Dynein at kinetochores: Making the connection

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Dynein removes the checkpoint proteins from kinetochores once chromosomes are bioriented. In this issue, Gama et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201610108) and Mosalaganti et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201611060) reveal the molecular basis for how dynein and its adaptor protein Spindly are recruited to the ROD–Zw10–Zwilch complex in the fibrous corona of unattached kinetochores.

As the cell enters mitosis, its chromosomes need to achieve biorientation so they can be segregated to the opposite poles of daughter cells. Each sister chromatid is connected at the centromeric constriction to its kinetochore, a large macromolecular structure that attaches the spindle microtubules to the chromosomes. The kinetochore harnesses the energy of microtubule depolymerization to drive chromosome movement. To ensure accurate chromosome segregation, the kinetochore must both act as a structural scaffold to bind microtubules and monitor and signal the attachment status. The composition and architecture of the kinetochore is now well defined (Cheeseman, 2014). At mitotic onset, the outer kinetochore, consisting of the Knl1–Mis12–Ndc80 (KMN) complex network, assembles onto the inner kinetochore constitutive centromere-associated network (Cheeseman, 2014). Unattached kinetochores have an additional layer termed the fibrous corona. The corona expands into crescents around the unattached outer kinetochore, which facilitates the capture of kinetochores by incoming microtubules. The fibrous corona consists of the ROD–Zw10–Zwilch (RZZ) complex and Mad1/Mad2 proteins, whose function is to sense and signal unattached kinetochores. Additionally, CENP-E and CENP-F localize to the corona and capture incoming microtubules to generate an initial kinetochore–microtubule attachment. The RZZ complex also recruits Spindly, a kinetochore-specific dynein adaptor that itself brings in dynein (Griffis et al., 2007), but the molecular details of RZZ complex formation were unknown.

The RZZ complex plays a pivotal role in coordinating checkpoint silencing and stabilization of the kinetochore–microtubule attachment. To reduce the probability of merotely, when a kinetochore becomes erroneously attached to the two poles, both Aurora B kinase phosphorylation and the RZZ complex initially inhibit the KMN network. As the correct lateral kinetochore–microtubule attachment is stabilized, the KMN network is dephosphorylated and binds directly to microtubules to generate a robust end-on kinetochore–microtubule attachment. In parallel, Spindly and dynein play critical roles in disassembling the corona and silencing the spindle checkpoint (Griffis et al., 2007). The Spindly–dynein complex uses its minus end–directed properties to transport the kinetochore-localized checkpoint proteins along the microtubules toward the centrosomes. However, mammalian dynein alone cannot walk on microtubules. Dynein associates with its essential cofactor, dynactin, but also requires the binding of cargo adaptors for processive movement (McKenney et al., 2014; Schlager et al., 2014). Structural work on the cargo adaptor BicD2 has shown that a long coiled-coil domain of BicD2 runs along the length of the dynactin filament and stabilizes the dynein–dynactin complex (Urnavicius et al., 2015). Spindly shares sequence similarities with BicD2 and other cargo adaptors, suggesting a shared function, and Spindly can activate mammalian dynein motility in vitro (McKenney et al., 2014).

How the RZZ complex mechanistically contributes to corona assembly at kinetochores and the recruitment of dynein has been unclear because of our limited understanding of the structural organization of the fibrous corona proteins. In this issue, Gama et al. and Mosalaganti et al. now define the molecular mechanism that underlies the RZZ-dependent recruitment of Spindly and dynein to kinetochores. They dissect the molecular architecture of the RZZ complex and demonstrate the direct binding of Spindly to the RZZ complex in vitro in human cells and in *Caenorhabditis elegans*. Mosalaganti et al. (2017) obtained a structural model of the RZZ complex from cryo-EM at 10.4 Å. To further understand the structural arrangement of the RZZ complex, they docked the previously determined Zwilch crystal structure (Çivril et al., 2010) as well as the homology models they generated for ROD and Zw10 into the cryo-EM–derived envelop of the full RZZ complex. Combining cryo-EM, cross-linking mass spectrometry, and structural modeling studies, Mosalaganti et al. (2017) show that the RZZ complex has a 2:2:2 stoichiometry. It forms an elongated complex of ∼42 × 10 × 10 nm with twofold symmetry perpendicular to the coiled-coil of ROD. Two ROD molecules associate in an antiparallel fashion, with their β-propellers on either end of an extended α-helical coil domain. Each Zwilch molecule directly contacts one ROD β-propeller domain and the C terminus of the other ROD in the complex. ZW10 lies in the center of the RZZ complex, interacting with the coiled-coil of ROD. Structural homology modeling of ROD found it to be structurally related to Clathrin, COP1, Sec31, and the nucleopore proteins Nup155 and Nup145, which all use their β-propeller domains to form self-assembling scaffolds at or near cellular membranes. This structural arrangement of ROD suggests it may oligomerize to assemble and expand the fibrous corona

