SAS-6 oligomerization: the key to the centriole?

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Centrioles are among the most beautiful of biological structures. How their highly conserved nine-fold symmetry is generated is a question that has intrigued cell biologists for decades. Two recent structural studies provide the tantalizing suggestion that the self-organizing properties of the SAS-6 protein hold the answer.

Centrioles are highly ordered structures that organize two key cellular organelles: the centrosome and the cilium. Defects in both organelles are now firmly linked to a wide variety of human diseases[1]. Centrioles are composed of nine blade-like microtubule triplets, arranged at the end of nine spokes that radiate from a central hub (**Fig. 1a**). The central hub and spokes are collectively referred to as the central cartwheel: this structure seems to be an essential and early intermediate in the centriole assembly pathway, and it is widely assumed to be responsible for generating the nine-fold symmetry of centrioles (**Fig. 1b**)[2,3].

Although proteomic studies indicate that centrioles consist of many proteins[4,5], centriole replication seems to depend on a small 'core' of proteins (reviewed in refs. 1, 6 and 7). These proteins were first identified in a series of genetic and RNA interference screens in *Caenorhabditis elegans*; they showed that SPD-2 recruits the kinase ZYG-1 to the mother centriole, which, in turn, recruits a complex of SAS-6 and SAS-5 to form a central tube (a structural variant of the central cartwheel that lacks detectable spokes). This complex recruits SAS-4, which is required for the addition of microtubules around the central tube. The central role of SPD-2 in centriole duplication in other species remains controversial[8–11], but a functional equivalent of ZYG-1 (the Polo-like kinase Plk4, also known as Sak) and clear functional orthologs of SAS-4, SAS-5 and SAS-6 have been identified, and they have been shown to be intimately involved in centriole duplication in other species.

Though Plk-4 is likely to be the crucial initiator of centriole duplication, accumulating evidence suggests that SAS-6 is a key structural component in the early assembly pathway. SAS-6 homologs in *Chlamydomonas reinhardtii* (Bld-12) and *Tetrahymena thermophila* (Sas6a) localize to the central cartwheel; *Bld12* mutants lack detectable cartwheels and show defects in the nine-fold symmetry of centrioles[3,5]. Moreover, *Drosophila* SAS-6 can self-assemble into oligomers *in vitro*[12], and, when overexpressed in embryos, it forms large, irregular tube-like structures that can recruit other centriolar and centrosomal proteins[13,14]. Intriguingly, when *Drosophila* SAS-6 is overexpressed together with Ana2 (the *Drosophila* functional ortholog of SAS-5), the two proteins can assemble into well-ordered tubules that bear a striking resemblance to the cartwheel[15]. Now, in two landmark papers, the crystal structures of SAS-6 from various species have been solved[16,17] (**Table 1**). These structures provide clues as to how the SAS-6 protein may be organized into a central-cartwheel structure to dictate the nine-fold symmetry of the centriole.

