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Cartwheels create symmetry and asymmetry

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Summary

In this issue of Structure, Kantsadi et al. (2022) present the crystal structures of coiled-coil bundles from SAS-6, a core component of the centriole cartwheel, and reveal that two coiled-coil domains interact asymmetrically. This work provides insights into how the polarity of centrioles is established.

The centriole is one of the largest membrane-less protein complexes in eukaryotic cells. Centrioles organise centrosomes in animal cells and are essential for generating cilia and flagella in many eukaryotes. A proteomic analysis of centrioles indicates that this organelle is comprised of over 60 different proteins[1]. Multiple copies of these proteins assemble into a cylinder (~250 nm diameter and 500 nm height) with a characteristic ninefold rotational symmetry[2] (**Figure 1**). This symmetry is created by nine copies of microtubule blades, which are triplets of one complete and two incomplete microtubules in most organisms. These microtubules are aligned with the same +/- polarity and are held together by protein linkers. The minus and plus ends of the centriole are called the proximal and distal ends respectively. Thus, the centriole is a polarised multi-protein assembly with a ninefold rotational symmetry.

A major question for the field is how the symmetry and polarity of centrioles are established correctly. A clue to answering this question lies within a substructure of centrioles called the cartwheel. It is probably the first recognisable substructure that appears during centriole biogenesis and itself has a ninefold rotational symmetry. The core of cartwheels is formed by Spindle assembly abnormal protein 6 (SAS-6), which can self-assemble into a ring structure with a ninefold symmetry[3,4]. The SAS-6 ring is created by two homo-dimerisation domains of the protein; a globular head and parallel coiled-coil domains, which correspond to the central hub and radial spoke respectively within cartwheels (**Figure 1**). The tip of the spoke is attached to a multi-protein complex known as the pinhead, which directly interacts with microtubule blades. Thus, SAS-6 rings join together with pinheads to scaffold nine microtubule blades[2]. The central role of SAS-6 in defining the symmetry of the centriole is evident because SAS-6 mutants that formed rings without ninefold symmetry resulted in centrioles with abnormal rotational symmetries[5]. This scaffolding role of SAS-6 seems to require more than one cartwheel. Electron microscopy (EM) images revealed that multiple cartwheels stack on top of each other at the proximal end of centrioles[6,7]. The stacking is likely to be an intrinsic feature of SAS-6 rings because it can be reconstituted *in vitro* using purified SAS-6 protein[8]. Recent cryo-electron tomography (cryo-ET) studies of centrioles from various organisms showed that coiled-coil domains of SAS-6 from distinct cartwheels form α-helix bundles, suggesting that an interaction between the coiled-coil domains facilitates the cartwheel stacking. However, the exact nature of this interaction has remained unclear so far.

In this issue of Structure, Kantsadi *et al* provide further insight into this question[9]. They observed how two coiled-coil domains of SAS-6 interact to form a bundle of four α-helices by determining the crystal structure of the coiled-coil domain. An intriguing twist of this study is that two different α-helical bundles were observed in the asymmetric unit: a bundle in which the interactions between two SAS-6 coiled-coil domains is symmetric, and an asymmetric bundle that has no rotational symmetry axes. These two structures raise the question of which interaction, either symmetric or asymmetric, contributes to SAS-6 ring stacking. To answer this question, the authors made two SAS-6 mutants, in which either the symmetric or asymmetric interaction was disrupted, and used these mutants for a cartwheel reconstitution experiment, in which SAS-6 ring stacks were observed by EM[8]. The SAS-6 mutant that lacks the asymmetric interaction did not form the ring stacks, whereas the mutant without the symmetric interaction did, suggesting that the asymmetric interaction is important for SAS-6 ring stacking. This asymmetric coiled-coil interaction results in a ~10º offset between two SAS-6 rings in a stack. Strikingly, a similar offset between cartwheels in a stack was indeed observed within centrioles by cryo-ET[7], further supporting the finding that the asymmetric SAS-6 coiled-coil interaction is required for generating SAS-6 stacks. Another important finding of this study is that the offset between SAS-6 rings introduces a polarity to the cartwheel stacks. The SAS-6 ring polarity might propagate to the pinhead, which is also polar along the microtubule blade within centrioles, and further contributes to establishing the proximal-distal polarity of centrioles[9]. Thus, the asymmetric coiled-coil interaction of SAS-6 might be central to build centrioles with correct polarity.

