**Caenorhabditis elegans** centriolar protein SAS-6 forms a spiral that is consistent with imparting a ninefold symmetry

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Centrioles are evolutionary conserved organelles that give rise to cilia and flagella as well as centrosomes. Centrioles display a characteristic ninefold symmetry imposed by the spindle assembly abnormal protein 6 (SAS-6) family. SAS-6 from *Chlamydomonas reinhardtii* and *Danio rerio* was shown to form ninefold symmetric, ring-shaped oligomers in vitro that were similar to the cartwheels observed in vivo during early steps of centriole assembly in most species. Here, we report crystallographic and EM analyses showing that, instead, *Caenorhabditis elegans* SAS-6 self-assembles into a spiral arrangement. Remarkably, we showed that these proteins can assemble into ninefold symmetric rings, reminiscent of the cartwheels observed in centrioles (19, 20). It was, therefore, proposed that SAS-6 functions by forming rings in vivo, through which it establishes the ninefold symmetry of centrioles (19, 20). This model is consistent with the recent demonstration of cartwheels in *Trichonympha sp.* basal bodies by electron tomography (21).

The most significant difference to this consensus model of centriole assembly is found in *C. elegans*, the very system in which SAS-6 was originally discovered. Although they also exhibit the signature ninefold radial symmetric arrangement of microtubules, *C. elegans* centrioles are smaller compared with other metazoans (4), and many of their components show a significant level of sequence divergence (22), raising the question of whether they assemble in the same manner. Intriguingly, instead of cartwheels, *C. elegans* centrioles have been reported to be organized around a central tube (4), which is initially ∼60 nm in length and then increases to ∼110 nm as the centriole grows. Whereas ceSAS-6 exhibits the same domain composition as other SAS-6 family members (19) and is recruited to centrioles concomitant with elongation of the central tube (4, 23), it remains unclear how such a SAS-6–containing central tube could establish a ninefold symmetrical arrangement. We sought to address this question by studying the oligomerization properties of ceSAS-6 and comparing it with SAS-6 variants from *C. reinhardtii* and *D. rerio* (19, 20). Strikingly, we find that ceSAS-6 differs from other members of this protein family, because it self-assembles into filamentous spiral oligomers instead of rings. Our findings lead us to propose that the function of SAS-6 proteins in determining centriolar ninefold symmetry is conserved across evolution but that the structural details of how this symmetry is achieved may fundamentally differ in certain species.

**X-ray crystallography | electron microscopy | structure | centriolar architecture | SAS-5**

Centrioles are essential for organizing cilia and flagella in most eukaryotes as well as forming the centrosome of animal cells (1). As such, they play a crucial role in cell signaling, cell motility, embryonic development, and genome stability. Aberrations in centriole structure and function are linked to a number of diseases, such as ciliopathies, male sterility, primary microcephaly, and cancer (2). *Caenorhabditis elegans* has proven instrumental for the discovery of five components essential for centriole formation (3, 4): the coiled coil-containing proteins spindle defective protein 2 (5, 6), SAS-4 (7, 8), SAS-5 (9), and SAS-6 (10, 11) as well as the kinase zygote defective protein 1 (12, 13). Related proteins have since been found across the eukaryotic domain (14, 15), indicating a common evolutionary route for centriole formation.

In most species, centrioles and the related basal bodies are organized around a cartwheel composed of a circular central hub with outward facing spokes that are connected to microtubules (1). Centrioles are characterized by a universal ninefold symmetric arrangement of microtubules, and proteins of the SAS-6 family, one of the earliest components recruited during centriole formation (11, 16–18), are critical for establishing this arrangement (16). SAS-6 proteins from *C. elegans*, *Chlamydomonas reinhardtii*, and *Danio rerio* (ceSAS-6, crSAS-6, and drSAS-6, respectively) were deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 4GFC, 4GFA, 4G79, 4GEK, and 4GEU).

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Data deposition: The crystallography, atomic coordinates, and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 4GFC, 4GFA, 4G79, 4GEK, and 4GEU).

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Results

Crystal Structure of the Coiled Coil-Mediated ceSAS-6 Dimer. To assess the oligomERIC conformation of ceSAS-6, the structure of a homodimer mediated by the C-C interface was necessary. For C. reinhardtii and D. rerio, this structure was obtained by crystallizing SAS-6 constructs that included a dimerization-deficient N-terminal domain and a small stretch of the coiled coil (19, 20). However, analogous fragments of ceSAS-6 evaded crystallization. Given that the long α2-β5 loop of the ceSAS-6 N-terminal domain (residues 1–168) was not observed in the crystal structure of this construct (19) (ceN[S123E]), we hypothesized that disorder in this loop would adversely affect crystal packing. Removing this loop by excising residues N103 to P130 did not alter the structure of the remainder of the N-terminal domain, which was shown by the 1.8-Å resolution structure of ceN[Δ103–130] (Fig. S1 and Table S1). Thus, we designed ceSAS-6 constructs lacking this loop and were able to crystallize two variants that included the N-terminal domain and the first 47 residues of the coiled coil. These variants harbored a single amino acid substitution (ceN-CC[Δ103–130/I154G]) or a short deletion (ceN-CC[Δ103–130/Δ151–156]) to disrupt dimerization through the N-N interface, whereas coiled-coil-mediated dimerization was maintained as shown by multistage light scattering experiments (Fig. S2).

