formerly called Kin I kinesins) [56], which display microtubule-end-stimulated ATPase activity to induce depolymerization from either end of the microtubule [57–59,60••]. During mitosis, kinesin-13 proteins are localized to kinetochores and to the centromeric region between sister kinetochores [61,62•]. Kinesin-13 proteins are clearly required for proper positioning of chromosomes on the spindle in both vertebrate and invertebrate species [61,62•,63,64••,65], although their precise role at kinetochores remains controversial. Analysis in *Drosophila* embryos suggests that Klp59C, one of two kinesin-13 proteins in this organism, remains associated with anaphase kinetochores where it may play a role in depolymerizing kinetochore-associated microtubules [64••] (Figure 2b). However, inhibition of MCAK, a kinetochore-localized kinesin-13 in vertebrate cells, does not perturb the rate of poleward chromatid movement during anaphase [62•]. Future work analyzing all kinesin-13 family proteins in vertebrates is necessary to reconcile these observations and to understand how kinesin-13 proteins contribute to poleward chromosome movement.

During mitosis in budding yeast, depletion of the microtubule-binding protein Stu2 suppresses the dynamics of spindle microtubules, including kinetochore microtubules, and results in non-motile centromeres [66•,67]. Kinetochores are not required for the stabilization of spindle microtubules observed in Stu2-depleted cells, suggesting that Stu2 affects microtubule dynamics through a kinetochore-independent mechanism [66•]. These findings imply that Stu2 can destabilize microtubules *in vivo,* an idea which is supported by

in vitro studies demonstrating that recombinant Stu2 can promote microtubule depolymerization [68•] despite its similarity to the XMAP215/TOG family of proteins, which strongly promote microtubule growth *in vivo* and *in vitro* [69]. Surprisingly, XMAP215 has also been shown to destabilize microtubules under specific *in vitro* conditions [70•], highlighting the diversity of the potential interactions between microtubules and members of this MAP family [71]. Whether vertebrate orthologs of Stu2 affect kinetochore-microtubule dynamics is an important question to address in the future. It is also important to note that proteins such as kinesin-13 depolymerases and XMAP215/TOG are prominent global regulators of microtubule dynamics and spindle bipolarity [65,72–79]. Consequently, potential indirect effects of their inhibition must be taken into consideration during phenotypic analysis, and may underlie superficially contradictory results from studies in different systems or studies using different methods of inhibition.

Ensuring proper chromosome orientation through regulation of kinetochore-microtubule attachments

Throughout mitosis, there exists the potential for improper kinetochore-microtubule attachments, which must be resolved to prevent missegregation and aneuploidy [80•]. Proper attachments result in a bi-oriented chromosome, wherein one kinetochore is connected exclusively to microtubules emanating from one spindle pole, while its sister kinetochore is connected to the opposite spindle pole. Such bi-oriented attachments result in tension between the two sister kinetochores [81]. The Aurora B kinase (Ipl1 in budding yeast) has emerged from recent work as a major regulator involved in correcting inappropriate kinetochore-microtubule attachments. Aurora B^{Ipl1} is thought to promote bi-orientation by selectively detaching kinetochore-microtubule attachments that are not under tension [82,83••,84•,85]. Antibody inhibition and small molecule inhibition studies have suggested that this role for Aurora B is conserved in vertebrate cells [86•,87•,88,89,90•]. It is less clear how Aurora B^{Ipl1} recognizes the tension created when proper bi-orientation is achieved, although micromanipulation of spermatocyte kinetochores support the idea that chemical properties of kinetochores, such as phosphorylation, can change in response to tension [91,92]. Biochemical and genetic analyses indicate that the multi-protein Dam1 complex, which is conserved within fungi but not identified elsewhere to date, is the key target of Aurora B^{Ipl1} [93]. Precisely how Aurora B^{Ipl1}-mediated phosphorylation of the Daml complex affects kinetochore-microtubule attachments and how this regulation is selectively targeted to attachments that are not under tension remain important future questions.

