

SPOTLIGHT

Centriolar appendages evolve into the inner sheath of mammalian flagella

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The annulus, a septin-based structure in vertebrate sperm connecting the MP and PP, has unclear migration mechanics. In this issue, Hoque et al. (<https://doi.org/10.1083/jcb.202307147>) report that the CBY3/CIBAR1 complex ensures its precise positioning by regulating membrane properties.

Cilia and flagella, conserved cellular organelles widespread in the biological world, have a core microtubule-based axoneme structure emanating from the basal body, a transformation of the centriole. They are crucial for movement, sensory functions, and various cellular activities across numerous organisms. The basal body connects to the ciliary axoneme through the transition zone (TZ), forming an independent ciliary compartment. Mature centrioles possess distal appendages (DAs) and subdistal appendages (SDAs), corresponding to the basal body's transition fibers and basal feet, respectively (1). DAs recruit ciliary vesicles for fusion with the cell membrane, anchoring the basal body for ciliogenesis, and form part of the ciliary gate with TZ, septin ring barrier, and nucleoporins, controlling protein traffic in the ciliary compartment (2). SDAs anchor microtubules during interphase and are essential for coordinated motile cilia beating. In the specific context of sperm flagella, the role of DAs and SDAs takes on additional complexity. Significantly, during sperm flagellum formation, the proximal end of the axoneme becomes exposed to the cytoplasm as the TZ migrates (3), with the distal centriole ultimately reshaped or degraded in mammalian sperm. The fate and specific roles of DAs and SDAs in sperm flagellum formation remain largely unknown.

The mammalian sperm tail, comprising neck, midpiece (MP), principal piece (PP), and end piece, is adorned with various sperm-specific structures: its 9+2 axonemal is encased in outer dense fibers (ODFs), a spiral mitochondrial sheath surrounds the MP forming a mitochondrial sheath, and a fibrous sheath envelops the PP. The annulus, a septin-based ring structure prevalent in vertebrate sperm, along with the mitochondrial and fibrous sheaths, constitutes the sperm's "outer sheath." In the development of mammalian sperm, the annulus initially positions itself at the distal end of the neck centriole, segregating the axonemal compartment, but as the axoneme grows, it migrates and precisely locates itself at the MP/PP junction. The annulus guides flagellar growth and mitochondrial alignment along the axoneme, acting as a diffusion barrier, confining proteins to specific compartments within the sperm tail (4). However, the specifics of its migration and exact localization mechanism remain unclear.

In fruit flies, *Cby* is essential for TZ assembly, with *Cby* mutants exhibiting sensory transduction defects and reduced male fertility. During sperm development, *Cby* relocates to the ring centriole, a structure similar to the annulus. Interestingly, TZ proteins CEP290 and MKS1 also localize to the annulus in mature mouse sperm flagella (3), suggesting a close relationship between annulus and TZ

migration, representing a conserved process in establishing sperm tail sub-compartments. In mice, three CBY homologs exist. In airway ciliary cells, DA protein CEP164 recruits the CBY1/CIBAR complex to DAs, facilitating ciliary vesicle formation for efficient basal body-cell membrane docking (5).

In this issue (6), Takemaru and colleagues, using ultrastructure expansion microscopy and structured illumination microscopy, revealed that CBY1 and CIBAR1 are initially recruited to DAs during sperm development. As the annulus assembles, CBY3 is recruited, replacing CBY1 and forming the CBY3/CIBAR1 complex, accompanying the annulus to the MP/PP junction. *Cby3* and *ciBARI* knockout mice exhibit abnormally located annuli in the PP and severe kinked tail deformities, impacting male fertility. Unlike the annulus, the CBY3/CIBAR1 complex, closer to the axoneme, binds the curved membrane regions of the flagellar pocket, increasing rigidity rather than regulating membrane curvature, stopping annulus migration upon reaching the MP/PP junction. These findings propose a model explaining the migration of the sperm-specific flagellar structure annulus to its designated position for precise flagellar compartmentalization, providing new evidence for distinct roles of centriolar distal appendage proteins in sperm flagellum formation compared to ciliogenesis.