The structures were solved by molecular replacement and refined to 2.85- and 3.55-Å resolution for ceN-CC[Δ103–130/I154G] and ceN-CC[Δ103–130/Δ151–156], respectively (Table S1). In total, we obtained three ceN-CC dimer models (Fig. 1A) with an average pairwise Cα rmsd of 0.55 Å; analysis henceforth focuses on the highest resolution structure. The N-terminal domain is not affected by inclusion of the coiled coil, with a Cα rmsd of just 0.42 Å compared with the previously resolved ceN[S123E] variant (19). The overall topology of the ceSAS-6 dimer is also comparable with the topology of crSAS-6 and drSAS-6 (19, 20), with the coiled coil yielding a parallel in-register dimer and the N-N dimerization interfaces facing in opposite directions (Fig. 1A).

ceSAS-6 Models Reveal a Spiral Conformation That Is Consistent with Ninefold Symmetry. Surprisingly, ceSAS-6 oligomers modeled using the coiled-coil dimer and the previously determined N-N interface dimer (19) display a steep right-handed spiral with a 33-nm pitch and ~5 dimeric units per helix turn (Fig. 1B and C). This arrangement stands in marked contrast to the rings of crSAS-6 (19) and drSAS-6 (20). The spiral is not driven by either the N-N interface, which is nearly identical between the three SAS-6 variants (19, 20), or the conformation of the coiled coil itself, the first three heptad repeats of which can be superimposed between ceSAS-6 and crSAS-6 with just 0.97-Å Cα rmsd. Rather, it is the relative orientation of the coiled-coil helix-α3 with respect to the N-terminal domain that differs between ceSAS-6 and crSAS-6 by ~38° (Fig. 1D). This displacement is responsible for moving successive ceSAS-6 dimers away from the plane of a SAS-6 ring (Fig. S3); a similar displacement can be seen upon superimposing the ceSAS-6 and drSAS-6 structures.

This intriguing ceSAS-6 spiral model compelled us to consider whether it is compatible with the ninefold symmetry of centrioles. Interestingly, we were able to model a ceSAS-6 spiral that features 4.5 dimeric units per turn by simply adjusting the ψ-angle of V167 at the pivot between the N-terminal domain and the coiled-coil helix from the observed 124° to 128° (Fig. S4A). This minor change creates no significant steric clashes. The resulting spiral is ninefold symmetric every two turns and displays a ~31-nm pitch (Fig. S4 C and D). In contrast, modeling a ninefold symmetric ceSAS-6 ring would necessitate larger changes in the backbone dihedrals of the pivot residues, most significantly ~30° adjustments of the H165 ψ- and R168 ϕ-angles, as well as the adoption of an α-helical conformation by G169 (Fig. S4B). Moreover, the α3 backbone would clash substantially with the side chain of V167 (Fig. S4E).

To better explore the limits of ceSAS-6 conformations, we performed atomistic molecular dynamic simulations starting from the ceN-CC[Δ103–130/Δ151–156] structure (Fig. S5). OligomERIC
models produced during a 20-ns simulation show spirals with an average periodicity of 4.9 ± 0.6 ceSAS-6 dimers per turn and a pitch of 31 ± 3 nm. A significant degree of protein flexibility is evident in these simulations, because the oligomeric models explore symmetries in the range of ~3.5–6.5 dimers per turn and pitches in the range of 25–39 nm. We conclude that crystallographic and modeling data strongly suggest a ceSAS-6 spiral oligomer as opposed to a ring and that ninefold symmetry is easily discernable within the conformational reach of a spiral arrangement.

Stabilization of the ceSAS-6 N-N Interface. We set out to directly visualize the ceSAS-6-oligomers by EM. However, neither negative stain EM nor rotary shadowing preparations showed the presence of large oligomers (Fig. S6 A and B); in contrast, crSAS-6 displayed clear rings under similar conditions (19). We hypothesized that an open-ended spiral assembly may not be stably populated under the relatively dilute (~20–40 μM) protein concentrations suitable for EM, because dimerization between N-terminal domains is weak (K_D ~ 100 μM) (19).

The N-N interface of ceSAS-6 critically depends on an iso-leucine at the tip of the β6-β7 loop (I154) (19), which buries in a hydrophobic cavity upon N-N dimer formation (Fig. 2E). Substitution at this position by a charged residue (I154E) disrupted dimerization (19), whereas substitution with tryptophan improves N-N dimerization affinity ~20-fold compared with WT (I154W, K_D = 4.7 ± 0.2 μM) (Fig. 2A). Two different crystal structures of ceN[S123E/I154W] at 2.65- and 2.8-Å resolutions (Table S1) showed only small changes in the N-terminal domain compared with WT, with an average Cα rmsd of 1.55 Å against ceN[S123E] (Fig. 2 B and C). The structural changes primarily involve repositioning of α1 and partial closure of the hydrophobic cavity originally occupied by I154 (Fig. 2 C and D). Crucially, the relative orientation and position of domains in the N-N dimer remain unaltered, and ceSAS-6 oligomers modeled using the ceN[S123E/I154W] structure show a spiral arrangement similar to the WT.

