Chromosome orientation

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Precise chromosome segregation during cell division results from the attachment of chromosomes to microtubules emanating from both poles of the spindle apparatus. The molecular machinery involved in establishing and maintaining properly oriented microtubule attachments remains murky. Some clarity is now emerging with the identification of Bod1 (Biorientation Defective 1), a protein that promotes chromosome biorientation by unleashing chromosomes from improperly oriented microtubule attachments.

Accurate chromosome segregation is required for cell and organism viability because errors are irreversible and cause aneuploidy. The microtubule-based structure called the spindle is responsible for chromosome segregation during mitosis. Spindle microtubules attach to chromosomes through unique structures called kinetochores that form as paired structures adjacent to centromeric heterochromatin (Fig. 1). To segregate faithfully, sister kinetochores on each chromosome must attach to microtubules from opposite poles of the spindle so that sister chromatids move to opposite daughter cells when they disjoin in anaphase. The process of establishing and maintaining the proper bioriented attachment of chromosomes to spindle microtubules is complex, and it frequently proceeds through intermediate stages of inappropriate attachment (Fig. 1). The intermediates are transient, but little is known about the molecular machinery that promotes their conversion to correct bioriented attachments. Insight into the problem is now provided by new data (see Porter et al. on p. 187 of this issue) that identify Bod1, a protein that promotes the correction of inappropriate kinetochore–microtubule attachments.

During mitosis in mammalian cells, chromosomes hold no microtubule attachment until they are released into the cytosol at the transition from prophase to prometaphase. This transition is marked by breakdown of the nuclear envelope, and two complementary mechanisms work to generate microtubule attachment to kinetochores (Wadsworth and Khodjakov, 2004). In one mechanism, the dynamic plus ends of microtubules emanating from centrosomes contact kinetochores to form relatively stable attachments. In the other, short microtubule stubs associate with kinetochores and are subsequently stabilized and grow outward to be focused at spindle poles. These two mechanisms are sufficiently robust to ensure efficient kinetochore–microtubule attachment, but they are both inherently stochastic. Consequently, improper attachments are frequently made during early stages of spindle assembly, as shown in Fig. 1. Monotely has been directly visualized in living cells and arises when one kinetochore forms microtubule attachments before its sister (Rieder and Salmon, 1998). Merotely is relatively common in early mitosis and, if uncorrected, leads to lagging chromatids at anaphase that are easily detected in the spindle midzone after all other chromatids have disjoined and moved poleward (Cimini and Degrassi, 2005). Syntely can arise if chromosome orientation favors the capture of centrosomal microtubule plus ends from the same pole or if pole-focusing mechanisms pull microtubules of sister kinetochores toward the same pole. The frequency of syntely in unperturbed cells is currently unknown, in part because syntelic attachments are difficult to visualize in live cells.

Porter et al. (2007) used proteomics to identify chromosome-associated proteins. This approach will surely identify proteins involved in chromosome structure, but one of the first unique proteins identified through this approach localizes to kinetochores and spindle poles during mitosis. When cells are depleted of this protein using RNAi, they are delayed in exiting mitosis and display persistent, unaligned chromosomes on bipolar mitotic spindles. Careful imaging revealed that many unaligned chromosomes possess syntelic attachments to spindle microtubules, leading Porter et al. (2007) to name this protein Bod1 for Biorientation Defective 1. The syntelic chromosomes in Bod1-deficient cells oscillate poleward and antipoleward, validating their persistent attachment to spindle microtubules and demonstrating that kinetochores retain force-generating capacity. However, syntelic attachments fail to resolve into amphitelic attachments. The striking aspect of these findings is that, to date, the deficiency of no other protein is known to cause persistent syntelic chromosome attachments in mitosis. It is highly likely that Bod1-deficient cells also have persistent merotelic attachments based on the defective anaphases presented, but the authors did not score that explicitly. The frequency of chromosome alignment defects in Bod1-deficient mitotic cells was variable from cell to cell, but that is expected because kinetochore–microtubule attachment is a stochastic process.

An open question is whether Bod1 actively discourages syntelic attachments at kinetochores or whether it works to correct syntelics that ordinarily arise out of the stochastic process of kinetochore–microtubule attachment. This distinction is not

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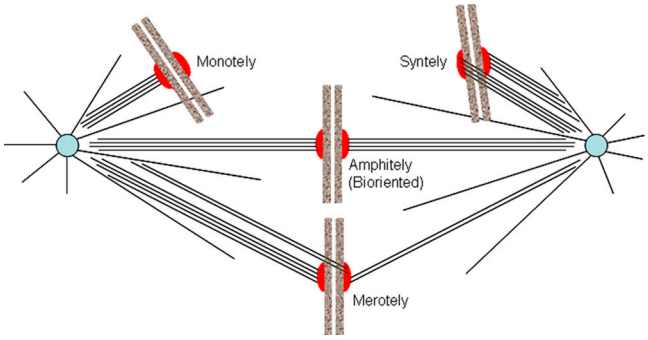

Figure 1. **Chromosome orientation on mitotic spindles.** In mammalian cells, multiple microtubules attach to each kinetochore (red). Precise chromosome segregation requires sister kinetochores on chromosomes to attach to microtubules from opposite spindle poles, leading to biorientation or amphitely. Incorrect orientations can range from single unattached kinetochores (montely) or single kinetochores with microtubule attachments to both spindle poles (merotely) to both sister kinetochores attached to the same spindle pole (syntely).

