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**Formin mDia3**

A novel target for Aurora B kinase

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**Stable attachment of kinetochores to spindle microtubules is essential for accurate chromosome segregation.** We have shown that a kinetochore-associated formin protein, mDia3, contributes to the generation of stable kinetochore-microtubule attachment. The published report reviewed here shows an essential role of mDia3 in achieving metaphase chromosome alignment, and this function is directly regulated by Aurora B phosphorylation. Aurora B is a central component during the capture of spindle microtubules by kinetochores, in which it selectively eliminates incorrect attachments by phosphorylating a group of microtubule binding proteins at kinetochores to reduce their microtubule binding affinity. Here, we discuss the roles of Aurora B kinase and its substrates in achieving proper kinetochore-microtubule attachment.

Accurate chromosome segregation requires proper stable attachment of spindle microtubules to kinetochores, in which one sister kinetochore attaches microtubules from one pole of a bipolar spindle while the other sister kinetochore is attached to microtubules from the opposite pole. Improper kinetochore-microtubule attachments occur in mitosis. A mitotic kinase, Aurora B, has been shown to be critical for correcting improper attachments. In budding yeast, Ipl1, the yeast homolog of Aurora B, facilitates biorientation by promoting turnover of kinetochore microtubules until tension is generated when sister kinetochores are attached to opposite spindle poles. In vertebrates, inhibiting Aurora B kinase activity with small molecules or depleting Aurora B with siRNA results in an increase of numerous monooriented chromosomes with syntelic attachment. Aurora B exists in a complex called the chromosomal passenger complex (CPC), which also includes INCENP, Survivin and Borealin/DasraB. Aurora B kinase activity requires interaction with its binding partner INCENP. Further, TD-60, an inner centromere protein, has also been suggested to be essential for Aurora B activation. In addition, it has been shown that Mps1, a mitotic checkpoint kinase, directly phosphorylates Borealin/DasraB and this phosphorylation has been proposed to be crucial for full Aurora B kinase activity in chromosome alignment.

Although specific functions in the control of Aurora B activity have been suggested for many of the auxiliary proteins, a current model has suggested that a spatial separation of Aurora B relative to its substrates, rather than an intrinsic kinase activity change, is how the proper attachments are stabilized and the improper attachments are corrected. Under this “spatial separation” model, a pair of bioriented sister kinetochores will produce enough force (interkinetochore stretch or tension) to separate Aurora B kinase at the inner centromere from its outer kinetochore substrates. In contrast, when sister kinetochores are attached to the same spindle pole (syntelic attachment) without interkinetochore stretch (tension), the outer kinetochore is constitutively phosphorylated by Aurora B and microtubule attachments cannot be stabilized. The caveat of this model is how kinetochore can form correct stable microtubule
attachments with a constitutively high level of Aurora B kinase activity before they are able to produce interkinetochore stretch (tension).

Another key unsolved issue is what are downstream players that help correct chromosome attachment errors? One of the first key substrates proposed for Aurora B kinase in this process is mitotic centromere-associated kinesin (MCAK), a microtubule depolymerase. Depletion of centromeric MCAK with a centromere dominant-negative protein in mammalian cultured cells results in kinetochore-microtubule attachment defects, including merotelic and syntelic attachments. Further, MCAK is enriched at merotelic attachments. These results would make MCAK an attractive candidate to depolymerize improperly attached microtubules upon Aurora B activation were it not that Aurora B phosphorylation of MCAK actually inhibits its microtubule depolymerization activity. Other substrates of Aurora B involved in attachment error correction include the KMN (KL1/Mis12 complex/Ndc80 complex) network, which has been shown to constitute the core microtubule binding sites at the kinetochore. The phosphorylation of these microtubule binding proteins by Aurora B reduces their affinities for microtubules to facilitate destabilization of improper attachments.

Using purified components and cultured cells, we have shown that a kinetochore-associated formin protein, mDia3, is a novel Aurora B substrate at the kinetochore. The formin proteins are well known for their actin nucleation and filament elongation activities in mediating actin dynamics, and are implicated in numerous actin-based cellular functions, including cytokinesis, cell morphogenesis, cell polarity and cell migration. The mDia3 formins have also recently emerged as prominent regulators of the microtubule cytoskeleton involving interphase and mitotic cells. We have demonstrated an essential role for mDia3 in stable kinetochore-microtubule attachment and this function is directly regulated by Aurora B phosphorylation of mDia3. Aurora B phosphorylates mDia3 in vitro, and this phosphorylation reduces mDia3 abilities to bind microtubules and stabilize microtubules against cold-induced disassembly. Using a phosphospecific antibody against one of the Aurora B phosphorylation sites, Ser196, phosphorylated mDia3 are observed on unattached kinetochores during prometaphase, and this phosphorylation level is substantially reduced upon treatment of ZM447439, a small molecule inhibitor of Aurora B kinase, and on metaphase aligned kinetochores. Furthermore, similar to what has been shown for Hec1 (a component of the Ndc80 complex), cells expressing nonphosphorylatable mutants of mDia3 exhibit many unaligned chromosomes.

Characterization of cells expressing a phosphomimetic mDia3 mutant provides more insights into functional roles of mDia3 at the kinetochore. These cells are able to form end-on kinetochore-microtubule attachment and congress to the metaphase plate; however, they can only generate about half the tension compared to cells expressing wild-type mDia3, resulting in a delay of anaphase onset with BubR1, an essential mitotic checkpoint kinase, present at metaphase kinetochores. The reduced tension suggests that the nonphosphorylated mDia3 plays a role in force generation between sister kinetochores upon microtubule attachment. Furthermore, using a chemical based assay in combination with live cell imaging, we have shown that about 30% of cells expressing the 4E-mDia3 mutant were able to form a metaphase plate without any obvious misaligned chromosomes even in the presence of an Aurora B inhibitor (ZM447439); whereas none of cells expressing the wild-type mDia3 could position all chromosomes at the metaphase plate (Zhang J, Cheng L, Mao Y, unpublished data). This result does not suggest that mDia3 is the only substrate for Aurora B in attachment error correction, since about 70% of cells expressing the 4E-mDia3 mutant still cannot position all chromosome to the metaphase plate. However, this result does argue that the formin mDia3 is one of the essential components at the kinetochore to stabilize microtubule attachment. Kinetochore-microtubule attachment and subsequent tension generation have not been assessed in detail in cells expressing phosphomimetic mutants of the KMN network, though expression of a phosphomimetic Ndc80 mutant results in an accumulation of cells undergoing a mitotic arrest. Therefore, one challenge for future studies will be to understand how Aurora B kinase integrates different functions from these kinetochore-associated microtubule binding proteins.

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