To evaluate the potential biological effect of the I154W substitution, we generated transgenic worms expressing GFP fused to SAS-6[I154W] that were engineered to be resistant to RNAi directed against endogenous SAS-6 (GFP-SAS-6RR[I154W]) (19). Whereas most WT embryos assemble a monopolar spindle in each blastomere on sas-6(RNAi) at the end of the second cell cycle as a result of defective centriole formation (Fig. 3A and Movie S1), 75% of embryos expressing GFP-SAS-6RR[I154W] undergo bipolar spindle assembly in each blastomere under the same RNAi conditions (Fig. 3 C and D and Movie S3). This level of rescue is comparable with the level observed in embryos expressing WT GFP-SAS-6RR (Fig. 3 B and D and Movie S2), despite the relatively low levels of GFP-SAS-6RR[I154W] expression (Fig. 3 B, D, and E). It is likely that the increased N-N affinity of GFP-SAS-6RR[I154W] compensates for the reduced expression level of this construct. We conclude that I154W represents a functionally competent substitution, thereby supporting the crystallographic data that I154W does not alter ceSAS-6 structure in a significant manner.

EM of ceSAS-6 Spiral Assemblies. Analytical ultracentrifugation (AUC) sedimentation velocity experiments of ceN-CC[S123E/I154W] confirmed that this construct, with two strong dimerization interfaces, forms larger oligomers compared with the WT (Fig. 4A). Whereas ceN-CC[S123E/I154E] migrated as a dimer and ceN-CC[S123E/I154E] migrated as a mixture of dimeric, tetrameric, and hexameric species at concentrations of 50 μM, ceN-CC[S123E/I154W] formed 8–10 mer assemblies already at 25 μM (1 mg/mL). However, these assemblies, if elongated, would be hard to visualize by EM. To increase the probability of observing large oligomers, we prepared EM grids using highly concentrated (5–7.5 mg/mL) ceN-CC[S123E/I154W] samples, which were first cleared of pelletable material by centrifugation, rapidly diluted to 1 mg/mL, placed on grids and stained with uranyl acetate. Under these conditions, ceN-CC[S123E/I154W] showed spirals of variable length (Fig. 4B and Fig. S6 D–F), albeit in the presence of significant protein.

Fig. 2. 1154W increases the ceSAS-6 N-N dimerization affinity. (A) Fluorescence polarization experiments of WT- (purple squares), I154E- (orange crosses), or I154W-substituted (green triangles) ceN constructs to determine the N-N dimerization affinity. The I154E substitution disrupts N-N dimerization (19), and the K_D of WT ceN matches the value estimated from isothermal titration calorimetry experiments (110 ± 30 μM) (19). The I154W substitution leads to a 20-fold increase in affinity compared with the WT. Error bars derive from five independent measurements. (B) Superposition of the four N-N dimer models from the two crystal structures of ceN[S123E/I154W]. The average rmsd for all Cα atoms is 0.51 Å. The N-N dimer interface is essentially unchanged compared with the interface of ceN[S123E], with the β6-β7 loop from one subunit contacting a hydrophobic patch between α1 and α2 of the second molecule. (C) Superposition of one copy of ceN[S123E/I154W] (gold) onto ceN[S123E] (red). The main difference in the two structures is the slight displacement of α1 in ceN[S123E/I154W]. Note that the relative position and orientation of the head domains are unaffected. (D and E) Close-up view of the area around residue 154 reveals the reason for α1 displacement. Whereas I154 in ceN[S123E] fits tightly into a cavity formed between primarily hydrophobic residues (E, green), the aromatic ring of W154 sits on top of this hydrophobic patch, leading to a partial collapse of the cavity (D).
Discussion

Our crystallographic and EM studies established that ceSAS-6 self-assembles into a spiral arrangement. This spiral model stands in marked contrast to the SAS-6 rings that form cartwheels in *C. reinhardtii* (19), *D. rerio* (20), and *Trichonympha sp.* (21) centrioles. Our analysis suggests that the spiral conformation is compatible with a ninefold symmetric output; however, oligomer flexibility yields a broad distribution of symmetry outputs by ceSAS-6 alone. Similar flexibility was observed in ring-forming SAS-6 variants, which yield a variety of symmetry levels.
(8- to 11-fold) in vitro (19, 20). Although the role of SAS-6 in dictating centriolar ninefold symmetry is not disputed (16), we, therefore, surmise that additional components may assist in robustly enforcing this symmetry level in vivo. Thus, from a mechanistic perspective, centriolar architecture could be built upon either a SAS-6 spiral or ring foundation.

In contrast to 4.5-fold symmetric ceSAS-6 spirals, which could be modeled from the crystallographic data with minimal adjustments, modeling ninefold symmetric ceSAS-6 rings required substantial distortions of the ceSAS-6 structure (Fig. S4 B and E). Given the near-identity of the N-N interfaces of ceSAS-6, crSAS-6, and drSAS-6 (19, 20) as well as the high similarity of the C-C interfaces seen in this study, ceSAS-6 rings could be created only by reversing the displacement of the coiled coil helix-α3 (Fig. 1D). This reversal requires both large backbone changes at the pivot residues between the ceSAS-6 N-terminal domain and the coiled coil and overcoming strong steric clashes from residue V167. This residue is highly interesting, because V167 is conserved in most nematode species but substituted by small amino acids (G or A) in other SAS-6 variants (Fig. S8A).

