The Ciliary Membrane

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Abstract

Cilia and flagella function as important organizing centers for signaling in both development and disease. A key to their function is a poorly characterized functional barrier at their base that allows the protein and lipid composition of the ciliary membrane to be distinct from that of the plasma membrane. We review current models on the biogenesis of the ciliary membrane, highlighting several structures, including the ciliary necklace and ciliary pocket, that appear during biogenesis and that likely contribute to the barrier. The regulated movement of membrane proteins and lipids across this barrier is central to the sensory function of these organelles.

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Cilia and flagella are ancient organelles. With their complement of 800-1000 proteins composing an intricate structural core of nine cylindrically arranged microtubule doublets enveloped by a highly specialized extension of the cell membrane, they are also arguably the cell's most complex. Although once thought to be important on only a few cells specialized for moving themselves or other cells or fluids, we now know that many cells in multicellular organisms possess a single, non-motile primary cilium whose principal function is to detect and transmit optical, mechanical, or chemical signals. Cilia are highly dynamic organelles. Trains of intraflagellar transport (IFT) particles within them are moved by microtubule motors bidirectionally, ferrying ciliary components between the tip of the organelle and the cell body. The entire organelle is assembled and disassembled each time the cell divides -- its core set of microtubule doublets is templated upon microtubules within the older, mother centriole [1]. Even in post-mitotic cells, the ciliary membrane is undergoing constant turnover [2]. In this short review, we focus on the ciliary membrane, outlining several of its conserved features, and highlighting the ability of cells to regulate ciliary membrane composition in response to signals. We have tried to assimilate data from the study of diverse organisms as well as from different types of cilia and flagella, with the hope of emphasizing common themes. We will use the terms cilia and flagella interchangeably given that these organelles that are functionally and structurally similar.

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The cilium is an ancient signaling compartment for the receipt of extracellular signals

In many ways, we experience our environment through cilia. The outer segments of photoreceptor cells, which approach single photon sensitivity, are modified cilia; the kinocilium in hair cells of the ear organizes the stereociliary bundles that detect sound waves; and odorant reception occurs on the cilia in the olfactory epithelium. Cilia are also involved in the detection of signals produced within the organism, suggested by the enrichment of various receptors in the ciliary membrane. Some examples of such receptors include mechanosensory proteins such as the Polycystic Kidney Disease proteins 1 and 2 (PKD1 and 2), receptors for peptides and monoamines, and receptors for morphogenetic signals such as Patched 1 (Ptc1) (reviewed in [3]). In some cases, there is evidence that the localization of these receptors to the ciliary membrane is essential for their function [4-6].

Although these observations may suggest that cilia-based signal transduction is new in the evolutionary play, the use of these organelles for signaling is an ancient invention, functioning prominently in the flirtation with multicellularity that many protists undergo during sexual reproduction. For example, during fertilization in the biflagellated green alga *Chlamydomonas*, interactions between adhesion molecules (agglutinins) on the flagella of *plus* and *minus* gametes activate a signaling pathway[7] within the flagella that includes a protein tyrosine kinase, a cGMP-dependent protein kinase, and an adenylyl cyclase [8].

We still do not understand the biochemical logic that underlies the organization of signaling reactions within cilia. An important principle is that the regulated trafficking of signaling proteins into and out of cilia can be used to control steps in signal transduction cascades. For instance, the initiating event in Hedgehog (Hh) signaling involves the reciprocal movement of two transmembrane proteins, Patched 1 (Ptc1) and Smoothened (Smo), at cilia. In the absence of the ligand Sonic Hedgehog (Shh), the receptor Ptc1 is localized in the ciliary membrane and in a collar around the base of the cilium [6]. In some way, Ptc1 prevents the enrichment of Smo within the ciliary membrane, which is required for signal propagation. When Shh binds to Ptc1, the Ptc1 is lost from the cilium, allowing Smo to accumulate in the ciliary membrane and activate signaling [9]. A major challenge is to uncover the molecular mechanisms that drive such finely choreographed movements of proteins at the ciliary membrane. We begin by considering the structure, regional differentiations, and biogenesis of the ciliary membrane, all of which are critical to understanding membrane protein transport to this organelle.

