Cilia and flagella are closely related centriole-nucleated protrusions of the cell with roles in motility and signal transduction. Two of the best-studied signalling pathways organized by cilia are the transduction cascade for the morphogen Hedgehog in vertebrates and the mating pathway that initiates gamete fusion in the unicellular green alga *Chlamydomonas reinhardtii*. What is the role of cilia in these signalling transduction cascades? In both Hedgehog and mating pathways, all signalling intermediates have been found to localize to cilia, and, for some signalling factors, ciliary localization is regulated by pathway activation. Given a concentration factor of three orders of magnitude provided by translocating a protein into the cilium, the compartment model proposes that cilia act as miniaturized reaction tubes bringing signalling factors and processing enzymes in close proximity. On the other hand, the scaffolding model views the intraflagellar transport machinery, whose primary function is to build cilia and flagella, as a molecular scaffold for the mating transduction cascade at the flagellar membrane. While these models may coexist, it is hoped that a precise understanding of the mechanisms that govern signalling inside cilia will provide a satisfying answer to the question ‘how do cilia organize signalling?’ This review covers the evidence supporting each model of signalling and outlines future directions that may address which model applies in given biological settings.

1. Introduction

Cilia and flagella are closely related structures (the terms will be used interchangeably in this review) that form slender projections at the surface of nearly every cell in the human body. Unlike motile cilia found on specialized epithelia (airways, oviduct) and flagella on motile cells (spermatozoa), primary cilia are not endowed with motile properties. Instead, primary cilia are limited to providing the cell with an extension of a few micrometres that pokes into the milieu. Fascinatingly, primary cilia, motile cilia and flagella play critical roles in the transduction of several signals. Among the best-characterized signalling pathways with a strict requirement for cilia are the Sonic Hedgehog pathway in vertebrates [1–4] and the mating pathway in the unicellular green alga *Chlamydomonas reinhardtii* [5]. For other ciliary signalling pathways, the reader is referred to [6–10]. Superficially, the finding that signalling intermediates localize to cilia provides an explanation as to why cilia are required for signalling. Increasing chemical activities by concentrating molecules in cilia constitutes an attractive hypothesis for how cilia could participate in signalling. Indeed, Mahjoub & Stearns [11] found that diluting Hedgehog signalling intermediates into multiple primary cilia decreased pathway activation. An alternative model views cilia as the assembly point for specific scaffolds that bring together entire signalling modules in close proximity. Such scaffolds may have evolved from ciliary trafficking complexes responsible for assembling the cilium that were later co-opted by signalling pathways. In this later model, the cilium is a bystander where these dual-function complexes happen to reside. Thus, at the extremes, signalling cascades may either be concentrated inside cilia or organized on scaffolds inside cilia. It should be noted that this dichotomy is not exclusive as some components of a pathway (e.g. enzymes) may be scaffolded while others (e.g. second messengers) may be distributed diffusely inside cilia. Nonetheless, if a complete understanding of how cilia organize signalling pathways is to be gained, one needs to precisely determine the contribution of the ciliary transport machinery in terms of scaffolding and trafficking.
2. Building cilia and moving inside cilia

(a) The intraflagellar transport machinery

The assembly of cilia is a multistep process that entails docking of the mother centriole to the plasma membrane, assembly of the diffusion barriers that will separate the cilium from the rest of the cell and elongation of the axoneme (reviewed in [12]). Axoneme growth necessitates that building blocks be transported from the site of protein synthesis (the cytoplasm) to the site of incorporation (the growing tip of the axoneme) by an active process termed intraflagellar transport (IFT), which describes the processive movement of cargo-laden ‘trains’ inside flagella [13]. IFT trains are moved from base to tip (anterograde transport) by the motor kinesin II [14] and become remodelled at the tip before retrograde transport back to the base by cytoplasmic dynein 2 [15] (figure 1). The precise composition of IFT trains is not known, but the primary constituents are the complexes IFT-A and IFT-B ([18,19], see [20] for an extensive review of IFT). As IFT-B mutants only make very short or no cilia [17,21–27] while most IFT-A mutants assemble swollen cilia filled with IFT-B particles [27–30], it has been generally assumed that IFT-B mediates anterograde transport while IFT-A mediates retrograde transport. However, recent evidence supporting a role for IFT-A in anterograde trafficking [30,31] suggests that the retrograde defects of IFT-A mutants might be indirectly caused by a failure to traffic dynein 2 to the tip of the cilium. In addition, IFT-A has been proposed to ferry G protein-coupled receptors (GPCRs) into cilia [32]. Yet, the paucity of biochemically validated cargoes of IFT-A and IFT-B makes it difficult to assign the specific transport step (entry, exit, anterograde IFT, retrograde IFT) carried out by each complex.