Chromosome bi-orientation is also promoted by the cohesin protein complex, which connects sister chromatids and sustains inter-kinetochore tension [94,95]. Surprisingly, in budding yeast any type of connection between sister chromatids that is capable of resisting tension can promote bi-orientation [83••]. Similarly, in vertebrate cells, chromosome alignment defects caused by depletion of cohesin are rescued by concomitant inhibition of topoisomerase II to generate a distinct type of physical linkage by catenation of sister DNA strands [96•]. These findings indicate a direct link between the mechanics and biochemistry of the kinetochore-microtubule interface, an idea further strengthened by recent data identifying the kinetochore protein Sgo as a microtubule-stabilizing protein and a protector

of the cohesive forces between sister centromeres [97•]. Understanding how centromeric tension is generated and detected will be key to defining how Aurora B^{Ipl1p} acts at the kinetochore-microtubule interface to promote detachment (in budding yeast, [83••]) or kinetochore fiber depolymerization (in vertebrate cells, [90•]) as a mechanism to resolve incorrect microtubule attachments.

In all eukaryotes, Aurora B is found in a complex with INCENP and Survivin [98]. This highly conserved chromosomal passenger complex exhibits a dynamic localization at the inner centromere early in mitosis and at the spindle midzone following the metaphase-anaphase transition. New components of this complex have been identified in metazoans, termed CSC-1 in *C. elegans* [99] and Dasra A and Dasra B/Borealin in vertebrates [100•,101•]. Interestingly, recent work suggests that, in addition to its role in promoting bi-orientation, the chromosomal passenger complex also contributes to spindle assembly [89,100•,101•]. In vertebrates, a potential link between Aurora B and both correction of aberrant kinetochore-microtubule attachments and regulation of spindle morphology may be via regulation of the microtubule-depolymerizing kinesin MCAK, a member of the kinesin-13 family. Inactivation of MCAK leads to highly aberrant kinetochore-microtubule attachments [62•] and spindle morphology defects [102]. Aurora B phosphorylates MCAK, promoting its localization to the centromere and inhibiting its microtubule depolymerizing activity (Figure 3) [103••,104••,105••]. In addition to being negatively regulated by Aurora B, MCAK activity is positively regulated *in vitro* by ICIS, which may work in combination with MCAK to depolymerize microtubules contacting the centromeric domain between sister kinetochores [106•]. Taken together, these recent discoveries indicate that the depolymerizing activity of MCAK is subject to a high degree of local regulation in the vicinity of the kinetochore-microtubule interface (Figure 3). An exciting direction for further research will be to investigate how inactivation of MCAK by Aurora B and stimulation of MCAK by ICIS are dynamically used to stabilize or destabilize kinetochore microtubules during chromosome positioning and attachment correction.

Ironically, the mechanism of attachment regulation by Aurora B could be one reason why it is difficult to elucidate exactly how stable kinetochore-microtubule attachments are formed and maintained. This possibility is illustrated in budding yeast mutants of the conserved kinetochore protein $Mis12^{Mtw1}$, which contain unattached chromosomes [84•]. This defect is abolished if Aurora B^{Ipl1} is concomitantly inhibited, indicating that active Aurora B^{Ipl1} is detaching defective kinetochore-microtubule attachments in $Mis12^{Mtw1}$ mutants [84•]. This finding suggests that Aurora B^{Ipl1} inhibition may help to decipher the molecular basis for kinetochore-microtubule attachments. It also highlights the complexity of the regulatory processes at the kinetochore that must be taken into account when analyzing various chromosome segregation defects.

Conclusions and future directions

Dissecting the mechanisms that regulate kinetochore assembly and modulate kinetochore-microtubule attachments is essential to understand chromosome segregation. This will require the combination of precise molecular perturbations with high-resolution assays in living cells. Such efforts will probably be facilitated by recent advances in microscopy [107]

and by the emergence of RNA interference and chemical inhibition as new specific methods for disrupting the functions of essential proteins in metazoans. Together, advances in molecular perturbation techniques and the development of increasingly sophisticated assays are gradually bringing the complexities of kinetochore structure and function into focus.