easy to resolve experimentally because syntelic attachments are difficult to detect in live cells, but the data hint that Bod1 serves to correct improper attachments. For example, forcing spindle formation to proceed through a monopolar intermediate substantially increases the frequency of syntelic chromosomes (Lampson et al., 2004). Under those conditions, Bod1-deficient cells establish a bipolar spindle but display elevated numbers of syntelic chromosomes, indicating a failure to correct improper attachments. Moreover, there is persistent syntelic chromosome attachment in Bod1-deficient cells despite chromosome oscillation and movement. This suggests that correction mechanisms are lacking or weakened in Bod1-deficient cells, and possible pathways for Bod1-mediated syntelic correction are presented in Fig. 2. This point may seem subtle, but it is important because Bod1-deficient cells may provide a means to estimate the relative frequency of syntelic attachments during early stages of spindle formation.

Correction of improper kinetochore attachments is intimately tied to the rate at which kinetochores release microtubules (Nicklas and Ward, 1994) and is regulated by Aurora B kinase activity (Lampson et al., 2004; Pinsky and Biggins, 2005). The release rate is probably tied to tension such that strong tension across amphitelic kinetochores suppresses microtubule release, favoring the retention of kinetochore microtubules and stabilizing appropriate attachments (Pinsky and Biggins, 2005). Syntelic kinetochores do not experience the same level of tension and fail to suppress microtubule release, which favors the correction of improper attachments. In this context, it is interesting to note that the quantity of GFP-Bod1 at kinetochores decreases in metaphase and anaphase compared with prometaphase. If Bod1 plays a role in correcting improper attachments by influencing microtubule release, as suggested above, perhaps high levels at kinetochores are unnecessary once stable, amphitelic attachments are attained. This idea raises several testable hypotheses. First, Bod1-deficient cells should have reduced rates of kinetochore–microtubule turnover compared with control cells. Second, the abundance of Bod1 at kinetochores may respond to tension.

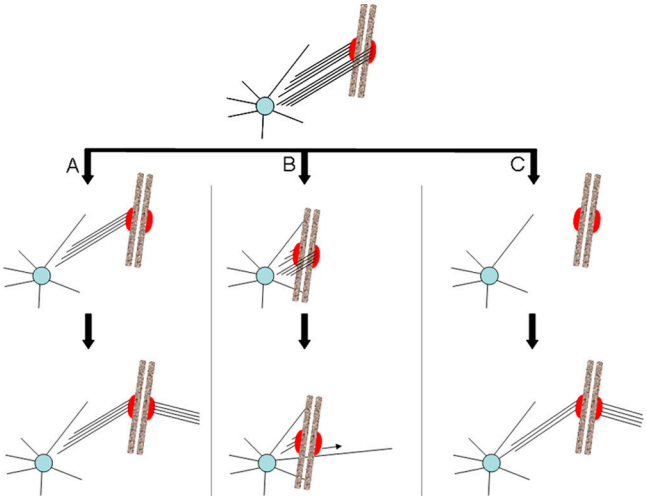

Figure 2. **Potential mechanisms for the correction of syntelic attachments by Bod1.** (A) Bod1 may induce the release of microtubules from one kinetochore so that appropriate attachments can be made to the opposite spindle pole. (B) Bod1 may promote the shortening of kinetochore microtubules, bringing the chromosome to the spindle pole. The chromosome could then release short microtubules and move to the metaphase plate for biorientation via kinetochore transport on adjacent microtubules (arrows), as demonstrated previously (Kapoor et al., 2006). (C) Bod1 may induce the release of microtubules from both kinetochores to permit new attachments with correct orientation toward both spindle poles.

To gain insight into why Bod1-deficient cells sustain improper kinetochore–microtubule attachments, Porter et al. (2007) examined the localization of the microtubule-depolymerizing kinesin-13 protein mitotic centromere-associated kinesin (MCAK). MCAK localizes to centromeres and inner kinetochores and participates in releasing microtubules from kinetochores through a mechanism regulated by the conserved Aurora B kinase (Gorbsky, 2004). Bod1-deficient cells display no substantial change in total MCAK at centromeres but are reduced in the quantity of phosphorylated MCAK. Moreover, localization of the remaining phosphorylated MCAK is disturbed at centromeres. Thus, Bod1 deficiency alters the behavior of MCAK, although this most likely represents an indirect effect because Bod1 and MCAK localize to different positions on the kinetochore. Bod1 may be an Aurora kinase substrate, influence the substrate selection of Aurora kinase, or alter the activity of one of the other kinesin-13 family members that are expressed in human cells (Manning et al., 2007).

Because of the stochastic nature of microtubule binding to kinetochores, the correction of inappropriate attachments is critical for faithful chromosome segregation. The identification of Bod1 opens new doors to the molecular analysis of this process. Bod1 is one component of a large complex, and the identities of its binding partners may reveal further molecular details and provide additional tools. Correction of kinetochore–microtubule attachment errors is likely to be a complex process for mammalian cells in which each kinetochore binds multiple microtubules. Bod1 appears to be a key piece that will help solve this complex puzzle.

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