(b) Intraflagellar transport cargoes: structural components of flagella

It is generally assumed that anterograde IFT trains bring axonemal building blocks from the cytoplasm to the tip of the axoneme, whereas retrograde IFT trains are laden with damaged proteins that must be recycled [33–35] and the first part of this hypothesis has now received significant support. The biochemical identification of numerous axonemal proteins associated with Chlamydomonas IFT-B was the first piece of evidence in support of the above hypothesis and motivated the search for the specific axonemal proteins that represent bona fide IFT cargoes [34]. One major axonemal component is the outer dynein arm, a gigantic complex pre-assembled in the cytoplasm that works in tandem with inner dynein arms to mediate flagellar waveform motility. It should be noted that, unlike cytoplasmic dyneins that move cargoes along microtubules, axonemal dyneins only function when stably incorporated in the axoneme. The protein ODA16 interacts directly with outer dynein arms but is not incorporated into the axoneme [36]. Since ODA16 interacts with the IFT-B subunit IFT46 and since an ift46 mutant fails to accumulate outer dynein arms in flagella [24], ODA16 appears to mediate the entry of outer dynein arms into flagella by bridging them to IFT trains. However, given the current data, it is unclear whether IFT46 also mediates intraflagellar transport of outer dynein arms once they have entered flagella. Another IFT-B subunit, IFT56, has been proposed to participate in the transport of some inner dynein arm subunits based on reduced amounts of those subunits in ift56 mutant flagella [37].

The best evidence to date for a bona fide IFT cargo comes from single molecule imaging of DRC4, a component of the dynein regulatory complex that regulates inner dynein arms. Here, about 1% of all IFT trains were found to harbour one molecule of DRC4 that co-moved with the IFT train until unloading at the tip (presumably during the IFT particle remodelling process) and diffusion to an unoccupied site on the axoneme [38]. Interference with IFT function affected both entry and intraflagellar movement of DRC4, thus demonstrating that DRC4 is an IFT cargo for entry and intraflagellar transport. Finally, it has been proposed that α/β-tubulin dimers are transported to

---

The alternative names of the vertebrate IFT polypeptides are shown with candidate IFT subunits awaiting confirmation italicized. (Online version in colour.)

Figure 1. Intraflagellar transport (IFT) and the cilium. The cilium and its diffusion barriers are shown together with the IFT machinery. The transition zone with its characteristic structures (Y-links) contains proteins required for restricting the diffusion of membrane proteins between the ciliary and plasma membrane [16]. By contrast, the precise location of the diffusion barrier for soluble proteins is not known and could be at the transition fibres or the transition zone. IFT trains composed of polymers of IFT-B and IFT-A complexes are believed to assemble on the transition fibres [17] and may capture their cargoes at this location. Upon loading onto the heterotrimeric kinesin-2, IFT trains move processively to the tip (anterograde transport) where train remodelling leads to loading on the microtubule minus end directed motor dynein 2 for retrograde transport. The alternative names of the vertebrate IFT polypeptides are shown with candidate IFT subunits awaiting confirmation italicized. (Online version in colour.)