The ciliary necklace and the ciliary pocket are sites of intimate membrane-basal body interactions

Membrane proteins and lipids that enter the cilium must traverse two distinct membrane specializations near the base of the cilium that are sites of membrane-basal body interactions. These regions constitute the functional barrier that separates the ciliary membrane from the plasma membrane. Both regions are near the transition zone, the site in the basal body at which the triplet microtubules of the basal body transition to the doublet microtubules of the axoneme. The most distal is the "ciliary necklace," which is visualized by freeze-fracture electron microscopy (EM) as multiple rows of intramembranous particles [10]. Transmission electron microscopy (Figure 1A) shows champagne-glass shaped structures that link this site of the membrane to the underlying microtubules of the basal body.

A second specialization of the ciliary membrane is a deep cleft that forms a double membrane sheath encircling the base of the cilium in cells from diverse organisms (Figure 1). In Trypanosomatid parasites, this region has been called the flagellar "pocket" membrane and we will adopt this terminology to refer to this region [11]. Though usually not

emphasized in most discussions of vertebrate cells, the ciliary pocket is a common feature in electron micrographs of mammalian cilia [12-15](Figure 1). A distinctive feature of the pocket is the interaction of its highly curved base with extensions from the basal body. These extensions, called transitional fibers or alar sheets [16], appear in transmission EM as struts that project at an angle from the basal body and connect to the base of the pocket (Figure 1) . These transitional fibers are likely derived from the nine distal appendages that mark the mother centriole prior to ciliation [1]. In fact, loss of distal appendages induced by depletion of the Odf2 protein in mouse cells is correlated with a defect in ciliation [17]. Although *Chlamydomonas* does not have a prominent flagellar pocket, the site where the transitional fibers link to the membrane is highly enriched in proteins of the IFT system [18]. Rosenbaum and colleagues have proposed that this IFT staging area functions as a flagellar pore that regulates entry of IFT particles into flagella (reviewed in [19]).

Interestingly, the ciliary pocket often marks the base of a deep invagination in the plasma membrane - - the ciliary sheath - - that can envelop a substantial fraction of the cilium. In some cases this sheath leads to the formation of "non-emergent" cilia that are almost entirely contained within the cytoplasm (Figure 1B)[12,13]. It is important to consider this topological property when interpreting immunofluorescence localization studies of membrane proteins at cilia because if the cilium being examined is non-emergent, it would be impossible to tell which of the two membranes layers contained the protein. The presence of non-emergent cilia suggests that the function of cilia as signaling centers may not always depend on their protrusion into the extracellular space, often considered a cardinal feature of these organelles.

Much of our current view of biogenesis of the membrane of the primary cilium is derived from seminal EM studies performed by Sorokin [13,20]. Although studies have suggested that the motile cilia of multi-ciliated epithelial cells and many protists form by docking of centrioles to the apical surface [14,20], Sorokin's work suggested that formation of the membrane of the primary cilium begins deep within the cell. The initial event in ciliary membrane biogenesis is the recruitment of membrane vesicles to encapsulate the distal end of the older mother centriole (Figure 2). This unusual, highly selective interaction between the incipient ciliary membrane and one centriole is a poorly understood but critical step in ciliogenesis that likely depends on interactions between specific proteins on the vesicles and those on the mother centriole. On the vesicle side, no candidate proteins are known that would mediate this interaction. Proteins in the planar cell polarity pathway have been recently implicated in specialized vesicle transport processes required for ciliary membrane biogenesis [21-24]. On the centriole side, several centriolar proteins required for ciliogenesis have been described, including those that selectively mark the mother centriole or have been implicated in membrane interactions [17,25-27]. However, the definitive identification of proteins that mediate this initial mother centriole-primary vesicle interaction will require the development of an assay that allows this event to be monitored in isolation from later steps in ciliogenesis.

After this initial step, the interaction between the centriole and the primary vesicle is likely stabilized by formation of the ciliary necklace, a site of close interaction between the membrane and newly assembled doublet microtubules. The primary vesicle adds additional membrane by fusion of secondary vesicles and simultaneously undergoes deformation into an invaginated sac that forms a double membrane sheath around the apical end (Figure 2). Morphologically, formation of this double membrane structure resembles reformation of the nuclear envelope around a chromosome or formation of the isolation membrane of an autophagosome. At this step, the interactions between the membrane and transitional fibers (presumably modified distal appendages) of the centriole stabilize the curved base of what will become the ciliary pocket.

