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SPOTLIGHT

Building the centrosome: PLK-1 controls multimerization of SPD-5

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Centrosome maturation relies on the assembly of an underlying molecular scaffold. In this issue of *JCB*, Rios et al. (https://doi.org/10.1083/jcb.202306142) use cross-linking mass spectrometry to reveal how PLK-1 phosphorylation promotes intermolecular SPD-5 self-association that is essential for scaffold formation.

What is the molecule that forms the centrosome scaffold? That was the big question in 1998 when salt-stripped centrosomes were shown to display an electron-dense fibrous network that could recruit factors necessary for microtubule nucleation (1). It took 16 years, but we now know the answer: *Drosophila* Centrosomin (Cnn) and its *Caenorhabditis elegans* homologue Spindle-defective-5 (SPD-5). But how do these molecules form the scaffold? This is the next big question that Jeffrey Woodruff and colleagues tackle in this issue of *JCB*.

Centrosomes are important microtubule organizers, and mutations in centrosome genes, including the human homolog of Cnn and SPD-5, CDK5RAP2, are associated with neurodevelopmental disorders, such as primary microcephaly (2). Centrosomes are also amplified in many cancers and may provide therapeutic targets (3). Understanding how centrosome assembly is regulated has therefore remained an important goal.

Centrosomes comprise a pair of ninefold symmetrical centrioles that are surrounded by a large collection of proteins known as the pericentriolar material (PCM) (2). The PCM expands during mitosis in a phosphorylation-dependent process called centrosome maturation, increasing the centrosome's ability to nucleate and organize microtubules. While our understanding of centriole assembly has progressed rapidly since the early 2000s, the details of how the "cloud-like" PCM assembles have been harder to come by, despite various screens identifying key components.

Two papers in 2014 and 2015 finally showed that Cnn in flies and SPD-5 in worms were the homologous proteins capable of forming the centrosome scaffold in their respective species (4, 5). Both proteins were already known to be essential for proper PCM assembly, but these papers showed that they were capable of oligomerizing into scaffolds. In the first study, a phospho-regulated multimerization (PReM) domain essential for scaffold formation was identified within the middle of *Drosophila* Cnn that, after the addition of phosphomimetic mutations, promoted Cnn within eggs to oligomerize into centrosome-like scaffolds that could organize microtubules (4). The second study showed how PLK-1 phosphorylation stimulated *C. elegans* SPD-5 to assemble into centrosome-like scaffolds in vitro that could also recruit other PCM components and organize microtubules (5). Thus, the molecular identity of the long-sought centrosomal scaffold was established.

We then needed to better understand the molecular details of scaffold assembly. This was no easy task as Cnn and SPD-5 are large multi-domain proteins with many coiled-coils. The first insight came in 2017 when it was shown that mixing Cnn's centrally located PReM domain with C-terminal fragments containing the conserved Centrosomin Motif 2 (CM2) led to the formation of micron-scale assemblies in vitro whose growth was stimulated by Plk1 (6). Moreover, mutations affecting the PReM-CM2 interaction abolished scaffold assembly in vivo (6). Then in 2022, a study showed that SPD-5's central and C-terminal regions interacted in a PLK-1-promoting manner and that this interaction was important for scaffold assembly (7). Now, in this edition of *JCB*, Woodruff and his team have performed a tour de force in cross-linking mass spectrometry to identify SPD-5 self-interactions and to show how phosphorylation by PLK-1 redefines this interaction landscape to promote scaffold formation (8).

To make firm conclusions about interactions within (intramolecular) or between (intermolecular) molecules, it was important to analyze either monomeric or multimeric forms of SPD-5. For this, the authors ran cross-linked samples on SDS-PAGE gels and cut out bands of specific size before performing mass spectrometry. Monomeric unphosphorylated SPD-5 had a few high-confidence and several low-confidence long-range intramolecular interactions, most involving coiled-coil domains and presumably reflecting a folded state that can transition between configurations. Strikingly, most of these long-range interactions, particularly those involving coiled-coils,

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were lost after phosphorylation by PLK-1. For SPD-5 multimers, PLK-1 promoted many intermolecular interactions between coiled–coils and reduced interactions between disordered regions. Most coiled–coil interactions were between the same coiled–coil domains of different molecules, suggesting that SPD-5 multimerizes in a parallel fashion, but intermolecular interactions between different domains were also identified, notably between the PReM and a CM2-like domain in the C-terminal region. Importantly, it is these interdomain interactions that are crucial to form a molecular network. Thus, phosphorylation by PLK-1 appears to promote the unfolding of SPD-5 and then promote intermolecular interactions between coiled–coils to stimulate scaffold assembly.

The authors then characterized three of their identified interactions involving regions toward the N-terminal (including the γ-TuRC–binding CM1 domain), the middle (PReM domain), and the C-terminal (including the CM2-like domain). They tested various mutant forms of SPD-5 for their ability to form functional PCM scaffolds in vivo that could resist microtubule pulling forces and came to the conclusion that each region was necessary for proper scaffold assembly. Using an in vitro assay of scaffold assembly, they found that the entire SPD-5 sequence was required for full scaffold assembly. Individual regions could only form smaller scaffolds to varying extents, with the PReM and C-terminal regions being the most important. Thus, it appears that multivalency is required for scaffold assembly.

Given the importance of the PReM domain, the authors also used various in silico and biophysical techniques, together with the cross-linking data, to conclude that the PReM domain exists as a helical hairpin that dimerizes to form a tetrameric coiled–coil. This structure was phosphorylation independent, as has been observed for PReM domain dimers in flies (4). A point mutation on the outside of the tetrameric coiled–coil inhibited PCM assembly, consistent with the PReM domain needing to interact with other regions of SPD-5 to mediate scaffold assembly.

In summary, Rios et al. (8) shed new light on how the centrosome scaffold forms via PLK-1–mediated interactions between coiled–coils from different molecules. An exciting possibility is that PLK-1 phosphorylation causes the unfolding of SPD-5 to allow scaffold formation, but it is not clear whether unfolding is necessary for scaffold formation or whether it is required for other processes. Indeed, it has been speculated that unfolding of the N-terminal region of Cnn and SPD-5 is necessary for γ-TuRC binding (9, 10), which could be independent of scaffold assembly.

It is striking that most discoveries regarding centrosome scaffold assembly have been made in flies and worms. While this highlights the value of model organisms, it also begs the question as to whether the Cnn/SPD-5 mammalian homolog CDK5RAP2 is also capable of forming a scaffold. Evidence would suggest the answer is yes, as CDK5RAP2 contains a CM2 domain and over-expression results in cytosolic aggregates that recruit other PCM components and organize microtubules (11). Nevertheless, experiments in vitro will be needed to show that CDK5RAP2 is sufficient to form a scaffold and whether this is promoted by Plk1.

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