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Permeability barriers for generating a unique ciliary protein and lipid composition

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Cilia (and flagella) are microtubule-based protrusions that are found in single or multiple copies on the surface of most eukaryotic cells. Defects in cilia formation and/or function have now been correlated with an expanding spectrum of human genetic diseases termed ciliopathies. Recent work indicates that cilia are indeed a *bona fide* organelle with a unique protein and lipid content that enables specific cellular functions. Despite the physiological and clinical relevance of cilia, our understanding of how a unique protein and lipid composition is generated for this organelle remains poor. Here we review recent work on the mechanisms that determine the protein and lipid content, and thus the functional outputs, of this unique organelle.

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Introduction

Cilia (and flagella) are microtubule-based protrusions that are found in single or multiple copies on the surface of most eukaryotic cells. When present in multiple copies, cilia are generally motile organelles that beat to move fluid over the surface of the cell or to move the cell itself (review, [1]). When present as a single protrusion, cilia are generally sensory organelles that play a large number of essential roles in eukaryotic biology ranging from regulating embryonic development to sensing the extracellular environment to tissue homeostasis (reviews, [2–4]). Defects in cilia formation and/or function have now been correlated with an expanding spectrum of human genetic diseases termed ciliopathies (reviews, [5–7]). Since cilia are found on the surface of nearly all cells in the human body, most ciliopathies occur as syndromic disorders that affect many systems during development and/or adult life including the skeletal, kidney, central nervous, olfactory, and visual systems. Recent work indicates that the cilium is indeed a *bona fide* organelle with a unique protein and lipid content that enables specific cellular functions. Here we review recent work on the mechanisms that determine the protein and lipid content, and thus the functional outputs, of this unique organelle.