Update

Recent analyses in *S.pombe* and human cells [108] support work in *C. elegans* and human cells [21••] indicating the existence of a complex of $Mis12^{Mtw1}$-interacting proteins required for chromosome segregation. In humans, a complex isolated from interphase nuclear extracts included the heterochromatic proteins HP1a and HP1g, suggesting a molecular link between kinetochore proteins and centromeric heterochromatin [108].

Two recent papers extend analyses of kinetochore-driven kinetochore fiber assembly. Photobleaching and lasermediated kinetochore fiber cutting experiments in *Drosophila* tissue culture cells indicate that fiber growth can initiate from ‘naked’ kinetochores and connect the kinetochore to the spindle pole via kinetochore-proximal tubulin addition [109]. Interestingly, the microtubule-associated protein CLASP is not required for the initial formation or elongation of kinetochore fibers but is required for polymerization at kinetochores once the fibers reach the pole and initiate flux [110]. This finding indicates the existence of distinct mechanisms that contribute to tubulin addition at kinetochores, and establish CLASPs as integral players in kinetochore fiber dynamics [110].

Acknowledgements

We thank K Oegema for comments on the manuscript. Work in this laboratory is supported by funding from the Ludwig Institute for Cancer Research. SS is supported by an NCI Predoctoral Cancer Cell Biology training grant. AD is a Damon Runyan Scholar supported by the Damon Runyan Cancer Research Foundation (DRS 38-04).

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genomics and mass-spectrometry-based protein identification, the authors identify a network of 10 interacting kinetochore proteins in C. elegans that includes the conserved components Ndc80Hec1, Nuf2HIM10 and Mis12. Functional analysis partitioned this network into three classes and suggested potential roles for each class at the kinetochore. The authors also describe the existence of a related protein network in human cells that includes members of each functional class and four new human kinetochore proteins. For related work, see [18•].

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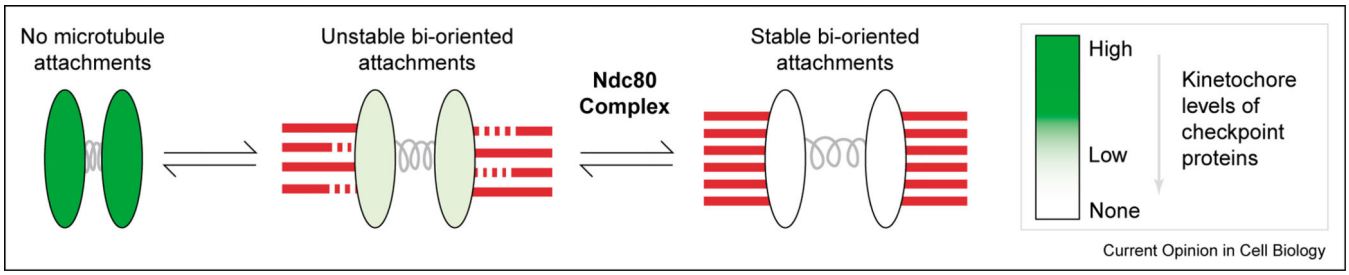

Figure 1.

Formation of stable kinetochore–microtubule attachments. Microtubules are in red and centromeric chromatin is in gray. Kinetochores are indicated by ovals in various shades of green corresponding to the level of kinetochore-localized spindle checkpoint proteins, which are progressively depleted as microtubules attach to kinetochores. With 95% depletion of Ndc80 complex subunits, significant depletion of checkpoint proteins still occurs, suggesting the existence of some type of attachment between kinetochores and spindle microtubules. However, stable kinetochore fibers do not form, and chromosome alignment and segregation is severely perturbed. Thus, the Ndc80 complex plays a critical role in stabilization of microtubule attachments, allowing the formation of mature kinetochore fibers capable of aligning and segregating chromosomes properly.