The new structures in detail

Kitagawa *et al.*[16] purified several recombinant constructs of *C. elegans*

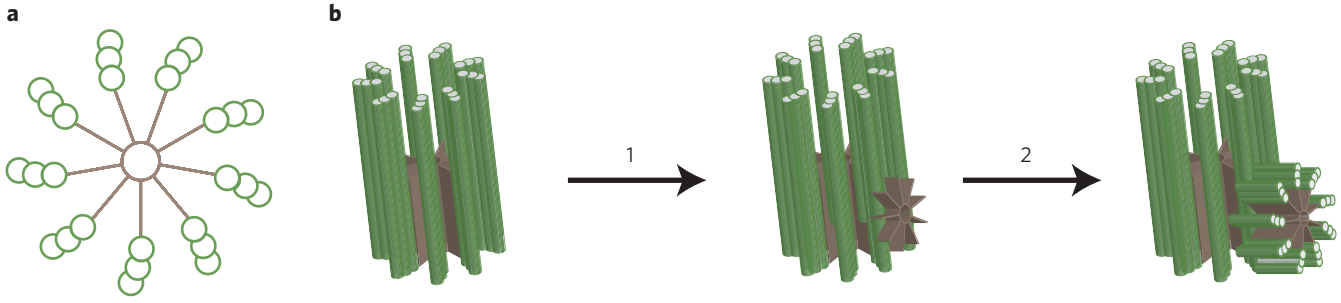

Figure 1 | A schematic view of centrioles and the early stages of centriole duplication. (**a**) An end-on view of a centriole showing the nine-fold symmetry of the central hub and spokes of the cartwheel structure (gray) and the surrounding 'blades' of triplet microtubules (green). In some species and cell types, only doublet or singlet microtubules surround the cartwheel: nine-fold symmetry is conserved in virtually all centrioles. (**b**) During S phase, the two centrioles in a cell each duplicate (for simplicity, a single centriole is shown here). The precise structural details of centriole duplication are poorly understood, but the formation of a new cartwheel structure at right angles to the existing 'mother' centriole is thought to be an important early step (1), and this cartwheel is then rapidly surrounded by new microtubules (2). During S and G2 phases, the new centrioles elongate until they are approximately the same size as the original mother centriole (not shown), and in most species the cartwheel structure is ultimately restricted to the proximal end of the centrioles (as illustrated for the mother centriole shown here).

Table 1 | Information available in the SAS-6 structures

Structure	PDB code	Interactions observed	Dimers in asymmetric unit
CeSAS6 N terminus	3PYI	Head-group interaction	1
CrSAS6 N terminus	3Q0Y	Head-group interaction	3
CrSAS6 N terminus-CC	3Q0X	Coiled-coil dimer	1
DrSAS6 N terminus	2Y3V	Head-group interaction	2
DrSAS6 N terminus-CC	2Y3W	Coiled-coil dimer	1 (plus fragment of N terminus)

All structures revealed self-interactions of SAS-6. In PDB entries 3Q0Y and 2Y3V, multiple copies of the dimer are present, but superimposition of the copies reveals slight differences in the orientations between the monomers. These differences have consequences when the structures are used to model oligomers, as shown in **Figure 3b,d**.

SAS-6 (CeSAS-6). The overall topology of SAS-6, which was assessed *in vitro* via EM, revealed that the protein comprises an N-terminal globular head followed by a long stretch of coil (**Fig. 2a**). Coupled with cross-linking experiments, this analysis showed that CeSAS-6 can use this region to dimerize via an extended parallel coiled-coil interaction.

The crystal structure of the N-terminal globular domain revealed that SAS-6 can further interact via the head group, which potentially explains how SAS-6 dimers can assemble into higher-order oligomers (**Fig. 2b,c**). This head-group interaction depends on a single residue, Ile154, which forms a hydrophobic plug that inserts into a socket located toward the end of the conserved PISA (present in SAS-6) motif. Analytical ultracentrifugation showed that mutating this residue (I154E) blocked the head-group interaction. Moreover, the inability of full-length CeSAS-6 containing the I154E mutation to rescue SAS-6–deficient *C. elegans* embryos suggests that the head-group association of SAS-6 coiled-coil dimers is of central importance to SAS-6 function at the centriole.

C. elegans centrioles are slightly unusual in that they appear to form a central tube rather than a central cartwheel, so Kitagawa *et al.*[3] also solved two structures of the SAS-6 ortholog in *C. reinhardtii* (CrSAS-6), an organism in which the organization of the central cartwheel is particularly well defined. Despite the limited sequence homology between these proteins (~20% identity between the N-terminal head groups), the structure of the equivalent N-terminal fragment is remarkably similar to that seen in *C. elegans* (average r.m.s. deviation = 1.4 ± 0.03 Å over 123 ± 3 Cα atoms). A single hydrophobic residue, Phe145, mediates dimerization by inserting into a hydrophobic pocket in the PISA motif, and mutation of Phe145 (F145E) disrupted this interaction. The structure of a version of this mutant protein extended to include a length of coil illustrates the coiled-coil interaction via ~55 residues of helix (**Fig. 2b,c**).

Interestingly, a range of higher-order oligomers formed when recombinant protein containing both coiled-coil and head-group interfaces was expressed, and EM studies revealed that some of these structures adopted ring-like conformations.