<table>
<thead>
<tr>
<th>IFT-B</th>
<th>IFT-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFT20</td>
<td>IFT43</td>
</tr>
<tr>
<td>IFT22/RabL5</td>
<td>IFT121/WDR10</td>
</tr>
<tr>
<td>IFT25/C1orf41</td>
<td>IFT122/WDR35</td>
</tr>
<tr>
<td>IFT27/RabL4</td>
<td>IFT139/TTC21B/THM1</td>
</tr>
<tr>
<td>IFT38/Qilin/Claap1</td>
<td>IFT140</td>
</tr>
<tr>
<td>IFT46</td>
<td>IFT144/WDR19</td>
</tr>
<tr>
<td>IFT52</td>
<td></td>
</tr>
<tr>
<td>IFT54/MIP-T3</td>
<td></td>
</tr>
<tr>
<td>IFT57/Hippi</td>
<td></td>
</tr>
<tr>
<td>IFT56/TTC26</td>
<td></td>
</tr>
<tr>
<td>IFT70/Fier/TTC30B</td>
<td></td>
</tr>
<tr>
<td>IFT74/IFT72/IFT71</td>
<td></td>
</tr>
<tr>
<td>IFT80/WDR56</td>
<td></td>
</tr>
<tr>
<td>IFT81/CDV1</td>
<td></td>
</tr>
<tr>
<td>IFT88/Tg737/Polaris</td>
<td></td>
</tr>
<tr>
<td>IFT172</td>
<td></td>
</tr>
</tbody>
</table>

---

It is generally assumed that anterograde IFT trains bring axonemal building blocks from the cytoplasm to the tip of the axoneme, whereas retrograde IFT trains are laden with damaged proteins that must be recycled [33–35] and the first part of this hypothesis has now received significant support. The biochemical identification of numerous axonemal proteins associated with Chlamydomonas IFT-B was the first piece of evidence in support of the above hypothesis and motivated the search for the specific axonemal proteins that represent bona fide IFT cargoes [34]. One major axonemal component is the outer dynein arm, a gigantic complex pre-assembled in the cytoplasm that works in tandem with inner dynein arms to mediate flagellar waveform motility. It should be noted that, unlike cytoplasmic dyneins that move cargoes along microtubules, axonemal dyneins only function when stably incorporated in the axoneme. The protein ODA16 interacts directly with outer dynein arms but is not incorporated into the axoneme [36]. Since ODA16 interacts with the IFT-B subunit IFT46 and since an ift46 mutant fails to accumulate outer dynein arms in flagella [24], ODA16 appears to mediate the entry of outer dynein arms into flagella by bridging them to IFT trains. However, given the current data, it is unclear whether IFT46 also mediates intraflagellar transport of outer dynein arms once they have entered flagella. Another IFT-B subunit, IFT56, has been proposed to participate in the transport of some inner dynein arm subunits based on reduced amounts of those subunits in ift56 mutant flagella [37].

The best evidence to date for a bona fide IFT cargo comes from single molecule imaging of DRC4, a component of the dynein regulatory complex that regulates inner dynein arms. Here, about 1% of all IFT trains were found to harbour one molecule of DRC4 that co-moved with the IFT train until unloading at the tip (presumably during the IFT particle remodelling process) and diffusion to an unoccupied site on the axoneme [38]. Interference with IFT function affected both entry and intraflagellar movement of DRC4, thus demonstrating that DRC4 is an IFT cargo for entry and intraflagellar transport. Finally, it has been proposed that α/β-tubulin dimers are transported to
the tip of the cilium through a direct interaction between tubulin and the IFT-B subunits IFT74 and IFT81 [39]. However, since IFT74/81 binds robustly to microtubules and since the IFT motility rates of an IFT81 tubulin-binding mutant are affected [39], it remains conceivable that the function of the IFT74/81-tubulin interaction pertains to a recognition of axonemal microtubules by IFT-B to enhance the processivity of IFT train movements. Accordingly, it should be noted that the requirement for IFT in transporting tubulin to the tip of the cilium has been recently questioned based on the measurement of diffusion rates inside cilia [40].

