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SPOTLIGHT

SPIN(DLY)-OFF: A tale of conformational change to control DYNEIN

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Barbosa et al. discuss work by Mussachio and colleagues (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202206131) finding that conformational changes in the DYNEIN adaptor SPINDLY can precisely control DYNEIN activation at kinetochores.

Cytoplasmic dynein-1 (hereafter DYNEIN) is a microtubule (MT)-based motor complex responsible for the vast majority of minus-end-directed intracellular transport in eukaryotic cells. By driving the movement of a diverse array of cargos, including protein complexes, vesicles, membranous organelles, and chromosomes, DYNEIN is easily recognizable as an essential molecular player both in interphase and cell division (1, 2). Not surprisingly, malfunction of the DYNEIN transport machinery has been linked to the pathophysiology of human diseases, in particular to neurological disorders caused by impaired axonal transport (3).

DYNEIN is a multi-protein complex consisting of a motor subunit and several non-catalytic subunits, all of which are present in two copies (1). The ability of DYNEIN to move over long distances requires additional binding of co-factors, such as DYNACTIN, and adaptor proteins known as "activating adaptors" (1). Together, these binding partners induce a large structural rearrangement of the DYNEIN dimer that causes the otherwise autoinhibited motor domains to re-orient, effectively increasing their MT binding affinity and motility (4). Interestingly, activating adaptors also act as linkers between DYNEIN and cargos (1).

Several DYNEIN activating cargo adaptors have been identified (1). Despite limited sequence conservation, the domain organization of most activating cargo adaptors is similar. These proteins share common features such as a long coiled-coil domain with structural motifs that stabilize the interaction between DYNEIN and DYNACTIN. Most activating adaptors contain a "CC1 box" motif or a Hook domain close to the N-terminus of the coiled-coil and a downstream "Spindly-box" motif, whose roles are to mediate binding to the dynein light intermediate chain subunit of DYNEIN or the pointed-end subcomplex of DYNACTIN, respectively (5). Moreover, most cargo adaptors use their C-terminal domain to engage with specific cargos (1). The question then arises: How are DYNEIN-DYNACTIN–activating adaptor complexes controlled so that the transport of a specific cargo is appropriately elicited? Although DYNEIN motility is subjected to multi-level regulation, several studies have pointed to the regulation of cargo adaptors as a critical step toward proper activation of DYNEIN (6). For example, it has been proposed that cargo adaptors undergo activating structural rearrangements following their binding to cargo (6). However, the underlying molecular details remained poorly understood.

In this issue, d'Amico et al. (7) address this gap in knowledge by exploring the molecular basis for the regulation of SPINDLY, a mitotic activating adaptor of DYNEIN-DYNACTIN, and bring a new perspective to the mechanism of DYNEIN activation by cargo adaptors (Fig. 1; 7). During early mitosis, SPINDLY localizes to kinetochores (KTs), a supramolecular protein complex that assembles on the centromeric region of chromosomes, where it associates with the ROD–ZW10–ZWILCH (RZZ) complex. This interaction drives the oligomerization of SPINDLY–RZZ complexes at the outermost KT region to form the fibrous corona, a transient mesh expansion that aids in MT capture and contributes to the initial phases of chromosome alignment during mitosis. As DYNEIN promotes corona disassembly by removing both SPINDLY and RZZ from KTs following the establishment of stable end-on MT attachment, the RZZ complex is considered to be a DYNEIN cargo (8). Previous studies have proposed that SPINDLY can adopt an autoinhibited conformation, which reduces its binding affinity to RZZ (9). This is in agreement with the notion that SPINDLY establishes intramolecular contacts that must be disrupted to ultimately operate as a functional activating adaptor. However, direct molecular evidence validating this hypothesis was still lacking. d'Amico et al. (7) now report that the long coiled-coil domain (referred to as CC1) in isolated SPINDLY is organized in consecutive segments that fold back on each other, leading to an intramolecular folding consistent with an autoinhibited conformation (Fig. 1 A; 7). In