In silico substitutions of the corresponding residue in crSAS-6 (G158V) or drSAS-6 (G144V) yielded clashes between the valine methyl groups and the α3 main chain (Fig. S8C) similar to those clashes observed for a ceSAS-6 ring model (Fig. S8E). Thus, we consider V167 and the equivalent residues in other nematode variants as strong indicators for SAS-6 self-assembly into spiral conformations. Although such conformations could be overcome in vivo by, for example, strong protein–protein interactions involving the SAS-6 pivot region, no such SAS-6 binding proteins are known at this time.

Therefore, the intriguing possibility emerges that a SAS-6 spiral may be critical for building the central tube that is characteristic of C. elegans centrioles. Indeed, we presented a ceSAS-6 spiral model that displays ninefold symmetry every two turns, with an approximate length of 62 nm over this period (Fig. 5A and B). This size is comparable with the initial length of the C. elegans central tube (~60 nm) (4), whereas its full length observed on centriole maturation (~110 nm) fits well to four turns of the ceSAS-6 spiral. Furthermore, the diameter of the spiral arrangement including idealized ceSAS-6 coiled coils is ~72 nm, which is similar to the diameter observed for the central tube in vivo (4). Thus, we propose that C. elegans centrioles feature at their center a SAS-6 spiral (single or intertwined vide infra) that, in analogy to cartwheels in other species, is necessary for determining the conserved ninefold symmetry (Fig. 5D). Compared with cartwheel rings observed elsewhere, a ceSAS-6 spiral allows establishment of ninefold symmetry over considerable length using relatively few protein molecules. This scarcity of ceSAS-6 molecules may explain the seemingly hollow nature of the central tube observed in electron tomography (4); furthermore, a filament of ceSAS-6 N-terminal domains would be difficult to visualize by current in situ microscopy methods.

How widespread across evolution could a SAS-6 spiral be? The V167 pivot residue noted earlier is only present in certain nematode sequences (Fig. S8A) but absent from the SAS-6 of Trichinella spiralis, a worm that diverged early in the nematode evolutionary tree (24). This finding suggests that the SAS-6 spiral conformation is a relatively recent adaptation, restricted to parts of the nematode phylum. It is intriguing to speculate whether the allflagellate nature of the C. elegans sperm (25) and the lack of cilia in all C. elegans cells except sensory neurons (26) may have released a fundamental constraint in centriole assembly, which then allowed alternative SAS-6 conformations to arise.

What other differences would a spiral imply for centriolar architecture? Compared with cartwheel rings, a spiral may require different components to cap it at a predetermined length; likewise, the mechanical links between a spiral and microtubules will differ from the mechanical links of cartwheels. We note, for example, that a protein equivalent to C. reinhardtii Bld10p (27) or Homo sapiens Cept33 (28) which is thought to connect the cartwheel spokes with microtubule walls, has not been identified in nematodes. Furthermore, a SAS-6 spiral model would predict relatively few physical connections between the microtubule walls and the central tube, with direct implications on the mechanical stability of nematode centrioles. Importantly, by its very nature, an open-ended spiral oligomer of over 100 nm in length is inherently less stable than a closed ring arrangement. Interwining of SAS-6 spirals (Fig. 5 C and D) as observed in our electron micrographs (Fig. 4C and Fig. S7) could intrinsically stabilize these oligomeric arrangements. At the same time, we postulate that other centriolar components [for example, SAS-5 (9)] may stabilize the overall conformation. Indeed, the SAS-5 interaction site was identified very recently at approximately the middle of the ceSAS-6 coiled coil by Qiao et al. (29) (Fig. 5 A and B) and independently by us (Fig. S9). SAS-5 includes a predicted coiled coil dimerization interface (9), and it has been shown to oligomerize in yeast two-hybrid experiments (30). It is, thus, possible that SAS-5 can bridge two adjacent binding sites on ceSAS-6, thereby stabilizing the formation of a ceSAS-6 spiral; a similar function has been proposed for the Dro sophila melanogaster functional homolog of SAS-5 (31).

In summary, our findings show how small structural switches can drastically transform the architecture of SAS-6 oligomers, while retaining the same ninefold output symmetry. We believe that the fundamental role of SAS-6 in imposing centriolar ninefold symmetry is conserved in C. elegans like in other species.
The emerging and intriguing question now is whether ceSAS-6 forms spirals in vivo.

Materials and Methods

An extended description of all methods is provided in SI Materials and Methods; briefly, protein fragments harboring N-terminal His6-tags were expressed in Escherichia coli and purified by metal affinity and size exclusion chromatography. Protein identity was confirmed by mass spectrometry.

