Interplay between mitotic kinesins and the Aurora kinase–PP1 (protein phosphatase 1) axis

John C. Meadows*1
*Division of Biomedical Cell Biology, Warwick Medical School, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, U.K.

Abstract
Correct transmission of genetic information from mother to daughter cells is necessary for development and survival. Accurate segregation is achieved by bipolar attachment of sister centromeres in each chromatid pair to spindle microtubules emanating from opposite spindle poles, a process known as chromosome bi-orientation. Achieving this requires dynamic interplay between centromere proteins, kinesin motor proteins and cell cycle regulators. Chromosome bi-orientation is monitored by a surveillance mechanism known as the SAC (spindle assembly checkpoint). The Aurora B kinase, which is bound to the inner centromere during early mitosis, plays a central role in both chromosome bi-orientation and the spindle checkpoint. The application of tension across centromeres establishes a spatial gradient of high phosphorylation activity at the inner centromere and low phosphorylation activity at the outer kinetochore. This gradient is further refined by the association of PP1 (protein phosphatase 1) to the outer kinetochore, which stabilizes kinetochore–microtubule interactions and silences the spindle checkpoint by dephosphorylating Aurora B kinase targets when chromosome bi-orientation is achieved. In the present review, I discuss emerging evidence that bidirectional cross-talk between mitotic kinesins and the Aurora kinase–PP1 axis is crucial for co-ordinating chromosome bi-orientation and spindle checkpoint signalling in eukaryotes.

Spatial gradients of Aurora kinase and PP1
Somatic mammalian cells contain two Aurora kinases, Aurora A and Aurora B, whereas fission and budding yeast each contain only one Aurora B-like kinase. In mammalian cells, Aurora A localizes to centrosomes throughout mitosis where it is involved in centrosome maturation, chromosome congression and segregation, microtubule flux, spindle formation and stability [1]. Aurora B kinase, on the other hand, is the catalytic component of a complex of proteins known as the CPC (chromosomal passenger complex) which binds the inner centromere during prometaphase and metaphase [2].

Association of CPC to the inner centromere is thought to create a gradient of phosphorylation activity so that incorrect microtubule–kinetochore attachments bring substrates at the outer kinetochore into close proximity to Aurora B, promoting their phosphorylation [3]. Such events preferentially destabilize aberrant kinetochore–microtubule interactions (for example via phosphorylation of Ndc80/HEC1 [4]) and, secondly, activate the SAC (spindle assembly checkpoint). Inappropriate microtubule detachment is required for subsequent rounds of microtubule–kinetochore reattachment until chromosome bi-orientation is achieved. The application of tension not only causes both an increase in inter-kinetochore distance, but also induces intra-kinetochore stretch, which is thought to be the prime trigger for onset of anaphase [5,6]. However, spatial separation of Aurora B from the outer kinetochore alone is not sufficient, as substrates still need to be dephosphorylated. PP1 (protein phosphatase 1) has emerged as the key enzyme that antagonizes Aurora B kinase both to establish chromosome bi-orientation [7] and to silence the SAC [8]. Upon correct bipolar attachment it is thought that a specific pool of PP1 bound to the kinetochore component KNL1 [9] is important for both of these functions [10–12]. Inactivation of the SAC removes inhibition of the anaphase-promoting complex allowing for destruction of cyclin B and securin and the transition from metaphase to anaphase. At onset of anaphase CPC relocalizes away from the inner-centromere regions to the overlapping antiparallel microtubules of the spindle midzone [2].

Kinesins as regulators and effectors of the Aurora kinase–PP1 gradient
Kinesins are a superfamily containing 14 classes of molecular motors that hydrolyse ATP to carry out work [13]. In most kinesins this work translates as directional translocation along a polar microtubule. This movement is frequently coupled with an ability to carry cargo, which can range from a vesicle to a chromosome. Other kinesins are more specialized and instead of movement along microtubules they act catalytically to depolymerize tubulin subunits from microtubule ends, whereas others cross-link parallel or anti-parallel microtubules by forming dimers or tetramers. The following kinesin families play recognized roles in mitosis:
kinesin-5 and kinesin-14 in spindle formation, kinesin-7 and kinesin-8 in chromosome congression to the metaphase plate, kinesin-6 in spindle midzone formation, and kinesin-13 in controlling the dynamics of kinetochore-attached microtubules. In the present review, I describe emerging links between mitotic kinesins and the Aurora kinase–PP1 spatial activity gradient that shed light on how accurate and timely chromosome segregation is achieved in eukaryotic systems.