Biophysical analyses determined that the coiled-coil interaction (K_D = 0.5 ± 0.1 μM and 0.9 ± 0.1 μM for CrSAS-6 and CeSAS-6, respectively) was stronger than the head-group interaction (K_D = 60 ± 20 μM and 110 ± 30 μM for CrSAS-6 and Ce-SAS-6, respectively), suggesting that SAS-6 initially forms homodimers via the coiled-coil domain. These dimers then associate via their head groups into ring-like structures that show a striking resemblance to a cartwheel—with the coiled-coil domains forming spokes that radiate from a central hub (**Fig. 2c**). Kitagawa *et al.*[16] modeled the oligomerization of SAS-6 dimers into a nine-fold symmetric closed ring and observed head-group contacts broadly consistent with those in the crystal. Thus, the crystal structures are compatible with the idea

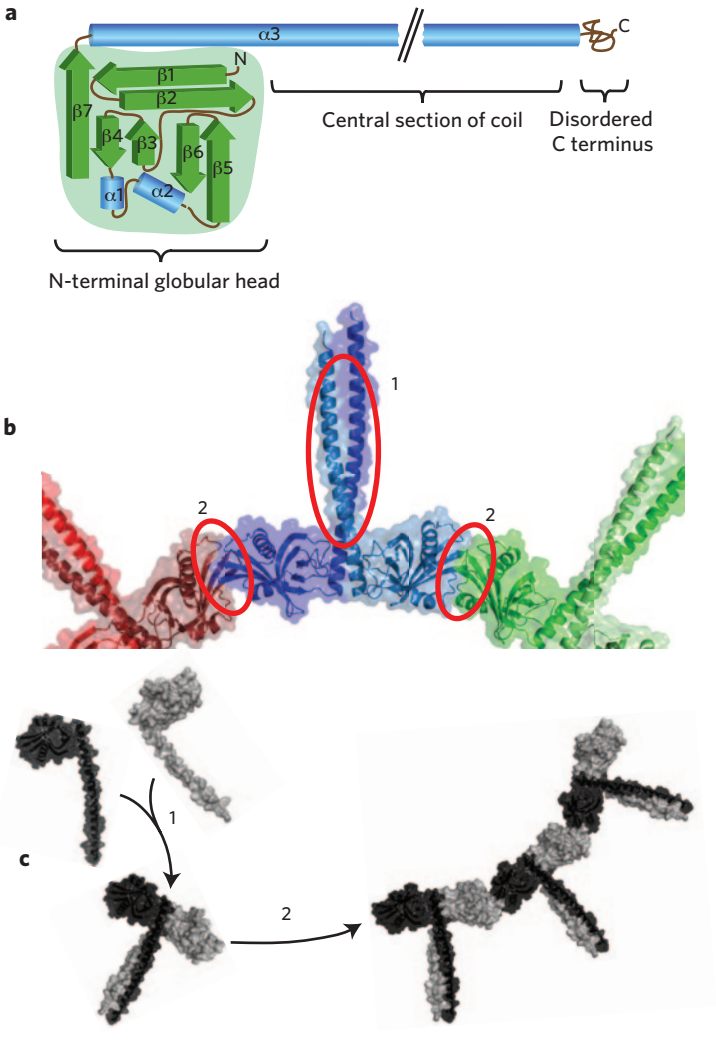

Figure 2 | The architecture and interactions of SAS-6. (**a**) Schematic representation of the overall topology of the SAS-6 family of proteins. (**b**) A schematic representation of the coiled-coil interaction (1) that occurs via the knob-and-holes packing of the coiled-coil tails and the head-group interaction (2) that is mediated by the hydrophobic plug residue. (**c**) A model of SAS-6 oligomerization: CrSAS-6 monomers initially dimerize via the coiled-coil interaction, and the resulting dimers can then associate via the head-group interaction to form circular oligomers.

that SAS-6 oligomers can form a nine-fold symmetric central-cartwheel structure.

Crystal structures of the equivalent coiled-coil and head-group interactions of the zebrafish *Danio rerio* ortholog (DrSAS-6) were independently solved by van Breugal *et al.*[17]. DrSAS-6 is highly divergent from its CeSAS-6 and CrSAS-6 counterparts (with only ~15% sequence identity between the three orthologous head groups), but the structural conservation between them was remarkably high (average r.m.s. deviation = 1.3 ± 0.1 Å over 127 ± 5 Cα atoms). The main dimerization-mediating residue in DrSAS-6 is Phe131. F131D mutation abolished dimerization *in vitro*, and an F131D mutation in human SAS-6 (HsSAS-6), in combination with mutations in residues comprising the hydrophobic pocket, abolished the centrosomal localization of HsSAS-6, demonstrating that self-association is crucial to SAS-6 function in human cells. van Breugal *et al.* also investigated the coiled-coil interaction by removing 90% of the coil, and doing so once again abolished centriolar localization. Thus, both interfaces are necessary for SAS-6 function.