Size exclusion chromatography/multilight scattering experiments were performed on 2–4 mg/mL protein samples in PBS using the same setup as described previously (19). AUC velocity experiments were performed on 25–100 μM protein samples using an AUC tomography XL-i analytical ultracentrifuge (Beckman). Sedimentation velocities were recorded by measuring either absorbance at 282 nm, with 200 scans every 4 min at 10 °C and 35,000 rpm, (Beckman). Sedimentation velocities were recorded by measuring either absorbance at 280 nm, λex = 340 nm, λem = 520 nm; MBM LaBtech) or for measurements of ceN[S123E] and ceN[S123E/1154W], the intrinsic fluorescence of the single tryptophan residue using an M5 Spectrmax fluorimeter (λex = 280 nm, λem = 340 nm; Molecular Devices).

Molecular dynamics of the ceSAS-6 coiled coil dimer were simulated for 20 ns at 310 K in explicit solvent with spherical boundary conditions using NAMD and the CHARMM22 force field. Protein crystals were obtained using the sitting drop vapor diffusion technique, data were recorded at Diamond Light Source or the European Synchrotron Radiation Facility, and structures were solved by molecular replacement using the ceN[S123E] cryo electron microscopy (Protein Data Bank ID code 3PYI) as the search model. Crystalllographic data processing and refinement statistics are provided in Table S1.

For EM, protein samples diluted to 0.1–1 mg/mL were transferred to freshly glow-discharged homemade carbon-coated copper grids and stained by uranyl acetate or ammonium molybdate solutions. To avoid disassembly of ceSAS-6 oligomers, all steps were carried out swiftly.

Generation of mutant nematode strains and analysis of resistance to SAS-6 RNAi were carried out as described previously (19).

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Supporting Information

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SI Materials and Methods

Protein Preparation. DNA encoding fragments of Caenorhabditis elegans spindle assembly abnormal protein 6 (Uniprot ID 062479) were cloned in a modified pET15b vector containing an N-terminal His-tag. All proteins were expressed in Escherichia coli BL21 (DE3) cells in LB medium. Protein expression was induced with 0.25 mM isopropyl β-D-1-thiogalactopyranoside and allowed to proceed at 18 °C overnight. Cell pellets were resuspended in lysis buffer containing 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 0.5% Triton X-100, and Complete protease inhibitors (Roche) and lysed by sonication. Protein purification involved an initial metal affinity purification step using a His-Trap HP column (GE Healthcare) and gradual elution by imidazole. After thrombin cleavage of the His-Tag, the proteins were further purified by size exclusion chromatography using Sephadex G75 or G200 columns (GE Healthcare). The final buffer was either 10 mM Heps, pH 7.0, 150 mM NaCl, and 2 mM DTT for crystallization or 20 mM sodium phosphate, pH 7.0, 150 mM NaCl (PBS), and 2 mM DTT for biophysical experiments. For use in analytical ultracentrifugation experiments, DTT was replaced by 2 mM Tris(2-carboxyethyl)phosphine. For EM experiments, the proteins were dialyzed against 150 mM NaCl, 50 mM Mes/Imidazole buffer, pH 6.5, and 2 mM DTT buffer. For fluorescence polarization experiments, samples of ceN, ceN[S123E], ceN[S123E/I154E], and ceN[Δ103–130] in PBS and 1 mM Tris(2-carboxyethyl)phosphine were incubated overnight with 10-fold excess of 5-((2-((iodoacetetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid to produce single fluorophore conjugates at residue C100 followed by size exclusion chromatography in PBS. Proteins were concentrated by spin ultrafiltration, divided into aliquots, and flash frozen in liquid nitrogen. Protein identity was confirmed by electrospray ionization—time of flight mass spectrometry, and concentrations were estimated by UV absorption at 280 nm. All proteins retained their native behavior for biophysical studies (1).

Crystallization, Crystallographic Data Collection, and Refinement. Crystals were obtained using the sitting drop vapor diffusion technique at 4 °C. A mosquito robot (TTP LabTech) was used to setup 200-nL drops with varying ratios of protein to mother liquor. Crystallographic data were integrated in MOSFLM (2) or XDS (3) and scaled in SCALA (4). All structures were solved by molecular replacement using Phaser (5) with a monomer of the ceN[S123E] crystal structure (Protein Data Bank ID code 3PYI) as search model and refined in Buster 2.10 (6) using automatic translation/libration/screw and noncrystallographic symmetry restraints where applicable (7). Crystallographic data processing and refinement statistics are provided in Table S1. For graphical representation, we used PyMOL (DeLano Scientific) (8). The constructs are detailed below.