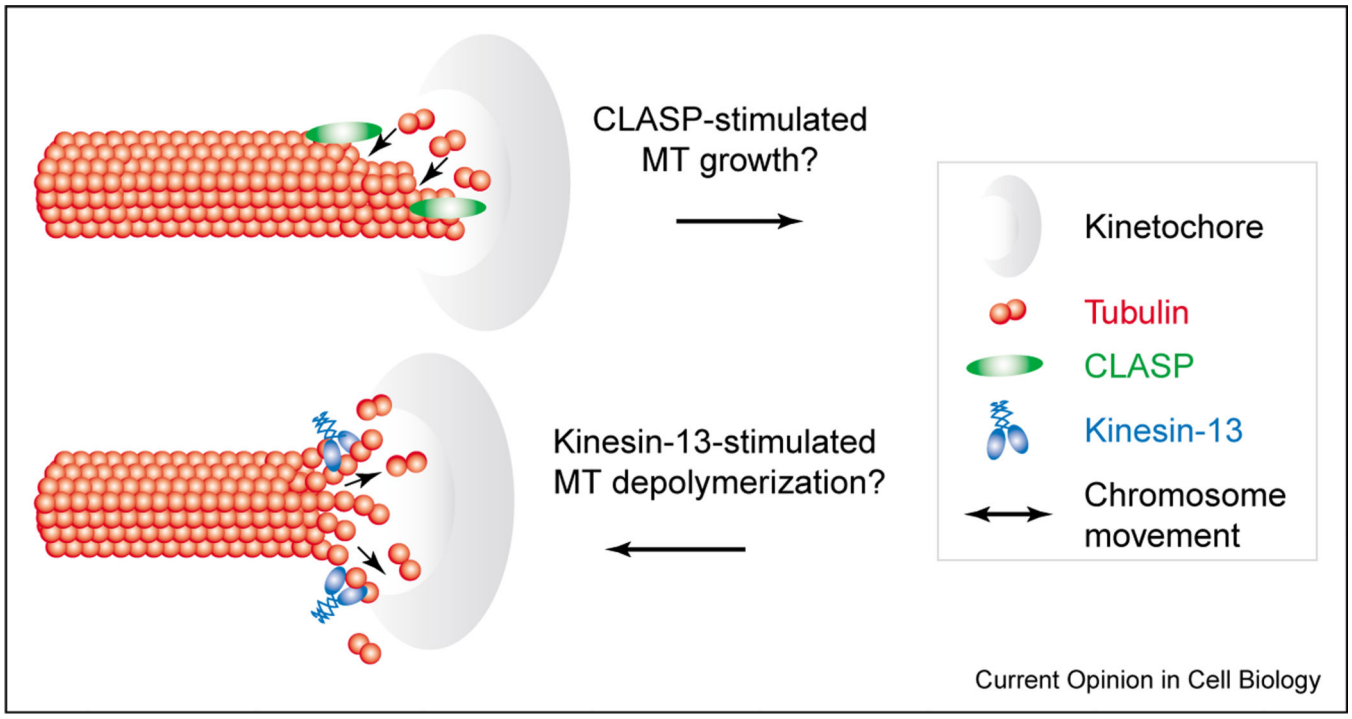

Figure 2.

Regulation of kinetochore–microtubule dynamics. The assembly dynamics of kinetochore-attached microtubules is coupled to chromosome movement on the spindle. Regulators of microtubule dynamics localized to kinetochores, such as the CLASP family of MAPs and the kinesin-13 family of microtubule depolymerases, are likely to play important roles in this process. Kinetochore-localized CLASPs (in green) may stimulate the growth of kinetochore-attached microtubules during anti-poleward movements that align chromosomes at the metaphase plate. Polymerization of kinetochore microtubule plus ends is also necessary for poleward microtubule flux during metaphase. In contrast, the microtubule depolymerase activity of kinesin-13 proteins (in blue) may contribute to poleward chromosome movement, although discrepancies between studies in different systems concerning this putative role need to be resolved. Arrows indicate the predicted direction of chromosome movement. MT, microtubule.