Note that an asymmetric protein-protein interaction within coiled-coil domains was also observed in the SAS-6 paralog XRCC4 (X-Ray Cross Complimentary 4), which is an essential protein for non-homologous end joining during DNA double-strand break repair. The coiled-coil domain of the XRCC4 homodimer asymmetrically interacts with its binding partner DNA ligase IV and forms a 2:1 complex of XRCC4/DNA ligase IV[10]. This interaction breaks the two-fold symmetry of the coiled-coil of XRCC4 by generating a kink in one of the α-helices. It is an interesting observation that the symmetric coiled-coil domain of SAS-6 and XRCC4 both participate in asymmetric protein-protein interactions that are key for their functions.

This report by Kantsadi *et al* also raises intriguing new questions. Is the observed asymmetric coiled-coil interaction an evolutionarily conserved mechanism for cartwheel stacking among SAS-6 orthologs? Since the key residues for the asymmetric coiled-coil interaction are not well conserved among different species, other organisms might use different residues / regions of the SAS-6 coiled-coil. Indeed, cryo-ET studies of cartwheels from three different organisms (*Paramecium*, *Trychonympa* and *Chlamydomonas*) showed that their radial spokes bundle differently. Thus, the asymmetric coiled-coil interaction might take place in different regions of the radial spoke. Is the asymmetric coiled-coil interaction the sole determinant of cartwheel polarity? In addition to polarised structures of SAS-6 ring stacks and pinheads, the cartwheel inner density (CID) is also polarised to the proximal-distal direction[6,7]. CID is an evolutionary conserved structure of cartwheels, but the protein that forms this structure is still unknown. It is possible that the polarity of the cartwheel stack might be promoted not only by SAS-6 itself but also its interacting proteins. Future studies on the cartwheel and its associating proteins including SAS-6 will help answering these questions.

It is also important to highlight that Kantsadi *et al.* captured and showed the importance of weak protein-protein interactions in the assembly of a macromolecular complex. The asymmetric interaction between two SAS-6 coiled-coil domains is in the mM range. However, since the SAS-6 rings are assembled from nine identical interactions, the overall avidity of the interaction is strongly increased and estimated to be in the pM range, according to Kantsadi *et al.*. This allowed the authors to probe the significance of the weak interaction using cartwheel reconstruction assays. Such weak protein-protein interactions are challenging to identify and verify and there is a tendency among researchers to consider them as non-physiological and thus might miss important biological insights. However, this paper shows that weak protein-protein interactions can be studied and can have critical biological functions. In the future, the development of new methodologies for identifying and characterizing weak and dynamic interactions is needed to fully unveil the whole picture of protein-protein interactions in cells.

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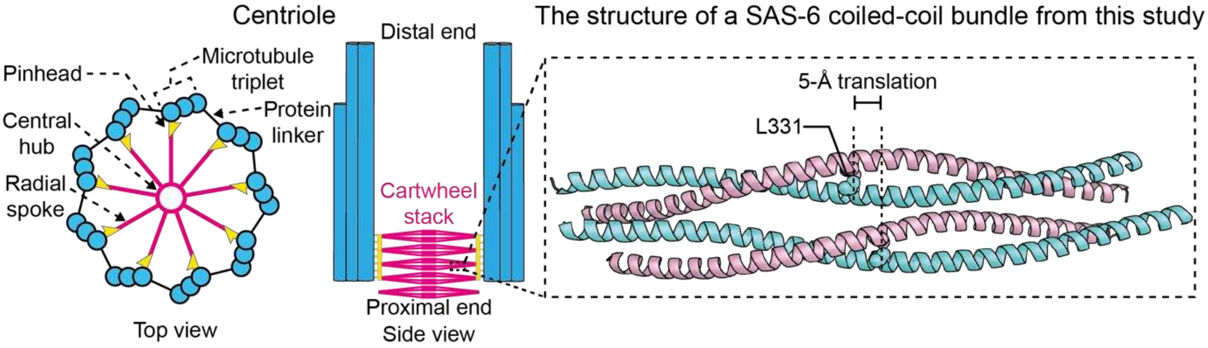

Figure 1. **An asymmetric interaction between two coiled-coil domains of SAS6 contributes to the formation of cartwheel stacks.** This schematic figure shows a centriole from the top and side[2]. Microtubules, SAS-6 rings and pinheads are shown in blue, magenta and yellow, respectively. On the right, the crystal structure of the coiled-coil domain of *Chlamydomonas* SAS-6 (PDB ID: 6YRN) is shown in a dotted rectangle. A homodimer of the coiled-coil (shown in cyan and pink) interacts with another copy of the homodimer asymmetrically with a 5-Å translation between the two homodimers. The side chains of L331 are shown in a ball representation to visualize the 5-Å translation.