(c) Intraflagellar transport cargoes: signalling components of cilia

While the literature offers strong evidence that the IFT machinery transports building blocks to the tip of the assembling axoneme, a role for IFT in moving signalling factors within the fully assembled cilium remains elusive. With the transient receptor potential channels OCR-9, OSM-9 and PKD2 tagged with fluorescent proteins, some foci of molecules move processively inside cilia at IFT motility rates [41,42]. However, the global contribution of IFT to the dynamics of these proteins remains untested. In the case of rhodopsin, a biochemical interaction has been described with the IFT-B subunit IFT20 [43] and the intraciliary mobility of rhodopsin heterologously expressed in cultured cell cilia is accelerated by kinesin 2 [44]. Meanwhile, imaging of single molecules of the signalling receptors SSTR3 and Smoothened (Smo) inside mammalian cilia enabled a systematic analysis of intraciliary dynamics. Somewhat surprisingly, Smo and SSTR3 spend only a quarter of their time undergoing active transport, and those active transport events span only a short distance within cilia [45]. However, this result may not be so surprising given that membrane proteins do not need to be rapidly delivered to the tip of the growing axoneme and may thus rely upon diffusive movements for their exploration of the ciliary space, while IFT-powered transport may only serve to bias their distribution.

3. The ciliary compartment model of signalling

(a) Biological evidence

The importance of IFT in signalling has remained a matter of debate. While IFT mutant mice show obvious Hedgehog signalling defects, cilia morphology in these mutants is also severely affected. The genetic requirement for IFT in Hedgehog signalling may thus result either from a direct requirement for the IFT proteins in organizing the Hedgehog cascade or from an indirect requirement in building an organelle that concentrates signalling molecules.

The Hedgehog signalling cascade is summarized in figure 2. For further details, the reader is directed to several excellent reviews covering the state of the art in Hedgehog signalling.
Box 1. Calculations

**Volume of the cilium**

Assuming an accessible cross section of 0.3 μm diameter, the ciliary volume $V_c$ is $5 \times \pi \times (0.15)^2 = 0.35 \mu m^3 = 0.35 \times 10^{-15} l$.

**Concentration of a single soluble protein inside the cilium**

The protein molarity inside cilia $[\text{Prot}]_c$ is expressed as $([\text{number of molecules}] / N_a) / V_c$, where $N_a$ is the Avogadro number. Thus for a single protein inside the cilium, $[\text{Prot}]_c = [1/6 \times 10^{23}] / (0.35 \times 10^{-15}) \approx 5$ nM.

**Ratio of cytoplasmic volume to ciliary lumen volume**

The cytoplasmic volumes of typical cultured mammalian cells are between 0.94 and 2.4 μm$^3$ [50], i.e. 2700–6800 greater than the cilium volume calculated above.

**Ratio of plasma membrane surface area to cilium membrane area**

A typical value for the surface area of the plasma membrane is 2000 μm$^2$ [51] and modelling the cilium as a 0.3 × 5 μm rod yields a surface area of $2 \times \pi \times 0.15 \times 5 = 4.7 \mu m^2$. The ratio of surface area is therefore close to 400.

**Resact receptor concentration on sperm flagella**

Here, it has been estimated that 14,000 molecules of the resact receptor are present on the 50 μm long flagellum, yielding a surface concentration of 14,000 / (50 × $\pi \times 0.15^2$) = 4000 receptors μm$^{-2}$.