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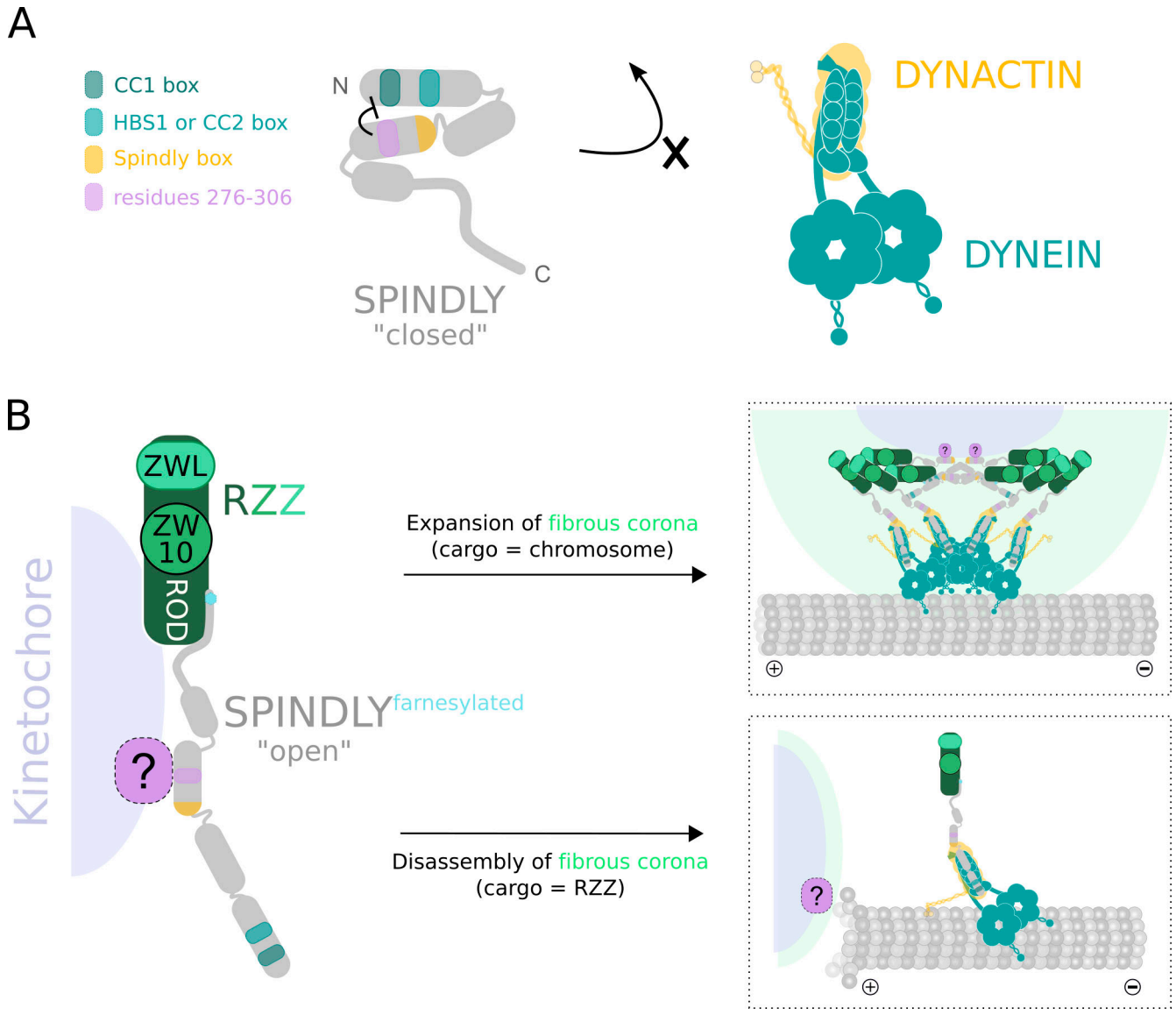

Figure 1. **Conformational changes in SPINDLY control DYNEIN activation at kinetochores. (A)** Schematic representation of native human SPINDLY according to the model by d'Amico et al. (7). Structural motifs in SPINDLY are highlighted (HBS1—heavy chain binding site 1; also referred to as CC2 box). Autoinhibited SPINDLY adopts a "closed" conformation that prevents its binding to DYNEIN-DYNACTIN. **(B)** During early mitosis, farnesylated SPINDLY localizes to kinetochores by binding to the RZZ complex and to a second receptor ("?"). Through this two-step mechanism, SPINDLY can interact with DYNEIN-DYNACTIN. As part of the fibrous corona, DYNEIN-DYNACTIN-SPINDLY assists in chromosome movement. Later in metaphase, the motor complex also contributes to corona disassembly by promoting RZZ removal from kinetochores.