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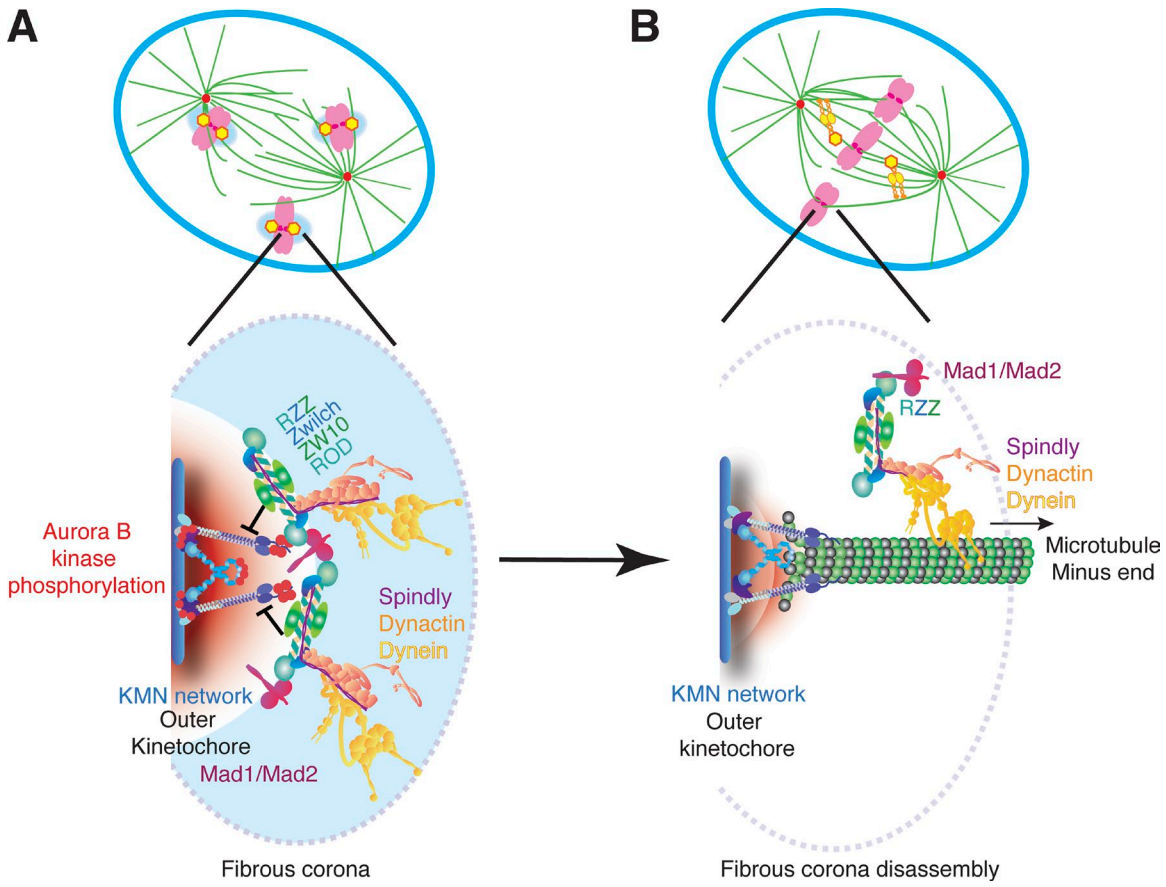

Figure 1. **The Spindly–dynein–dynactin complex binds to the RZZ complex and initiates the disassembly of the fibrous corona and removal of checkpoint proteins upon kinetochore–microtubule attachment.** (A) At unattached kinetochores, binding of the KMN network to microtubules is inhibited by the presence of the RZZ complex and by Aurora B kinase phosphorylation. The RZZ complex self-assembles into the fibrous corona and recruits the spindle checkpoint proteins Mad1/Mad2 and Spindly, which in turn recruit dynein. (B) Upon stabilization of a kinetochore–microtubule attachment, the Spindly–dynein–dynactin complex walks to the minus end of microtubules with its RZZ and spindle checkpoint cargo. Thus, dynein disassembles the fibrous corona and silences the spindle checkpoint by "stripping" the checkpoint proteins away from the outer kinetochore.

around the outer kinetochore in a similar manner to these cytosolic coats. Supporting this idea, Gama et al. (2017) show that in *C. elegans*, the ROD β-propeller and Zwilch are necessary for the expansion of the fibrous corona. Future work should test how the RZZ complex may oligomerize to assemble the corona, which then recruits the checkpoint proteins and other corona-associated proteins.