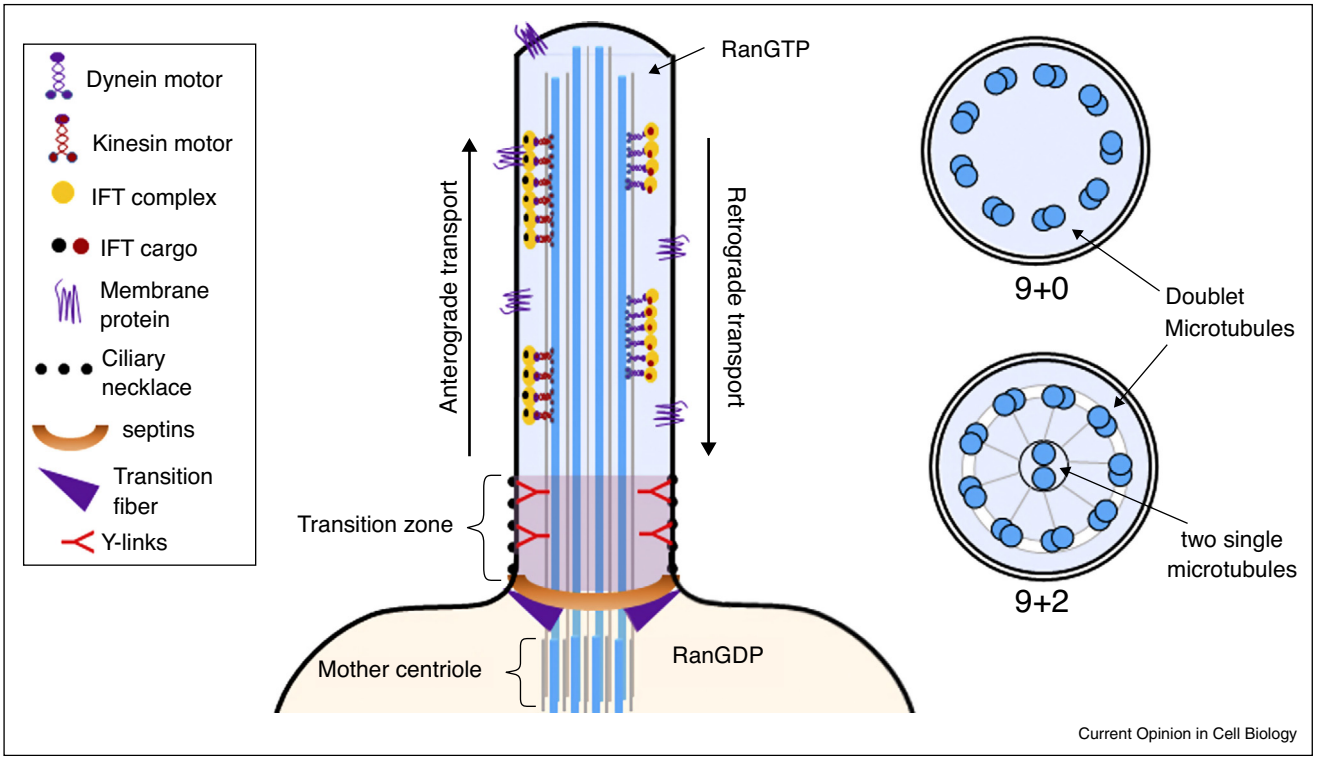
Cilium form and function

Cilia contain a microtubule core, the axoneme, which consists of a ring of nine doublet microtubules without (9+0) or with (9+2) two single microtubules in the center (Figure 1; for a review of cilium structure, see [8]). The axonemal microtubules serve not only a structural role but are also the 'tracks' for intraflagellar transport (IFT), a transport process driven by kinesin and cytoplasmic dynein motors that delivers building blocks and signaling molecules within the cilium (review, [9]). The doublet microtubules of the axoneme are extensions of the triplet microtubules of the mother centriole (the basal body).

The base of the cilium contains a specialized region called the transition zone (TZ) that contains structures that appear Y-shaped by electron microscopy (thus called Y-links) (Figure 1). Although the protein composition of the Y-links is not known, they serve to connect the doublet microtubules to the ciliary membrane. The Y-links may also organize a structure within the ciliary membrane called the ciliary necklace [10] (Figure 1). Proximal to the TZ are the transition fibers (TFs, derived from the distal appendages) that anchor the mother centriole to the periciliary membrane (the border between the plasma membrane and the ciliary membrane). The periciliary membrane is frequently infolded to produce a ciliary pocket that is an active site for exocytosis and endocytosis of ciliary membrane proteins [11,12].

Cilia require a unique protein complement for their function as motility-generating organelles, including axonemal dyneins, nexin/dynein regulatory complexes, and radial spoke complexes [13,14]. Cilia also require a unique protein complement for their function as sensory organelles, including receptor tyrosine kinases, ion channels, G-protein coupled receptors, and receptors for growth factors, Notch, Hedgehog, and extracellular matrix proteins (reviews, [15–18]). Understanding how these proteins are targeted to the ciliary compartment has implications for understanding a large number of cellular signaling pathways and the resultant diseases of their dysfunction. Unlike other organelles, the cilium is not

Figure 1



Cilium structure. The structural basis of the cilium is the axoneme which consists of a ring of nine doublet microtubules (blue) without (9+0) or with (9+2) two single microtubules in the center. The doublet microtubules also function as the tracks for intraflagellar transport (IFT) with anterograde IFT driven by kinesin motors (red) and retrograde IFT driven by cytoplasmic dynein (purple) motors. The transition zone (TZ) contains the ciliary necklace (black spots), Y-links (red), and septins (orange). The transition fibers (TFs, purple triangles) link the triplet microtubules of the mother centriole to the periciliary membrane. The cilium contains high levels of RanGTP whereas the cytosol contains high levels of RanGDP.

entirely enclosed by a membrane barrier but rather is open to the cytoplasm at the base. The ciliary base thus needs to control the selective entry of ciliary proteins and the TZ and the TFs are candidate gating complexes.

Generating a unique luminal protein composition

The prototype for an organelle that is not entirely enclosed by a membrane barrier is the nucleus where nuclear pore complexes (NPCs) embedded in the nuclear envelope provide the permeability barrier (reviews, [19–21]). NPCs are comprised of ~30 different nucleoporins (Nups) that assemble into defined subcomplexes such as the scaffold, the central channel, and the nuclear basket. The NPC has the remarkable property of being freely permeable to small molecules but becomes increasingly restrictive as the size of the molecule approaches or exceeds a diameter of ~5 nm, thus limiting the passage of proteins in a size-dependent and/or mass-dependent manner. Critical for this permeability is the presence of a specific class of nucleoporins (Nups) that contain intrinsically disordered domains rich in repeats of the dipeptide Phe-Gly (FG repeats). Large proteins and protein complexes containing nuclear import or export sequences are escorted through NPCs by karyopherins (importins and exportins, respectively) that interact with FG-containing Nups within the central channel. The association and dissociation of karyopherin-cargo complexes are regulated by a concentration gradient of RanGTP/GDP across the nuclear envelope.

The base of the cilium contains a permeability barrier with properties similar to that of the NPC. Small molecules are able to pass freely between the cytosolic and ciliary compartments but the access of larger molecules is restricted [22]. Like the NPC, the ciliary barrier is a 'kinetic barrier', meaning that increasingly larger objects take progressively longer to pass through ciliary pores [23,24]. It is not just overall molecular weight but the shape and thus the diameter of the molecule that determines its ability to enter the cilium by diffusion. For example, the increased mass of a GFP fusion does not impact the ability of soluble proteins to enter cilia [25–27], presumably because the GFP moiety does not alter the diameter of the molecule. Like the NPC, the ciliary barrier uses importins and a RanGTP/GDP gradient to regulate entry of large molecules [28–30] (Figure 1).