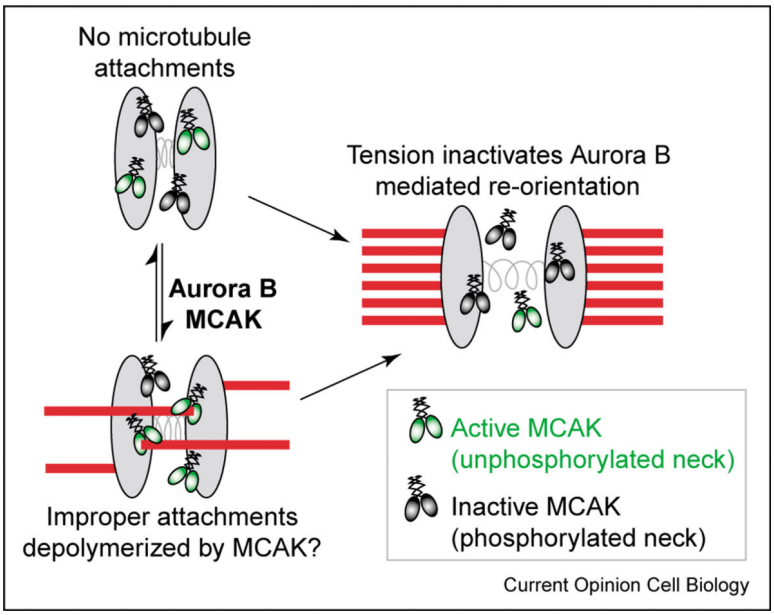

Figure 3.

Error correction mechanisms ensure chromosome bi-orientation on the spindle. Attachment of sister kinetochores to microtubules emanating from opposing spindle poles, a configuration referred to as bi-orientation, is critical to ensure chromosome alignment and segregation. This cartoon depicts how errors in microtubule attachments may be resolved in vertebrate cells by Aurora B kinase and the kinesin-13 protein MCAK, a newly identified substrate of Aurora B. Kinetochores and centromeric heterochromatin are in gray, unphosphorylated MCAK (active microtubule depolymerase) is in green, and MCAK phosphorylated in the neck region (inactive depolymerase) is in black. Throughout mitosis, populations of both phosphorylated and unphosphorylated MCAK exist at the centromere, although the precise localization of active versus inactive MCAK is controversial. One interpretation is that active MCAK is more prevalent when incorrect attachments are present and tension is low. MCAK can then depolymerize inappropriately attached microtubules. Upon attachment of sister kinetochores to opposite spindle poles, the resulting increase in tension prevents Aurora-B-mediated re-orientation, perhaps by affecting local regulation of MCAK activity. In fungi, the Dam1 complex is the key target of Aurora B^{Ipl1} implicated in this error correction process. Such a complex has not been identified in metazoans.

Table 1

Mitotic defects resulting from inactivation of Ndc80 complex proteins in eukaryotic systems.