Gama et al. (2017) and Mosalaganti et al. (2017) reveal how Spindly and dynein are targeted to the fibrous corona at unattached kinetochores. Both groups report that the C terminus of recombinant Spindly interacts directly with the N-terminal β-propeller region of the ROD subunit using *C. elegans* and human proteins. In human cells, Spindly binding to the RZZ complex requires the cysteine farnesylation of its C terminus (Moudgil et al., 2015). Mosalaganti et al. (2017) demonstrate that Spindly farnesylation strengthens an otherwise weak RZZ–Spindly interaction. They identify the β-propeller domain of ROD as the farnesylation receptor using an elegant chemical biology approach. However, short farnesylated peptides do not bind the RZZ complex, suggesting that the amino acids flanking the farnesylated cysteine are necessary but not sufficient for the binding of Spindly to the RZZ complex. Additional Spindly regions must contribute to its kinetochore targeting. Interestingly, Gama et al. (2017) find that in *C. elegans*, Zwilch additionally supports the binding of Spindly to the RZZ complex. Mutation of two conserved surface-exposed residues in Zwilch (E433A and E437A) prevents its binding to Spindly. These surface-exposed residues are conserved in humans, suggesting this Spindly–Zwilch interface may be conserved across species (Çivril et al., 2010). Although the C terminus of Spindly interacts with the RZZ complex to target to kinetochores, the N terminus of Spindly is necessary for the subsequent recruitment of dynein to kinetochores. The first coiled-coil of Spindly (CC1) is highly conserved in dynein adaptors. Mutation of two conserved alanines in the CC1 region abrogates the binding of BicD2 to dynein light intermediate chain (LIC; Schlager et al., 2014). Gama et al. (2017) tested whether the CC1 box of Spindly was also important for binding to dynein LIC1 by mutating the two key conserved alanines in the CC1 box to valines. They found that these mutations were sufficient to disrupt the binding of Spindly to dynein LIC1.

Previous work has indicated that an additional mutation in the Spindly motif, a region C-terminal to the CC1 box and conserved in other cargo adaptors, prevents the recruitment of dynein–dynactin to kinetochores in human cells (Cheerambathur et al., 2013). Gama et al. (2017) find that mutation of the Spindly motif does not affect the LIC–Spindly interaction in vitro. Using the structure of BicD2 bound to dynein and dynactin (Urnavicius et al., 2015), they hypothesized that the Spindly motif is close to the dynactin pointed-end complex, which is

capped by a complex of four proteins: Arp1, p62, p27, and p25. They reconstituted this dynactin pointed-end complex in vitro and showed that Spindly indeed binds to the pointed-end complex in a Spindly motif–dependent manner. Overall, Gama et al. (2017) demonstrate that Spindly binds to dynein LIC using the CC1 box and to the dynactin pointed-end complex using the Spindly motif. Both interactions are required for the formation of a stable Spindly–dynein–dynactin complex. This mechanism may be extended to other adaptor proteins, as BicD2 and Hook3 also contain a Spindly motif downstream from their LIC binding sites. The presence of both a dynein and a dynactin binding motif separated by ∼250 residues in several other functionally diverse cargo adaptors suggests this as a common mechanism of cargo recruitment to dynein.

Overall, this exciting work by Gama et al. (2017) and Mosalaganti et al. (2017) communicates a convincing model for the molecular basis of dynein recruitment to the RZZ complex at unattached kinetochores (Fig. 1). The RZZ complex is effectively a dynein cargo. This work has direct implications for the mechanism of spindle checkpoint silencing. Dynein is both recruited and primed by Spindly at unattached kinetochores. As a kinetochore–microtubule attachment is stabilized, dynein walks toward the minus end of the newly attached microtubule, stripping the RZZ complex and associated checkpoint proteins away from the kinetochore and thereby silencing the spindle checkpoint (Fig. 1). Questions remain concerning the composition and architecture of the fibrous corona. This structure at unattached kinetochores was first described over 25 years ago; however, little is known about its assembly and architecture because of the difficulty of isolating the fibrous corona for study. Mosalaganti et al. (2017) propose that the oligomerization properties of the RZZ complex would initiate the mesh-like fibrous corona assembly. Through multiple cooperative weak interactions, the RZZ complex would recruit other corona proteins such as Mad1, CENP-E, and CENP-F. However, how these proteins are recruited to the unattached kinetochores remains unclear. Mitotic kinases such as Aurora B, Mps1, and CDK1, which control the assembly of the kinetochore and regulate the kinetochore–microtubule interaction, are likely to locally stimulate the corona assembly. There may be additional species-specific differences in checkpoint signaling and silencing, as budding and fission yeast do not assemble a fibrous corona but have a Mad1/Mad2-dependent checkpoint.

These studies also contribute important insights into the role and regulation of the dynein motor by its cargoes. Gama et al. (2017) further define the mechanism by which Spindly and other adaptor proteins activate the human dynein–dynactin complex. The number of motile Spindly–dynein–dynactin complexes in in vitro assays is low when compared with other adaptors, such as BicD2 (McKenney et al., 2014). It is possible that the binding of the RZZ complex to the tail of Spindly and posttranslational modifications are required to fully activate dynein at kinetochores. Given that the RZZ complex, Spindly, and dynein–dynactin can all be purified for in vitro studies, future work should investigate the effect of the RZZ complex, Spindly, and mitotic phosphorylation on dynein motility and processivity. It will also be interesting to discover how dynein teams up with other cargoes to mediate distinct mitotic processes.

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