Kinesin-7

The mammalian kinesin-7, CENP-E (centromere protein E), is required for congression of chromosomes to the metaphase plate [14,15] following retrieval to the poles by dynein [16]. Kim et al. [17] found that CENP-E is phosphorylated on Thr\(^{422}\) by Aurora A and Aurora B, and that this phosphorylation both reduces the affinity for microtubules and decreases the processivity of the motor; however, it is required for chromosome congression. Phosphorylation of this site also opposes association of CENP-E to PP1 as a PP1-binding motif is nested within the Aurora site, indicating that CENP-E is either phosphorylated or bound to PP1. The proposed mechanism for CENP-E function relies on the Aurora A gradient being high at the poles and decreasing towards the middle of the spindle where the metaphase plate will be established. Following dynein-dependent retrieval to the pole, Aurora A phosphorylates CENP-E due to its proximity to the poles and this action displaces PP1. When Thr\(^{422}\) is phosphorylated CENP-E processivity decreases, ensuring that stable high-density k-fibres are preferred for binding and translocation, promoting movement of chromosomes to the metaphase plate on established k-fibres. At some distance from the pole, and thus the influence of Aurora A, CENP-E is dephosphorylated and able to bind PP1, resulting in an increase in processivity for the motor. Binding of PP1 to CENP-E also results in a high local density of PP1 at kinetochores which may promote dephosphorylation of key Aurora targets such as Ndc80 and KNL1, which in turn would increase their affinity for microtubules and promote end-on attachment.

The role of Aurora B in this mechanism remains largely mysterious as CPC bound at the inner centromeres could theoretically phosphorylate CENP-E at any time during this process. Indeed once the metaphase plate is established, regulation by Aurora B over processivity of CENP-E may be beneficial. Perhaps the large size of CENP-E (~250 nm) enables it to escape phosphorylation by Aurora B at the centromeres, while still being able to couple chromosome movement to the nascent metaphase plate. Importantly, neither budding nor fission yeast contains a kinesin-7 protein. It is tempting to speculate that the smaller size of the mitotic spindle precludes the requirement for such a lateral sliding chromosome congressor.

Kinesin-13

Members of the non-motile kinesin-13 family have their motor domain in the middle of the protein and are ATP-dependent microtubule depolymerases. Somatic mammalian cells usually contain three kinesins-13, Kif2A, Kif2B and Kif2C, or MCAK (mitotic centromere-associated kinesin), depletion of which fails to correct attachment errors [18]. Kif2A, which is negatively regulated by Aurora A [19], binds to centromeres where it is thought to be involved in microtubule flux and disassembly, although this is the subject of some debate [20–22]. Kif2B binds to centromeres, spindles and kinetochores [23]. Kif2C/MCAK binds to kinetochores and weakly to centrosomes [24] and is regulated by Aurora phosphorylation.

MCAK is phosphorylated on Ser\(^{192}\) by either Aurora A or Aurora B and this inhibits its microtubule depolymerase activity by reducing its affinity for microtubules [25–27]. MCAK localization is also controlled by Aurora phosphorylation, with phosphorylation by Aurora B targeting it to centromeres and dephosphorylation favouring kinetochores. However, the interplay between phosphorylation and localization is complex, with some sites needing to be phosphorylated (Ser\(^{125}\)) and others (Thr\(^{29}\)) dephosphorylated for centromere loading [28]. Therefore Aurora B phosphorylation appears to both inhibit the depolymerase activity and promote localization of MCAK to the centromeres. Perhaps this complexity hints at the spatial and temporal regulation necessary to preferentially correct erroneous k-fibre microtubule–kinetochore attachments. Indeed the local action of phosphatases could potentially activate MCAK without disrupting its localization by dephosphorylating certain sites but not others.

As well as direct regulation via Aurora kinase phosphorylation, MCAK is also indirectly controlled through the Aurora–PP1 axis. Aurora B phosphorylation of Sgo2 is required for centromeric MCAK localization [29], whereas PP1–Repo-Man dephosphorylates centromosomal histones to regulate Aurora B and MCAK targeting in an interaction that is itself regulated by Aurora B [30,31]. However, just like kinesin-7, neither budding nor fission yeast contains a member of the kinesin-13 family. This may be because yeast kinetochores bind far fewer microtubules (one in budding yeast, and three or four in fission yeast), so the risk of merotelic microtubule–kinetochore interactions is either lower or absent.

Kinesin-8

We recently found that, in addition to KNL1–PP1, association of PP1 to two members of the kinesin-8 family in fission yeast, Klk5 and Klk6, via conserved binding motifs in their C-terminal tails is important for establishing chromosome bi-orientation and silencing the spindle checkpoint [11]. Although it was previously thought that kinesin-8 family members were exclusively microtubule depolymerases [32–34], it is now believed that they act instead as dynamicity factors for microtubules, in that they can both increase the polymerization and depolymerization rate [35,36]. Several members of this class of kinesins (including mammalian Kif18A and budding yeast Kip3) have a second microtubule-binding site in the tail region that increases the processivity of the motor domain [37–39]. In all cell
types so far examined, kinesin-8 motors are crucial for regulating spindle length, chromosome congression, accurate segregation and the timing of onset of anaphase [40–43]. Although both Kif18A and Kip3 contain PP1-binding motifs in their C-terminal tails, it is currently unknown whether they bind PP1 or whether the role of kinesin-8–PP1 in spindle checkpoint silencing and chromosome segregation is conserved. Nevertheless, these results suggest that, at least in fission yeast, two distinct pools of PP1, one kinetochore-bound and one kinesin-bound, are needed to antagonize Aurora B activity to both establish chromosome bi-orientation and silence the SAC. It is presently unclear whether the CENP-E–PP1 also contributes to silencing of the SAC in mammalian cells.