Axoneme assembly can begin well before the cilium reaches the cell surface[13], suggesting that IFT and other processes that deliver materials to cilia become operational during the cytoplasmic biogenesis process and a functional barrier develops at this stage that separates the ciliary membrane from the outer sheath membrane (Figure 2). Lipids and membrane proteins delivered by vesicles to the outer sheath membrane would have to traverse this barrier before gaining access to the ciliary membrane. Eventually the sheath membrane at the distal end of this enveloped cilium fuses with the apical plasma membrane, allowing the cilium to emerge into the extracellular space (Figure 2). A natural consequence of this assembly pathway is formation of the ciliary pocket, which represents a remnant of the invaginated sac that initially formed around the centriole.

The ciliary pocket and its associated structures likely regulate membrane protein and lipid entry into the primary cilium

The ciliary pocket, necklace, and transitional fibers likely each make important contributions to the barrier that prevents the free mixing of membrane proteins between the plasma membrane and the ciliary membrane. The highly curved nature of the membrane at the base of the pocket could itself impose geometric constraints on the movement of lipids and membrane proteins across this region. The apparently stable, intimate interaction between the transitional fibers and the membrane could also hinder movement of membrane proteins through this site. In addition, based on studies of filipin-cholesterol complexes in freeze-fracture micrographs of multi-ciliated cells [28], the ciliary membrane in the necklace region is thought to possess a lipid composition different from the membrane covering the shaft of the cilium. The membrane over the main shaft is replete with filipin-sterol complexes (and thus perhaps more highly ordered than the plasma membrane), but the ciliary necklace region is devoid of these complexes. Sterols may play a role in protein localization at the ciliary membrane, since specific oxysterols can induce the movement of Smo to the ciliary membrane [6]. In keeping with the sterol studies, use of the membrane probe Laurdan as well the diffusion of a GPI-linked fluorescent protein have suggested that the base of the cilium has a unique lipid composition in the form of a condensed lipid zone that may form a diffusion barrier or "fence" between the plasma membrane and the membrane of the cilium [29]. The relative importance of these three features in overall barrier function remains to be determined.

Transport pathways for membrane protein movement to primary cilia

Current models suggest that membrane proteins targeted to cilia are deposited by vesicles near the base of the cilium (reviewed in [30]). The most likely place for vesicle fusion in this case is the membrane of the ciliary pocket itself (Figure 1D); however, this has not been rigorously established for primary cilia. Early insight into this process came from the study of ciliary appendages called mastigonemes from certain flagellates [31]. Using EM, these structures were found to be transported in Golgi-derived secretory vesicles to the flagellar pocket, from which they were the transferred to the ciliary membrane. In vertebrates, this model has been most prominently supported by the study of opsin transport to the outer segment of rod photoreceptors [32]. This directed, vesicle-mediated trafficking pathway from the Golgi to the base of the cilium remains the leading model for selective sorting of protein and lipid components to cilia (recently reviewed in [30] in this journal). It is important to emphasize, however, that this model is based largely on proteins that undergo constitutive rather than signal-induced enrichment in the ciliary membrane. Furthermore, the requirement for Golgi-derived vesicle trafficking has been explicitly tested (using drugs such as Brefeldin A) in only a few cases [33,34].

While the above model focuses on directed vesicle trafficking, an alternative possibility is that membrane proteins resident in the plasma membrane can simply move laterally into the

ciliary membrane by traversing the barrier imposed by the ciliary pocket and necklace. Initial evidence for such a route came from studies of adhesion molecules called agglutinins in Chlamydomonas [35-37]. In Chlamydomonas, fertilization is initiated when gametes of opposite mating types adhere to each other via agglutinins on their flagella. Adhesion leads to loss of active agglutinins from the flagella and subsequent replenishment by a pool from the cell body. Surprisingly, this adhesion-triggered movement was not due to the exocytosis of intracellular vesicles to the base of the cilium (as would be predicted by the vesicle trafficking model) but rather from the lateral transport of agglutinins from the plasma membrane to the flagellar membrane. The movement of agglutinins into the flagellar membrane was also independent of intraflagellar transport [38], further reinforcing the point that this represents a fundamentally different trafficking route from the canonical Golgi vesicle→ciliary base→IFT cargo model [30].

For almost two decades, flagellar agglutinins were the only example of lateral transport. In mammalian cells, the Hedgehog signaling protein Smoothened (Smo) has recently been shown to move via a strikingly similar lateral transport process from the plasma membrane to the membrane of the cilium when the pathway is activated by Shh [39]. A different study suggested that Smo moves from an intracellular pool so both pathways may be used in this instance [40]. Despite the evolutionary distance between mammals and Chlamydomonas, the lateral transport of both agglutinins and Smo can be regulated by the cAMP pathway [35,37,39,41]. One important similarity between agglutinins and Smo is that the enrichment of both proteins in the ciliary membranes is triggered by a signal.