When both dimerization interfaces were present in fragments of DrSAS-6, higher-order oligomers formed, with a range of stoichiometries. One fragment formed crystals that were not of high enough quality to analyze by X-ray diffraction but could be analyzed by cryo-EM. These studies revealed striking assemblies of rings in which eight SAS-6 dimers (joined by their head groups, from which the coiled-coil domains radiate) could be modeled to a structure remarkably similar to the central hub and spokes of the cartwheel, only with eight-fold symmetry. The authors also modeled oligomers containing 6–12 dimers and found that the eight- and nine-fold symmetric versions of these rings required the fewest orientation changes to the dimer structure[17]. Thus, both van Breugal *et al.*[17] and Kitagawa *et al.*[16] arrive at the same conclusion: the self-assembly properties of SAS-6 are essential for centriole formation and are compatible with the formation of a nine-fold symmetric SAS-6–based cartwheel structure.

What's behind and ahead

Although these structural studies illustrate how SAS-6 oligomers could form the main body of the cartwheel structure, many questions remain. Most obviously, *C. elegans* appears to form a central tube rather than a central cartwheel, yet the CeSAS-6 N-terminal structure appears virtually identical to its equivalents in *C. reinhardtii* and *D. rerio*. It is far from clear how such similar molecules can form such apparently diverse assemblies. Perhaps the central tube and cartwheel structures are not so different, but the cartwheel is just hard to visualize in *C. elegans*.

It is unclear whether SAS-6 alone is sufficient to establish the observed nine-fold symmetry of centrioles, or whether it merely has a propensity to oligomerize into circular structures, and the strict nine-fold symmetry is enforced by interactions with other factors. Data from several distinct methodologies have shown the potential for SAS-6 to adopt multiple oligomeric conformations. Indeed, using the interactions present in the various crystal structures (**Table 1**), one can build several distinct oligomer models (**Fig. 3**). When the CrSAS-6 crystal interaction angles are used, for example, the SAS-6 head groups form a left-handed spiral, whereas the *D. rerio* structures can be modeled into either a right-handed spiral or an almost flat ring with approximately eight-fold symmetry (**Fig. 3b**). For SAS-6 to be sufficient to establish nine-fold symmetry, the spoke dimers would have to associate rigidly with a precise geometry to generate a 40° angle. The head-group interaction is reported to have a K_D in the range of 60–110 μM, a weak value that perhaps strengthens the argument for the existence of other symmetry-ensuring factors.

Several independent observations indicate that proteins other than SAS-6 probably contribute to the establishment