ceN-CC[Δ103–130/Δ151–156] at a concentration of ~8 mg/mL was mixed in 1:1 ratios with mother liquor containing 10% (wt/vol) PEG 20,000, 20% (vol/vol) polyethylene glycol (PEG) monomethyl ether 550, 0.1 M Mes/Imidazole buffer, pH 6.5, and 0.02 M each d-Glucose, d-Mannose, d-Galactose, t-Fucose, d-Xylose, and N-Acetyl-d-Glucosamine. Rod-shaped crystals developed in 2–3 d, were flash frozen in liquid nitrogen (LN2) using the mother liquor as cryoprotectant, and diffraction to 2.85 Å at the Diamond Light Source beamline I02. The space group was determined as C121 with two molecules per asymmetric unit. The model and associated data have been deposited in the Research Collaboratory for Structural Bioinformatics databank under accession number 4GFC.
ceN-CC[Δ103–130/Δ151–156] at a concentration of ~5 mg/mL was mixed in 1:2 ratios with mother liquor containing 0.2 M LiSO4, 0.1 M Mes, pH 6.0, and 35% (vol/vol) 2-methyl-2,4-pentanediol. Initial crystal clusters developed in 1–2 d, these clusters were broken by vigorous mixing and used for seeding under the same conditions. Tetragonal plate crystals developed in 2–3 d, were flash frozen in LN2 using the mother liquor as cryoprotectant, and diffraction to 3.55 Å at Diamond Light Source beamline I02. The space group was determined as P212121 with four molecules per asymmetric unit. The model and associated data have been deposited in the RCSB databank under accession number 4GFA.
ceN[Δ103–130] at a concentration of ~12 mg/mL was mixed in 1:1 ratios with mother liquor containing 0.1 M Heps, pH 7.5 and 2.0 M ammonium sulfate. Thick plate crystals developed in ~5 d, were briefly immersed in mother liquor supplemented with 20% (vol/vol) glycerol, and were flash frozen in LN2. The crystals were diffracted to 1.8 Å using either the Diamond Light Source I04-1 beamline or the European Synchrotron Radiation Facility beamline ID14-4. The space group was determined as I222 with one molecule per asymmetric unit. The model and associated data have been deposited in the RCSB databank under accession number 4G79.
ceN[S123E/I154W] at a concentration of ~7 mg/mL was crystallized under two different conditions: a 1:1 mixture with mother liquor containing 0.2 M Lithium sulfate, 0.1 M Tris, pH 8.5, and 40% (vol/vol) PEG 400 (form A) or a 1:1 mixture with mother liquor containing 0.1 M calcium acetate hydrate and 20% (wt/vol) PEG 3350 (form B). Form A yielded ellipsoid crystals in ~7 d, which were flash frozen in LN2 using the mother liquor as cryoprotectant and diffracted to 2.8 Å at Diamond Light Source beamline I04-1. The space group was determined as P3121 with four molecules per asymmetric unit. Form B yielded crystal clusters in 2–3 d, which were broken and used for seeding under the same conditions. Trigonal plate crystals appeared after ~5 d. They were cryoprotected by brief immersion in mother liquor supplemented with 20% (vol/vol) glycerol and flash frozen in LN2. The crystals diffracted to 2.65 Å at Diamond Light Source beamline I24. The space group was determined as P1211 with four molecules per asymmetric unit. The models and associated data have been deposited in the RCSB databank under accession numbers 4GEX and 4GEU for forms A and B, respectively.

Molecular Dynamics Simulation. ceN-CC[Δ103–130/Δ151–156] (Protein Data Bank ID code 4GFA; residues 1–184, chains A and C) was simulated with spherical boundary conditions in an explicit water droplet at 310 K (Langevin Dynamics). Hydrogens and explicit water (TIP3P) were added with VMD (9). Simulation was performed in NAMD (10) with CHARMM27 force field (11) and a time step of 2 fs, while constraining all bonds between hydrogens and heavy atoms. The start geometry was optimized with 50,000 steps of energy minimization before it was heated to 310 K in 10-K intervals. During each interval, the system was simulated for 5,000 steps followed by 20,000 steps of energy minimization. After equilibrated, the productive simulation was run for 107 steps (20 ns). Snapshots of the trajectory were extracted every 0.22 ns with AmberTools12 (12), and every snapshot of the coiled coil-mediated dimer was used to model the oligomeric assembly with the ceN[S123E] dimer structure (Protein Data Bank ID...
Electron micrographs were taken in a Morgagni TEM (Philips) operated at 80 kV equipped with a Megaview III CCD camera and a JEM2200FS (JEOL) operated at 200 kV equipped with a TVIPS F416 camera. Individual filamentous specimens were observed after centrifuged sample to 1 mg/mL in 50 mM Tris Cl, pH 7.5, 150 mM NaCl, and 2 mM β-mercaptoethanol and immediately transferred to freshly glow-discharged homemade carbon-coated copper grids. Protein excess was quickly washed away with buffer followed by immediate drying by side blotting. Negative stain was applied by repeating this procedure three times with freshly prepared uranyl acetate (1% wt/vol). To avoid disassembly of ceSAS6 oligomers, all steps were carried out swiftly. Assemblies of intertwined helices were observed at 0.1 mg/mL without prior centrifugation and staining with ammonium molybdate solution (2% wt/vol). Similar structures could be observed, albeit more rarely, with uranyl acetate or uranyl formiate (2% wt/vol) staining. Mild cross-linking was archived by mixing the sample in 50 mM phosphate buffer (pH 7), 150 mM NaCl, and 2 mM β-mercaptoethanol with 0.02% (vol/vol) glutaraldehyde and incubating for 5 min at room temperature. Cross-linking was stopped by the addition of 20 mM Tris Cl, pH 7.5. Samples for rotary metal shadowing were supplemented with glycerol to a final concentration of 30% (vol/vol). Samples were subsequently sprayed onto freshly cleaved mica and rotary shadowed in a BA 511 Mfreeze-etch apparatus (Balzers) with platinum/carbon at an elevation angle of 3°–5° (13).