line with this, wild-type SPINDLY is unable to bind DYNEIN and DYNACTIN in solution. To identify structural determinants critical for SPINDLY compact conformation, the authors implemented cross-linking mass spectrometry assays and supported these with AlphaFold2 (AF2)-based structure predictions. In addition to a two-residue insertion (SPINDLY$^{154–155}$) that causes CC1 to split into two segments, d'Amico et al. (7) found a 20-residue-long region (SPINDLY$^{276–306}$) immediately downstream of the Spindly-box motif required to establish intramolecular contacts with the CC1 box (Fig. 1 A). Accordingly, mutation or deletion of key residues in these specialized domains is sufficient to relieve SPINDLY autoinhibition and allow binding to DYNEIN-DYNACTIN. These findings favor a model in which SPINDLY exists natively in a closed conformation that occludes binding of DYNEIN-DYNACTIN to its CC1 box and Spindly-box motif (Fig. 1 A).

SPINDLY's intramolecular folding and conformational transitions most likely reflect a common strategy for the activation of cargo adaptors. In fact, BICD2, a well-known DYNEIN activating adaptor, has also been previously shown to undergo conformational rearrangements, which are thought to limit its access to DYNEIN (6). Upon binding to a specific cargo, autoinhibited BICD2 unfolds and a functional tripartite DYNEIN-DYNACTIN-BICD2 motor is assembled (6). If a similar mechanism exists for SPINDLY auto-regulation, then the interaction with the RZZ complex (the KT cargo) should promote SPINDLY activation. However, and perhaps the most surprising finding from

d'Amico et al. (7), the release of SPINDLY autoinhibition—and consequent ability to interact with DYNEIN-DYNACTIN—was not facilitated upon binding to the KT cargo (7). This implies that, in addition to the RZZ complex, a second trigger is necessary to fully unleash the potential of SPINDLY to bind DYNEIN-DYNACTIN (Fig. 1 B). Curiously, SPINDLY mutants for key residues 276–306 failed both to reach KTs and promote corona expansion, despite being able to interact with RZZ in solution (7, 10). These results further suggest that the second trigger for SPINDLY activation probably lies at KTs and involves an interaction with the segment between residues 276–306 in SPINDLY (Fig. 1 B).

The study by d'Amico et al. (7) not only constitutes a leap forward toward understanding SPINDLY regulation, but also provides important insight into the molecular mechanism leading to appropriate DYNEIN activation (7). At KTs, SPINDLY is required for the RZZ-dependent expansion and DYNEIN-dependent disassembly of the fibrous corona (Fig. 1 B). Importantly, corona expansion and compaction temporally coincide with DYNEIN-mediated transport of either chromosomes or the RZZ complex, respectively (Fig. 1 B; 8). Although substantial advances have been made in the characterization of the dynamic behavior of the fibrous corona, the mechanistic details regarding how SPINDLY coordinates both processes are still poorly understood. It has been previously shown that farnesylation and phosphorylation of SPINDLY during mitosis contribute to the fine regulation of its function (9–12). The results now reported by d'Amico et al. (7) imply the existence of a yet unspecified KT component that also plays a crucial role in SPINDLY autoregulation by controlling its structural transition from a closed to an open conformation required for binding to DYNEIN-DYNACTIN (Fig. 1 B). Future studies should address the identity of this KT receptor, as results will certainly have significant implications for mechanisms ensuring the fidelity of mitosis.

Whether a multi-step activation mechanism exists for activating adaptors other than SPINDLY also remains to be addressed. Importantly, and despite undeniable advantages provided by AF2-based predictions, future work should include more direct structural evidence to validate new molecular models. Nonetheless, given the structural features shared among known DYNEIN activating adaptors, it is tempting to expand the findings from d'Amico et al. (7) and envisage that other adaptors may adopt similar autoinhibitory-dependent mechanisms to achieve precise spatiotemporal stimulation of DYNEIN function.

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