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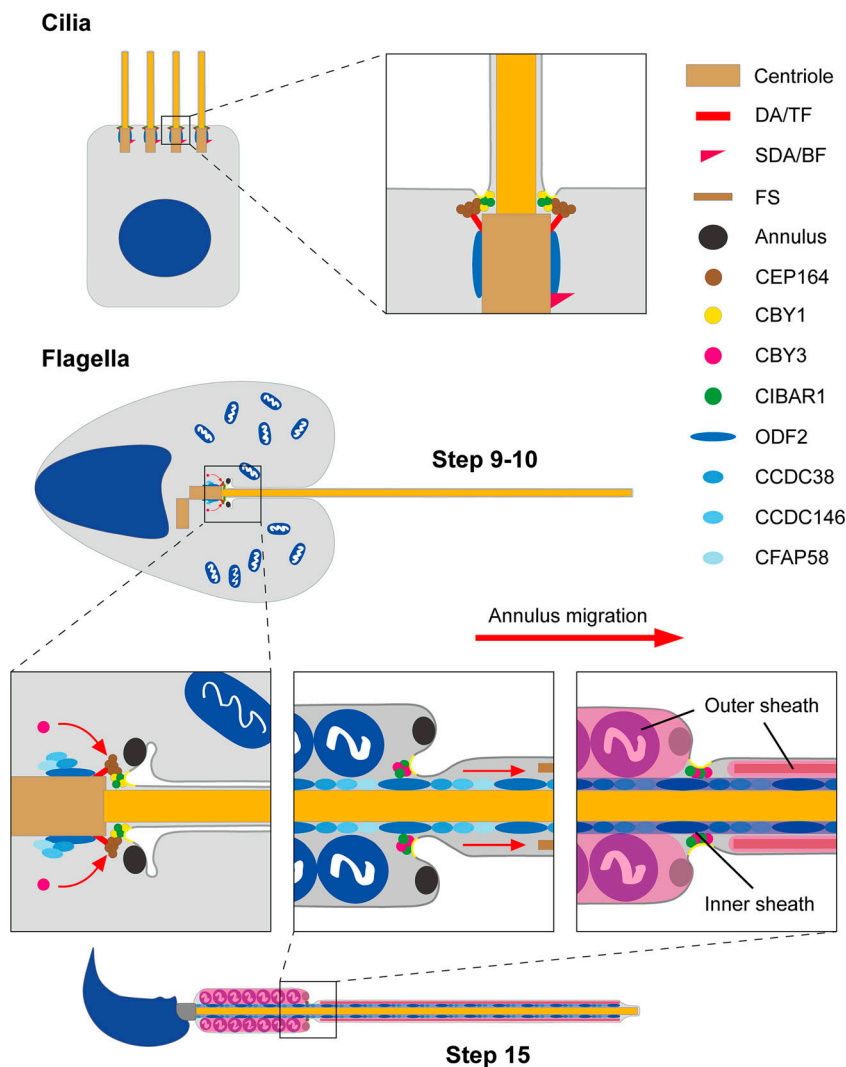


Figure 1. Centriolar appendages specialized as an inner sheath structure anchored between the flagellar axoneme and outer sheath. In mammals, the mitochondrial sheath, annulus, and fibrous sheath that envelop the outer side of the flagellar axoneme constitute an outer sheath. This configuration, not found in motile cilia, is considered the characteristic outer sheath of mammalian flagella. Within the flagellum, proteins associated with centriolar appendages form specialized structures. Examples include the CBY3/CIBAR1 complex and ODFs, which wrap around the exterior of the axoneme and are situated inside the outer sheath. These structures function as an inner sheath, playing a crucial role in the architecture of sperm flagella. TF, transition fibers; BF, basal feet; FS, fibrous sheath.

The CBY3/CIBAR1 complex, a derivative of the centriole, is retained in mammalian flagella, a phenomenon that is not isolated. Another interesting example of centriolar appendage proteins is ODF2, initially identified as a component of sperm's ODFs. Subsequent studies revealed *Odf2* transcripts encode the proteins CENEXIN and ODF2. CENEXIN, with an extended

C-terminus, is thought to be a shared component of the SDAs and DAs, crucial for centrosome and primary cilium formation. Male mice haploinsufficient for *Odf2* are infertile due to severe sperm deformities (7). Current antibodies cannot distinguish between CENEXIN and ODF2 signals, thus not excluding the possibility of CENEXIN's localization to ODFs within SDAs.

Consistently, ODF2 interacting proteins CCDC38, CCDC146, and CFAP58 also have dual localizations near the neck centriole and in mature sperm flagella (8–10). These findings suggest that some DA and SDA proteins persist in flagella after centriole degradation, specializing as flagellar structures, the inner sheath, and performing functions such as: (1) aiding in the formation of sperm-specific appendages, like the annulus, establishing flagellar subcompartments; and (2) acting directly as structural components of mature sperm tails, like ODFs (Fig. 1). Notably, the complete proteomic composition and molecular arrangement of these specialized centriolar appendage structures remain unclear. Future work could focus on isolating the sperm “inner sheath” and employing proteomic and structural biology techniques to unravel the secrets of its structure. These results broaden our understanding of centriolar protein functions, providing unique cases for understanding the structural specialization of classical organelles in terminally differentiated cells.

Acknowledgments

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