Nematode Strains, RNAi, and Western Blot Analysis. The RNAi-resistant strain GFP-SAS-6RR[I154W] was generated as previously described (1). Appropriate primers (forward primer: TGG TCG AAA GGA AAA ATC TTT GCC; reverse primer: AGG AGT CTT CGA GAA TAG TCG) were used to PCR-amplify sas-6rr cDNA, replacing the ATT that normally codes I154 with TGG and cloning the resulting fragment into pIC26, a pie-1–based vector. The obtained sequence-verified construct was then bombarded, and one integrated line was selected for additional analysis. RNAi-mediated inactivation was performed by soaking (14).

Brieﬂy, L3–L4 larvae were placed in a solution containing in vitro synthesized dsRNAs targeting a portion of sas-6 corresponding to the engineered RNAi-resistant construct (15), incubated for 24 h at 20 °C, and allowed to recover for 17 h at 24 °C before analysis. Cell cycle progression was monitored by differential interference contrast time-lapse microscopy, recording one image every 5 s.

Worm lysates were prepared by collecting 150 adult worms and resuspending them in SDS sample buffer before boiling. Equal amounts of worm lysates were loaded on a 10% SDS/PAGE gel before Western blot analysis. SAS-6 primary antibody was used at 1:200 (rabbit); HRP-conjugated anti-rabbit antibodies (Promega) were used at 1:5,000. The signal was detected with chemiluminescence (Roche).

Fig. S1. The ceSAS-6 α2–β5 loop is dispensable for the N-terminal domain structure. The N-terminal head domain of ceSAS-6 forms a robust globular entity that is not affected by the deletion of the α2–β5 loop. The Cα atoms of the previously published ce[N130E] structure (red; Protein Data Bank ID code 3PYI) (1) superpose onto the crystal structure of ce[N130–130] (green) with Cα rmsds of only 0.3 Å. The loop deletion site is shown in the foreground.


Fig. S2. \textit{ceN-CC} is a dimer in solution. Size exclusion chromatography/multiangle light scattering analysis of \textit{ceN-CC[Δ102–130/I154G]}. Despite disrupting dimerization through the N-N interface by substituting residue I154, the molar mass corresponds well to the 43.4 kDa of a dimer. This finding indicates that \textit{ceN-CC}, which stops at \textit{ceSAS-6} residue 215, forms a dimer in solution through the truncated coiled coil interface.

Fig. S3. Displacement of the \textit{SAS-6} coiled coil results in spiral conformations. (A and B) Shown here are two perpendicular views from a section of a \textit{crSAS-6} ninefold symmetry ring composed of five \textit{crSAS-6} dimeric units as indicated. Note the planar arrangement of all coiled coil dimerization interfaces. (C) Superposition of a single \textit{ceSAS-6} N-terminal domain to \textit{crSAS-6} as in Fig. 1D. (D) The \textit{ceSAS-6} coiled coil helix deviates from the ring plane, a change that is compounded as each \textit{ceSAS-6} dimeric unit is added to the model. The final result is a \textit{ceSAS-6} spiral as opposed to the flat \textit{crSAS-6} ring.
Fig. S4. Modeling of ninefold symmetric ceSAS-6 conformations. (A and B) Shown here are the adjustments required in modeling a ceSAS-6 structure that yields (A, green cartoon) a 4.5-fold symmetric spiral or (B, red cartoon) a ninefold symmetric ring. Changes in dihedral angles are shown as differences in degrees from the ceN-CC(Δ102–130/I154G) model (semitransparent blue cartoon). (C and D) Two orthogonal views of a ninefold symmetric ceN-CC spiral. The individual ceN-CC dimers are numbered. The outer diameter of the spiral was calculated using the ceN-CC(Δ102–130/I154G) model, where almost the entire coiled coil length is visible. E shows the strong steric clash between the V167 side chain and the α3 backbone resulting from the ceSAS-6 conformation of B.
Fig. S5. Molecular dynamics simulations of ceN-CC spirals. Shown here are the ceSAS-6 spiral oligomer (A) symmetry and (B) pitch values calculated from snapshots of a molecular dynamics simulation of ceN-CC[Δ103–130/Δ151–156]. The mean pitch and mean diameter during the simulation agree with our proposed spiral model, where clearly no flat rings or ninefold symmetric assemblies are sampled during the trajectory. The simulation was performed in explicit solvent at 310 K, and snapshots were extracted every 0.22 ns.
Fig. S6. Overview of ceN-CC spirals by EM. Shown here are large fields of (A) rotary metal shadowing preparations of ceN-CC[S123E], (B) negative staining preparations of the same sample, (C) rotary metal shadowing preparations of ceN-CC[S123E/I154W], and (D) negative staining preparations of the same sample. ceN-CC[S123E], with the relatively weak N-N dimerization interface, lacks distinct large-scale features in the EM (either spirals or rings). In contrast, crSAS-6 constructs similar to ceN-CC[S123E] show clear rings in equivalent rotary metal shadowing preparations (1). (D) ceN-CC[S123E/I154W], with the stabilized N-N interface, displays filaments under both preparations, the structural features of which are best observed under negative stain conditions. Note that even ceN-CC[S123E/I154W] depolymerizes rapidly under dilute conditions, necessitating relatively high concentrations for EM, which then lead to crowded and noisy negative stain fields. (E) shows the measured features and length distribution of ceN-CC[S123E/I154W] spirals. The length of the central hub in vivo (60–110 nm) (2) is well within the observed spiral length in vitro. For comparison, our crystallographic model of ceSAS-6 N-CC[S123E/I154W] spirals is shown in scale. (Scale bar: 10 nm.) (F) Examples of diameter, pitch, and length measurements from spirals. The values for length and diameter were determined from the length and height of a box enclosing a spiral (black box). The pitch was measured as the distance between succeeding maxima or minima in a spiral (orange box). (Scale bar: 10 nm.)