Proteins that move either by the vesicle-mediated or the lateral transport pathways must ultimately traverse the diffusion barrier at the base of the cilium before gaining access to the ciliary membrane. Their movement to the peri-ciliary membrane (or the pocket membrane), however, must be regulated in fundamentally different ways. For the former, regulatory mechanisms might control either the packaging of cargo into vesicles targeted to cilia or control the fusion of vesicles with the pocket membrane. For the latter, it is the movement of proteins laterally from the plasma membrane into the pocket membrane that must be regulated.

Concluding remarks

Many of our current ideas about the ciliary membrane rest on studies performed decades ago. An exciting frontier in ciliary biology is the integration of this largely ultrastructural information with the recent explosion in the discovery of molecular components required for cilia formation and function.

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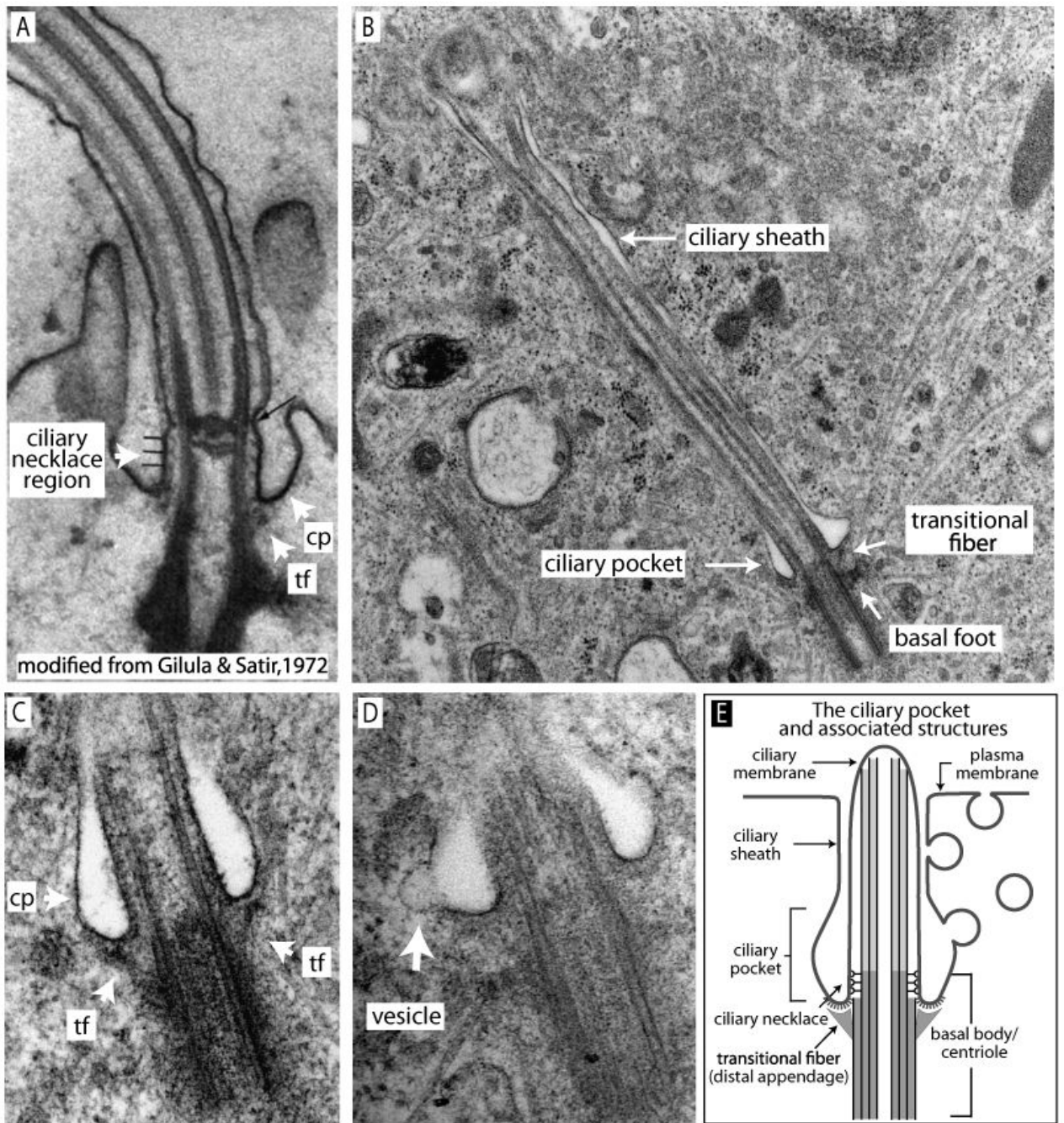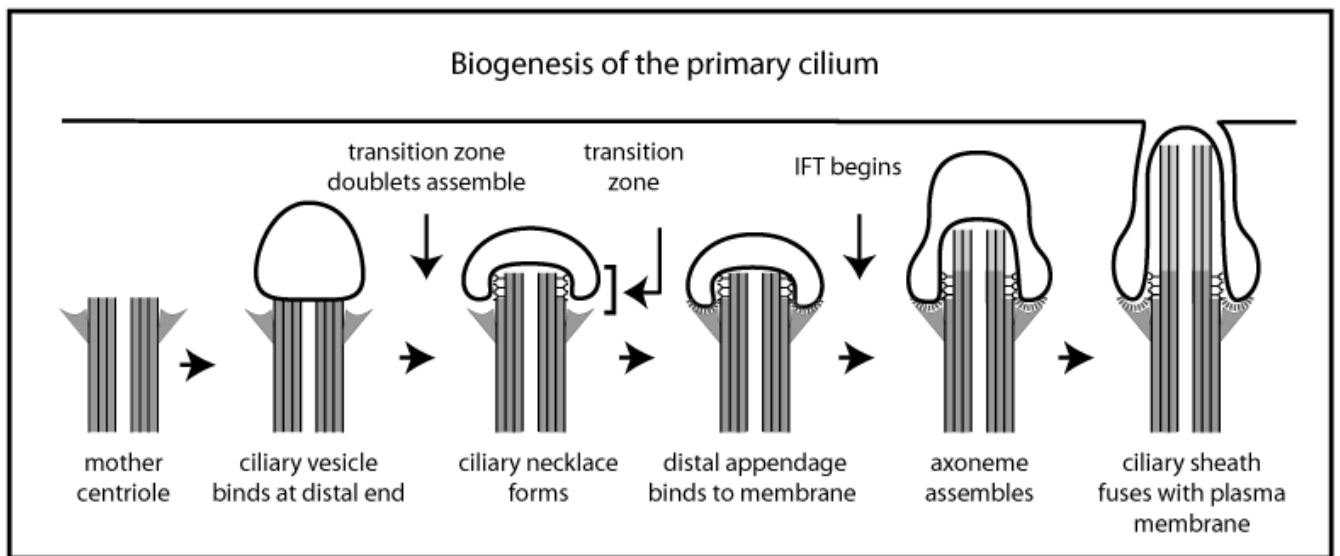
Figure 1.
Ciliary membrane structures. **A.** Modified from Gilula and Satir, 1972. Arrows indicate the
ciliary necklace and transitional fibers (tf), noted in the original report. We refer to the
invaginated membrane as the ciliary pocket (cp). **B-D** are images of mouse 3T3 cells in
culture and were kindly provided by Olivier Belzile, a graduate student in the Snell
laboratory. **B.** A cilium that appears to be entirely cytoplasmic (non-emergent) possesses a
ciliary pocket with transitional fibers attached at the base of the pocket. A basal foot is also
visible with microtubules emerging from it. **C.** A higher magnification view of a ciliary
pocket with transitional fibers (tf). **D.** A vesicle either budding or fusing with the ciliary

pocket membrane (see also Moser et al., 2009). **E.** Diagram of the ciliary pocket and associated structures.

Biogenesis of the primary cilium

transition zone   transition
doublets assemble   zone   IFT begins

mother   ciliary vesicle   ciliary necklace   distal appendage   axoneme   ciliary sheath
centriole   binds at distal end   forms   binds to membrane   assembles   fuses with plasma
                                                                                                                    membrane

Figure 2.
Biogenesis of the primary cilium. This model is derived from Sorokin [12]. Although the
ciliary necklace and the transitional fibers are well-documented structures, the timing of
their formation shown here is speculative as is the proposal that the doublet microtubules in
the transition zone form independently of IFT. Note the double membrane that envelops the
axoneme before the cilium reaches the cell surface. The inner membrane is the ciliary
membrane and the outer membrane is referred to as the sheath membrane in the text.