and cilia [46,47]. At one extreme, it has been proposed that the role of IFT in signalling is strictly owing to IFT being required for cilium assembly [30,31]. The ‘signalling compartment’ hypothesis posits that the cilium as a whole is a signalling centre and relegates the IFT machinery to the industrious task of building cilia (figure 4). In the support of the signalling compartment model, it was shown that defects in Hedgehog signalling caused by mutations in the retrograde IFT machinery can be rescued by mutations in anterograde IFT components, commensurately with the rescue of cilium morphology [30,31]. Specifically, cilia are severely malformed in a null mutant of dynein 2 and show characteristic bulges filled with IFT-B complexes, indicative of a failure to transport IFT-B complexes from tip to base and out of cilia. Consistent with previous observations [1,48], this dynein 2 mutant causes strong defects in Hedgehog signalling and abnormal accumulation of Hedgehog signalling components inside cilia. Remarkably, reducing the activity of the IFT-B subunit Ift122 or of the IFT-A subunits Ift122 or Ift144 in the dynein 2 null mutant ameliorates ciliary architecture and partially rescues defects in Hedgehog signalling and in trafficking of Hedgehog pathway components. These results suggest that reducing the anterograde transport of Hedgehog signalling components compensates for the lack of retrograde IFT in dynein 2 mutants and that it is the balance of directional transport fluxes inside cilia—rather than the activity of IFT complexes themselves—that is the critical determinant for Hedgehog signal transduction. A remarkable side conclusion of this study is that it is possible to assemble morphologically intact cilia in the complete absence of retrograde IFT. In the dynein 2$^{-/-}$ /Ift122$^{-/-}$ or dynein 2$^{-/-}$/Ift172$^{-/-}$ cilia, the removal of material from cilia must be through diffusion rather than active transport. While surprising, this conclusion is supported by the finding that flagellar shortening in *Chlamydomonas* still takes place with normal kinetics in a temperature-sensitive mutant of dynein 2 shifted to the restrictive temperature, even though retrograde IFT becomes rapidly undetectable under these conditions [49]. Thus, diffusion must be sufficiently efficient to remove proteins from the cilium, a prediction supported by our calculations that a typical protein of 100 kDa (e.g. the tubulin dimer) can move from one end of the cilium to the other by diffusion nearly as fast as it can by IFT [40].

(b) Physical considerations

(i) Soluble space

In the signalling compartment model, appropriate signal processing is mediated by the increased molecular crowding brought through concentration within cilia or by the specific physicochemical environment of the cilial interior [2,3]. A simple calculation of cell surface and volume shows that the volume of the cilium is approximately 5000 times less than the cytoplasmic volume and the surface area of the cilium is approximately 500 times smaller than that of the plasma membrane (box 1). Thus, translocating the entire pool of a protein into cilia would increase its concentration by two to three orders of magnitude. Along these lines, it is worth noting that the molarity of a single protein present in the lumen of a 5 μm cilium is calculated to be a remarkably high 5 nM (box 1). While the activity of a single molecule in solution cannot be formally assessed using ensemble theories, the nanomolar concentration of a single molecule present in the ciliary interior provides a sense of the extremely confined environment that the cilium represents.

(ii) Membrane

The highly confined surface of the cilary membrane is conducive to very high protein concentrations and has enabled the evolution of quantal detection systems in vision and sperm chemotaxis. In the example of phototransduction, the concentration of receptor molecules reaches the highest level known for membrane proteins. Rhodopsin is concentrated in pancake-shaped discs stacked inside the outer segment (equivalent of a ciliary shaft) of photoreceptors at a surface concentration estimated to be 27,000 rhodopsin μm$^{-2}$ in amphibian and mammalian rods [52]. While it may be difficult to gauge the magnitude of this number, it is worth considering the relative amount of space occupied by rhodopsin in disc membranes. Given that the cross-sectional area of rhodopsin is approximately 10 nm$^2$, rhodopsin occupies 270 000 nm$^2$ μm$^{-2}$ of membrane, or 27% of the total surface area [53]. With a theoretical packing limit of 78% space occupancy for rhodopsin, one realizes that disc membranes are near-crystalline arrays of rhodopsin. This extremely high concentration of rhodopsin is critical to the ability to detect single
photon intensity, a twofold reduction in rhodopsin levels results in a decrease of sensitivity in vision [53]. Thus, the outer segment represents a true collecting antenna for photons. Remarkably, rhodopsin mobility is extremely high [54,55], in large part because of the high fluidity of disc membranes rich in poly-unsaturated lipids (such as the ‘omega 3’ fatty acid DHA) [56].

Meanwhile, sea urchin spermatozoa exhibit chemotaxis towards the egg by sensing an egg-secreted peptide (named resact) at their flagella. Remarkably, sperm chemotaxis displays single molecule sensitivity to resact thanks to the relatively high concentration of resact receptors in sperm flagella [57]. Here, it has been estimated that the resact receptor is present at a surface concentration of 4000 receptors μm⁻² on sperm flagella [58]. While the concentration of resact receptor is considerably less that of rhodopsin, sensing a diffusible peptide does not require that every peptide colliding with the flagellar membrane be detected (unlike the case of photons travelling through the retina).