Fig. S7. Overview of ceN-CC[S123E/I154W] filaments by EM. (A) Negative stain micrographs of ceN-4HR[S123E/I154W] filaments observed after mild sample cross-linking by glutaraldehyde. Red arrows indicate the filament position. Protein background is high, possibly because of nonspecific cross-linking. (Scale bar: 200 nm.) (B) Similar filaments in noncross-linked ceN-4HR[S123E/I154W] obtained without prior sample centrifugation. The filaments appear as supramolecular assemblies of individual spirals illustrated by their repeated splitting into single spirals and reassembly (indicated by white arrowheads). Loosely wound instead of tightly intertwined spirals are also observed and may be of a transition state. (Scale bar: 200 nm.)
Fig. S8. Sequence diversity at the SAS-6 pivot region. (A) Alignment of SAS-6 amino acid sequences from strand-β6 to helix-α3 from a variety of different species, including a number of nematodes. Gray bars highlight conserved or conservatively substituted residues. The conserved hydrophobic residue responsible for dimerization across the N-N interface (I154 in *C. elegans*) is highlighted in blue. A pivot position between the N-terminal domain and the coiled coil is occupied by small residues (glycine or alanine; green highlight) in most phyla, but it is a larger hydrophobic residue in many nematodes (yellow highlight). Note that *Trichinella spiralis*, an early branch in nematode evolution (1), maintains the small residue at this position. (B and C) Close-up view of the hinge region between the N-terminal domain and α3 in ceN-CC and crSAS-6. (B) V167, the large pivot residue in *C. elegans*, yields large steric clashes on modeling ninefold symmetric ceSAS-6 rings (Fig. S4D), whereas it accommodates a displaced coiled coil helix well. (C) The equivalent position is occupied by G158 in crSAS-6, *in silico* substitution of which by valine yields similar steric clashes between the methyl groups (shown as wireframe) and the main chain of α3.

**Fig. 59.** *C. elegans* SAS-5 binds to the ceSAS-6 coiled coil between residues 260 and 290. (A) The binding site was identified by testing ceSAS-6 constructs for binding to a fluorescently labeled SAS-5 peptide (residues 384–404). Fluorescence detection was used to monitor peptide sedimentation in analytical ultracentrifugation velocity experiments. Removal of the ceSAS-6 region 260–290 (highlighted in green) abolishes binding. (B) Sedimentation distribution from analytical ultracentrifugation velocity experiments with 100 nM fluoresceine-labeled SAS-5 384–404 alone and mixed with 100 μM ceSAS-6 181–260 or ceSAS-6 181–290 (black, red, and green lines, respectively). As seen, ceSAS-6 181–290 (A, green box) binds to SAS-5 384–404, whereas no binding is observed with ceSAS-6 181–260 (A, red box). Note that, because of fluorescence detection, only the sedimentation behavior of the labeled peptide is observed. (C) Comparison of the SAS-5/ceSAS-6 binding site identified by analytical ultracentrifugation (green; residues 260–290) with the binding site recently reported by Qiao et al. (1) (blue; residues 275–288). The two regions overlap, thereby providing an independent verification of the recently published work (1).

Table S1. Crystallographic data and refinement statistics

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ESRF, European Synchrotron Radiation Facility; PDB, Protein Data Bank.
*Statistics for the higher-resolution dataset from Diamond I04-1.
†Values in parentheses correspond to high-resolution shell in data collections.
‡Statistics from Molprobity (1).

**Movie S1.** Time-lapse differential interference contrast (DIC) microscopy of WT embryos on sas-6(RNAi) two cell-stage embryos. Frames were captured every 5 s, and the movie is played at 10 frames/s. Embryos are oriented with the anterior to the left and the posterior to the right; elapsed time is shown in minutes and seconds. Time 0 corresponds to the onset of cytokinesis.

**Movie S2.** Time-lapse DIC microscopy of WT embryos on GFP-SAS-6RR on sas-6(RNAi) two cell-stage embryos. Frames were captured every 5 s, and the movie is played at 10 frames/s. Embryos are oriented with the anterior to the left and the posterior to the right; elapsed time is shown in minutes and seconds. Time 0 corresponds to the onset of cytokinesis.

**Movie S3.** Time-lapse DIC microscopy of WT embryos on GFP-SAS-6RR[1154W] on sas-6(RNAi) two cell-stage embryos. Frames were captured every 5 s, and the movie is played at 10 frames/s. Embryos are oriented with the anterior to the left and the posterior to the right; elapsed time is shown in minutes and seconds. Time 0 corresponds to the onset of cytokinesis.