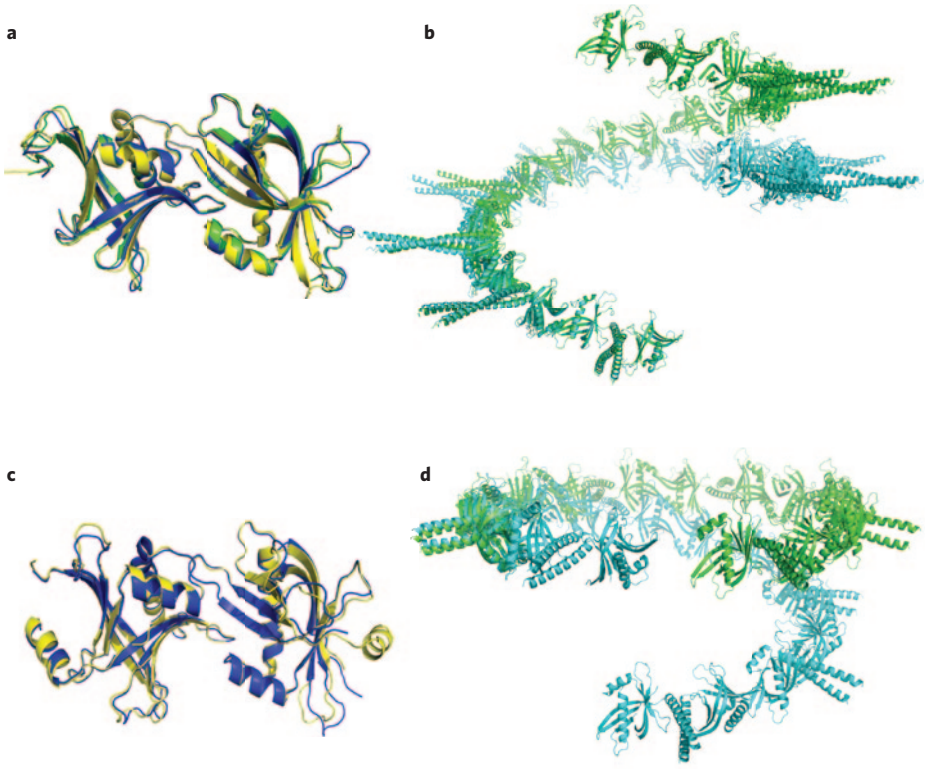

Figure 3 | An illustration of the distinct SAS-6 oligomer models that can be derived from slightly different dimer orientations observed in the SAS-6 crystal structures. All models are further described in **Table 1**. (**a**) Three slightly different head-group dimers were present in the CrSAS-6 N terminus (PDB code 3Q0Y) structure. Here, dimers CD (yellow) and EF (blue) are superimposed upon dimer AB (green). Average r.m.s. deviation between the dimers = 0.6 ± 0.1 Å over 1,876 ± 14 atoms. (**b**) Two models of CrSAS-6 dimer oligomers were assembled (in a clockwise manner) using the A→B orientation of the coiled-coil dimer shown in PDB entry 3Q0X and either the 3Q0Y C→D (green) or the 3Q0Y B→A (blue) orientation of the head group. Both models generate a left-handed helical structure with approximately ten dimers per helical turn, but each has a different helical pitch. (**c**) Similar overlays shown for the two head-group dimers (AC and BD) present in the DrSAS-6 N-terminus crystal (r.m.s. deviation = 0.8 Å over 1,975 atoms). (**d**) Two models of DrSAS-6 dimer oligomers were assembled (in a clockwise manner) using the 2Y3W A→B orientation of the coiled-coil dimer and either the 2Y3V A→C (green) or B→D (blue) orientation of the head group. The first model generates a nearly circular arrangement of dimers (eight dimers are shown), whereas the second model generates a left-handed helical structure with eight to nine dimers per helical turn.

of nine-fold symmetry. *Chlamydomonas* mutants that completely lack the SAS-6 ortholog Bld-12, for example, can still form centrioles and flagella under certain conditions; although the centrioles appear to lack the central cartwheel, ~90% of the axonemes that form retain nine-fold symmetry[3]. In *Drosophila*, overexpression of SAS-6 leads to the formation of irregular tube-like structures (that can be much larger than the centrioles)[14], and regular structures resembling cartwheels only form when SAS-6 is overexpressed together with Ana2 (the SAS-5 ortholog in flies)[15,18]. Thus, in both flies and worms, SAS-6 must interact directly with at least one other protein (SAS-5 or Ana2) to function properly in centriole duplication. The role of the spoke-tip protein Bld-10 (Cep135 in humans) in cartwheel formation also remains to be fully elucidated; in *Chlamydomonas*, this protein plays an essential part in cartwheel formation and centriole duplication[2,19], and RNA interference experiments suggest it is important for centriole duplication in flies and humans[20,21]. In flies, however, the *in vivo* centriole defects associated with the loss of Cep135 are surprisingly mild[22].

Finally, how SAS-6 is organized in the cartwheel remains a major structural question. The authors of both studies favor the idea that cartwheels are formed from rings of SAS-6 oligomers that are stacked on top of one another in register so that their spokes are in near-perfect alignment. It remains possible, however, that the cartwheel is arranged in a spiral that has a regular periodicity. Indeed, as described above, models based on the crystal structures can support either of these possibilities (**Fig. 3**). This problem may seem esoteric, but the difference can have important implications for centriole duplication. One could easily imagine, for example, that a spiral structure could be extended to seed the growth of a new centriole, thus explaining why daughter centrioles usually initially form in close contact with a mother. Further work is necessary to assess how closely the crystallographic interaction angles resemble those that form *in vivo*.

In summary, these two studies provide a major advance in our molecular understanding of the center of the centriole, the formation of the cartwheel and the importance of SAS-6 to the process of duplication. Future studies will focus on how SAS-6 interacts with the other 'core' duplication proteins and on how the assembly of SAS-6 is regulated in cells so that centrioles form in the close vicinity of an existing centriole. ■

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Competing financial interests

The authors declare no competing financial interests.