These features of high protein concentration in both the soluble and the membrane space of the cilium are likely to play a more general role in signal transduction in the cilium. However, unlike the example of rhodopsin and the resact receptor outlined above, no information is currently available on the absolute number of signalling molecules present in conventional cilia and flagella. Our efforts at measuring absolute numbers of the GPCR somatostatin receptor 3 (SSTR3) expressed in mouse kidney IMCD3 cells yielded a surface concentration of 5000 molecules μm⁻² corresponding to a 5% space occupancy [40]. While SSTR3 was heterologously expressed in these IMCD3 cells, care was taken to limit the levels of expression of SSTR3, and these data suggest that receptor concentration in the ciliary membrane may reach unexpectedly high levels.

(c) Chemical considerations

The existence of a specialized lipid composition in the ciliary membrane has received anecdotal evidence (see [59] for a detailed summary), and it is tempting to consider the ciliary membrane as a privileged lipid environment. Yet, the absence of quantitative data on the lipid composition of the ciliary membrane makes it difficult to precisely assess this hypothesis.

Another physico-chemical parameter that might be unique to the ciliary space is the redox potential. While the textbook view of a highly reducing environment being limited to the endoplasmic reticulum lumen remains largely true, the intermembrane space of mitochondria freely exchanges small molecules with the cytoplasm yet maintains a more reducing environment than the cytoplasm [60]. This reducing environment in turn permits the formation of disulfide bridges in the intermembrane space. Likewise, it is conceivable that the ciliary lumen could harbour a different redox potential than the cytoplasm. In Chlamydomonas, three subunits of the outer dynein arms harbour thioredoxin motifs consisting of closely spaced cysteine residues known to function in redox reactions [61]. Remarkably, these three subunits have been shown to form mixed disulfides with other flagellar proteins [62]. Whether similar redox-sensitive proteins exist in primary cilia and whether cilia harbour a unique redox potential remains to be seen.

Most recently, a technical tour de force has enabled the measurement of Ca²⁺ levels inside cilia. The possibility that cilia may constitute a privileged environment for Ca²⁺ was proposed more than a decade ago based on the localization of Ca²⁺-permeable channels in the ciliary membrane [63,64], but direct measurements of ciliary Ca²⁺ levels had thus far been lacking. Using classical electrophysiology as well as newly developed biosensors, the Clapham laboratory determined that the molarity of Ca²⁺ in cilia is close to 0.6 μM, more than five times greater than cytoplasmic [Ca²⁺] [65,66]. Interestingly, the authors of these studies proposed that this asymmetry in Ca²⁺ concentration is dynamically maintained and needs no diffusion barrier. Instead, the continuous entry of Ca²⁺ ions from the extracellular milieu into cilia through abundant Ca²⁺ channels in the ciliary membrane is sufficient to counteract the leaking of Ca²⁺ at the base of cilia. High steady-state levels of ciliary Ca²⁺ are thus achieved dynamically and the 5000-fold greater volume of the cytoplasm compared with the cilium makes any leakage of ciliary content into cytoplasm the equivalent of diluting a river into the ocean [65,66]. The important conclusion of these studies is that, even in the absence of a diffusion barrier, small molecules and ions can become enriched in cilia relative to cytoplasm, and one might wonder if other molecules than Ca²⁺ may similarly achieve high ciliary concentration in the absence of diffusion barriers.