Species	Complex components	Experimental method	Results	References
			Mitotic progression and morphology	
H. sapiens	Ndc80^{Hec1} Nuf2 Spc24 Spc25	RNAi Nuf2 in HeLa cells	Chromosome alignment and spindle defects. Reduced stability of kinetochore fibers to cold treatment. Reduced inter-kinetochore distances. Poor kinetochore morphology and microtubule binding at EM level [25••]. Active spindle checkpoint.	[15,25••]
		RNAi Spc24, Spc25, Ndc80^{Hec1} in HeLas	Chromosome alignment and spindle defects. Active spindle checkpoint.	[22•,23•,27]
		RNAi Nuf2 in HeLa cells	Chromosome alignment and spindle defects. Active spindle checkpoint with 95% depletion. Inactive spindle checkpoint with more penetrant depletions.	[36•]
			Targeting of kinetochore components	
		RNAi Nuf2, Ndc80^{Hec1} in HeLa cells	Decreased levels of Mad1 and Mad2 targeting to kinetochores restored by nocodazole depolymerization of microtubules.	[22•,30••]
		RNAi Nuf2 in HeLa cells	For penetrant depletions, Mad1 and Mad2 targeting is abolished. Kinetochore localization is not restored upon nocodazole treatment.	[36•]
		RNAi Spc24, Spc25 in HeLa cells	Loss of outer kinetochore proteins, including Mad1 and Mad2, is mostly restored after nocodazole treatment.	[23•]
		RNAi Nuf2, Spc24, Spc25 in HeLa cells	RNAi of Nuf2 [30••] or Spc25 [22•] abolishes Ndc80 binding at kinetochores. RNAi of Spc24 abolishes kinetochore targeting of Spc25 and vice versa [23•]. Localization is not restored upon nocodazole treatment.	[22•,23•,30••]
			Mitotic progression and morphology	
X. laevis	Ndc80 Nuf2 Spc24 Spc25	Injection of a-Nuf2, α-Ndc80 into XTC cells	Failure of chromosome alignment and segregation. Cells exit mitosis prematurely, presumably because spindle checkpoint is inactive. Spindle checkpoint is also inactivated in egg extracts following immunodepletion.	[20••]
		Injection of a-Spc24, α-Spc25 into S3 cells	Chromosome alignment and segregation defects. Decreased inter-kinetochore distances. Active spindle checkpoint.	[23•]
		Immunodepletion of Nuf2, Ndc80 from egg extracts	Targeting of kinetochore components Multiple outer kinetochore proteins fail to target in immunodepleted extracts.	[20••]
			Mitotic progression and morphology	
C. elegans	Ndc80 Nuf2^{HIM-10}, Spc25^{KBP-3}	RNAi of Ndc80, Nuf2^{HIM-10}	Chromosome-microtubule attachments form, but congression and segregation are aberrant. Ndc80, Nuf2, and Spc25 are part of a larger KNL-1/3 complex, which includes Mis12 and 6 novel kinetochore proteins [21••].	[18•,21 ••]
		nuf2^{him-10} mutant	Defective chromosome alignment and disrupted kinetochore structure, as assayed by EM of high pressure frozen embryos.	[11]
			Mitotic progression and morphology	

Species	Complex components	Experimental method	Results	References
S. cerevisiae	Ndc80 Nuf2 Spc24 Spc25	*ndc80, nuf2* mutant and degron alleles	Disrupted kinetochore-microtubule binding. Aberrant centromere movements. Active spindle checkpoint.	[9,10,12,20••,28]
		spc24, spc25 mutants	Disrupted kinetochore-microtubule binding. Spindle microtubule defects. Inactive spindle checkpoint.	[12,14,16]
			Targeting of kinetochore components:	
		ndc80 mutants	Most kinetochore proteins localize in ChIP assays, except Stu2 and the Dam complex (both require microtubules to target to kinetochores [34,35•].	[12,28,29•, 31 ••,32]
		spc24, spc25 mutants	Most kinetochore proteins localize in ChIP assays [12]; Bub1 and Mad2 do not in *spc25* mutants [32].	[12,32]
			Mitotic progression and morphology:	
S. pombe	Ndc80 Nuf2 Spc24 Spc25	*nuf2* mutants	Allele-specific effects on spindle length and spindle checkpoint activity. Defects in centromere motility and chromosome segregation.	[13,17]

Defects in mitotic progression, spindle and kinetochore morphology, and targeting of kinetochore components are organized above according to experimental system and inhibitory method. *H. sapiens, Homo sapiens; S. cerevisiae, Saccharomyces cerevisiae; S. pombe, Schizosaccharomyces pombe; X. laevis, Xenopus laevis*. Please see text for comments.