Although the pool of PP1 bound to kinesin-8 motors antagonizes Aurora B kinase, it is currently unknown whether Aurora B reciprocally regulates kinesin-8 PP1-binding and/or motor function. For this reason it is important to determine whether members of the kinesin-8 family are substrates of Aurora B kinase. To add to this complexity, kinesins-8 can also interact with members of other kinesin families. Kif18A has been reported to bind to and stabilize CENP-E [44], whereas Kif18B has been shown to deliver MCAK to the plus ends of microtubules [45] and, intriguingly, the interaction between Kif18B and MCAK is negatively regulated by Aurora activity.

Kinesin-6

At the metaphase-to-anaphase transition CPC relocalizes from the inner-centromere region to the overlapping anti-parallel microtubules of the mitotic spindle, effectively disestablishing the Aurora B–PP1 kinetochore gradient. This relocalization is dependent on the mammalian kinesin-6 family member Mklp2 and Aurora B activity, but is negatively regulated by Cdk1 activity [46,47]. In the absence of Mklp2, CPC is retained at centromeres during anaphase B, which triggers the rebinding of several SAC components, including Bub1, BubR1 and Mps1, to the kinetochore [48]. It has therefore been proposed that Mklp2-mediated relocalization of CPC from centromeres to the spindle midzone helps to prevent reactivation of the SAC after the onset of anaphase. Consequently both kinesin-8 and kinesin-6 family members can antagonize the spindle checkpoint, but by different mechanisms. Whereas kinesin-8 brings PP1 to the kinetochore to antagonize Aurora B-mediated phosphorylation, kinesin-6 takes Aurora B, and other components of the CPC, away from centromeres at the onset of anaphase. This latter mechanism is not conserved in budding yeast as this organism lacks a member of the kinesin-6 family. However, recent evidence suggests that in fission yeast the timing of anaphase onset is delayed in the absence of Klp9, the single kinesin-6 in Schizosaccharomyces pombe, and that Klp9, like Mklp2, binds the CPC at the spindle midzone during anaphase B (J.C. Meadows, unpublished work).

Figure 1 | Model highlighting the known interactions between mitotic kinesin families and the Aurora–PP1 axis

At least four families of kinesins are either differentially regulated by or regulate the Aurora–PP1 axis themselves. Kinesins-8, Klp5 and Klp6, recruit a population of PP1 to the kinetochore, whereas kinesin-13, MCAK, activity is directly regulated by Aurora phosphorylation. Kinesin-7, CENP-E, is both directly regulated by Aurora phosphorylation and also recruits PP1 to kinetochores in a mutually exclusive manner. Finally, kinesin-6, Mklp2, acts to remove Aurora B from the inner centromere at the metaphase-to-anaphase transition, thus disestablishing the Aurora B–PP1 axis.

Conclusions

In summary, it is clear that mitotic kinesin function can be altered by Aurora B-mediated phosphorylation (kinesin-7 and kinesin-13) and that kinesins can influence the cell-cycle-dependent localization of Aurora B (kinesin-6) and its antagonist PP1 (kinesin-8) (Figure 1). Thus reciprocal regulation of the Aurora–PP1 axis by mitotic motors provides a mechanism to couple force generation at the kinetochore with signal transmission to the cell cycle machinery. It is notable, however, that members of two of the kinesin families mentioned above (kinesin-7 and kinesin-13) exist in mammalian cells but not in yeast, and members of the kinesin-6 family are found in mammalian cells and fission yeast, but not in budding yeast. One possibility is that fungal kinesins-8 may perform some of the functions provided by kinesin-13 (in terms of depolymerase activity) and kinesin-7 (in terms of PP1 association). Regardless, this suggests that the interplay between Aurora B kinase and the kinesin-8–PP1 complex may lie at the heart of how chromosome bi-orientation and spindle checkpoint signalling are co-ordinated in all eukaryotes. Therefore further dissection of this relationship is clearly merited.

Importantly, Aurora kinases and several mitotic kinesins are overexpressed in specific types of human cancers and are thought to contribute to tumorigenesis. For this reason both classes of enzyme are of interest to the pharmaceutical industry as the targets of small-molecule inhibitor drug trials [49,50]. Although it is difficult to predict the full extent of the interactions between the Aurora–PP1 axis and
kinesins, the present review highlights the fact that their functions are intimately intertwined. However, to gain an understanding of how to preferentially perturb this system for therapeutic benefit, much further work is needed to dissect this fascinating and multifaceted relationship.

Acknowledgements
I apologize to those researchers whose work I could not cite due to space constraints. I thank Jonathan Millar and Andrew McMains for their critical reading of this paper.

Funding
J.C.M. is funded by a Global Research Fellowship from the Institute of Advanced Study, University of Warwick.

References


Received 9 August 2013
doi:10.1042/BST20130191