4. The scaffold model of signalling

(a) Mating

An active role of the IFT machinery in transducing signals has been suggested by studies of the mating response in Chlamydomonas. The availability of a conditional mutant of the anterograde IFT motor (Kinesin-2ts) in Chlamydomonas has enabled the rapid interruption of IFT in structurally normal flagella that were assembled at the permissive temperature [14]. Thus, unique to this system, a direct role for IFT in signalling can be assessed separately from the role of IFT in flagellum assembly. Chlamydomonas flagella endow this single-cell organism with motility and with phototropic and mating signalling. The mating cascade (figure 3, reviewed in [5]) is initiated when two gametes of opposite mating types adhere through association of their mating receptors (termed agglutinins) exposed in an active form on flagella (an impermeable cell wall covers the rest of the plasma membrane of Chlamydomonas). Subsequent to interaction of the mating receptors, a transduction cascade triggers tyrosine phosphorylation of a cGMP-dependent protein kinase (PKG) and activation of adenylate cyclase inside flagella. The ensuing production of cAMP generates a signal that can diffuse out of flagella to trigger cell wall shedding and assembly of a cell protrusion that promotes gamete fusion (figure 3). While shifting the Kinesin-2ts mutant from 20°C to 32°C does not result in any appreciable change in cilium morphology for at least an hour, IFT polypeptides rapidly disappear from flagella and the ability of gametes to fuse is entirely lost 40 min after the temperature shift [67]. Importantly, addition of the cell permeable cAMP analogue dibutyrate cAMP results in a complete rescue of gamete fusion in Kinesin-2ts cells shifted to 32°C [67], thus demonstrating that IFT per se is required for activation of the flagellar adenylate cyclase in response to mating signals. One interpretation of these results is that simple diffusion of molecules within flagella is not sufficient for transduction of the mating signals and that IFT is needed to scaffold the signalling components of the mating cascade. Alternatively, it remains possible that IFT is required for the import of a specific signal transduction intermediate into flagella. While both models remain viable, a series of experiments from the Snell laboratory
favour a direct role for IFT in the transduction of mating signals [68]. Upon mating pathway activation, the flagellar PKG and the IFT complexes translocate from a largely insoluble fraction into a membrane-associated fraction. These results suggest the formation of a supercomplex of IFT, PKG and possibly other signalling components (e.g. agglutinins) at the flagellar membrane upon engagement of the mating pathway. Together, these results point to a rather direct role for IFT in mating signalling and suggest that IFT trains may assemble signalling scaffolds at the membrane to bring all components of the mating cascade into close proximity and allow signals to be rapidly propagated from one molecule to the next (figure 4b). Inspiration for this model comes from the yeast mating cascade in which the Ste5 scaffold binds to MAPKKK, MAPKK and MAPK and optimally orients each substrate for phosphorylation by its upstream kinase [69,70].

(b) Hedgehog
While one might be tempted to conclude that different ciliary signalling pathways are simply organized differently, i.e. Hedgehog by the compartment model and mating by the scaffold model, some evidence may support the scaffold model for Hedgehog signalling. First, while deletion of most IFT-B subunits in mice leads to severe structural abnormalities of the cilium [22,25,26,71,72], knockout of Ift25 resulted in cilia that are morphologically indistinguishable from their wild-type counterparts [73]. Yet, Hedgehog signalling was as strongly affected in Ift25 mutants as in other IFT-B mutants. Similarly, a hypomorphic allele of Ift80 produced animals with morphologically intact cilia but compromised Hedgehog signalling [74]. Although these results demonstrate a role of IFT in Hedgehog signalling besides simply building cilia, the finding that Patched and Smo hyperaccumulate in cilia of

---

**Figure 3.** Mating signalling at a glance. The mating of haploid *Chlamydomonas* gametes is initiated by the adhesion between flagella of cells of opposite mating types. The interaction between opposite agglutinins (i.e. mating receptors) triggers the activation of a flagellar signalling cascade that culminates in the elevation of cytoplasmic cAMP and a series of morphological changes that enable gamete fusion. See text for details. (Adapted from [5].) (Online version in colour.)

**Figure 4.** The compartment and scaffold hypotheses of ciliary signalling. A diagram of the Hedgehog cascade fully contained inside cilia is depicted in the compartment model (a). The mating cascade is the exemplar chosen for the IFT scaffold model (b). See text for details. (Online version in colour.)
Ift25 knockout suggests that IFT-B may transport signalling molecules out of cilia, and these results are therefore compatible with the compartment model.

Second, examination of another trafficking complex, the BBSome [75], also lends support to the signalling scaffold model. The BBSome is a hetero-octameric complex of proteins defective in the ciliopathy Bardet–Biedl syndrome (BBS) [76], a disease characterized by obesity, polydactyly, retinal degeneration and kidney malformations. While the precise molecular basis of these symptoms remains elusive, polydactyly is frequently associated with hyperactive Hedgehog signalling during limb patterning, and it was thus thought that the BBSome participates in Hedgehog signalling. Interestingly, the BBSome shares several features with coat complexes such as clathrin, COPI and COPII [77]: recruitment to membranes by an Arf family GTPase (ARL6/BBS3 in the case of the BBSome), mixture of β-propellers and α-solenoid domains, direct recognition of sorting signals exposed on the cytoplasmic tails of membrane proteins and finally polymerization into coats apposed to membranes. The polymerization of canonical coats sculpts membranes. The polymerization of canonical coats sculpts membranes to generate a carrier vesicle filled with membranous cargoes [78] while BBSome polymerization is only thought to generate a planar coat bound to membrane proteins destined for cilia [77]. Intriguingly, the BBSome has been shown to mediate both the import and the export of transmembrane as well as membrane-associated proteins into and out of cilia [77,79–84]. Furthermore, the BBSome itself undergoes IFT [75,81] and it is conceivable—albeit undemonstrated—that the BBSome may promote active movements of specific proteins inside cilia. With respect to Hedgehog signalling, binding of the BBSome to the cytoplasmic tails of Smo and Patched suggests that the BBSome may serve as a scaffold for part of the Hedgehog signalling cascade [82–84]. While the signalling scaffold activity of the BBSome remains to be demonstrated, a requirement for the BBSome in exit of Patched and Smo out of cilia strongly suggest that the BBSome functions as a trafficking complex for these membrane proteins [83,84]. Interestingly, in all species tested to date, the BBSome appears to be required for signalling (phototropism in Chlamydomonas, sensory signalling in nematode) rather than cilium assembly and therefore constitutes an appealing candidate for either a signalling scaffold and/or a sorting complex specializing in signalling factors transport.

Although these results demonstrate that Hedgehog signalling defects can be present in cells with apparently normal cilia, they are compatible with both the compartment and the scaffold models as the ciliary levels of Hedgehog signalling intermediates are altered in the Ift25 and BBSome mutants. While it is conceivable that the primary defect in these mutants is improper loading of Smo or Patched onto specific scaffolds, it is also possible that Hedgehog signalling perturbations result from aberrant amounts of Patched or Smo inside cilia.

Third, an intriguing candidate scaffold molecule is the Evc complex [85], composed of the proteins Evc and Evc2, which are mutated in Ellis Van Creveld and Weyers syndromes together with the proteins EFCAB7 and IQCE. The Evc complex is localized distal to the transition zone and in some cell types is required for targeting of Gli and Sufu to cilia [85,86]. Given a biochemical interaction between the Evc complex and Smo, it has been proposed that it may participate in bringing Smo close to its downstream signalling partners [85,86].

5. Conclusion

In the end, one must conclude that both models of ciliary signalling are equally viable in the context of the Hedgehog pathway and may in fact not be mutually exclusive. Clearly, the tools available in Chlamydomonas have enabled a deeper analysis of the role of IFT in mating signalling and acute interference with IFT would help refine our understanding of how IFT contributes to Hedgehog signalling. Here, the recent discovery of the dynein inhibitor cilobrevin [87] holds some promise as this compound rapidly interrupts both anterograde and retrograde IFT [45]. However, Hedgehog signalling is typically assessed using a transcriptional readout of Gli activity, after several hours of pathway activation, and long-term treatment of cells with cilobrevin does ultimately lead to cillum resorption. Biosensors of Hedgehog signalling activity (e.g. through monitoring the processing of Gli using Förster resonance energy transfer pairs) together with rapid inactivation of IFT would enable a test of the signalling models.

Another line of research that may allow for a test of the signalling models is the mechanistic dissection of recruitment to IFT trains. As noted above, we know very little about the interaction of signalling factors with the IFT machinery. Here, if one could interfere with the recruitment of specific signalling factors to IFT trains without altering the levels of said signalling factors inside cilia, it should be possible to rigorously put the signalling scaffold model to test, as this model predicts that losing association with IFT trains will lead to defects in signalling. While the concern that interference with recruitment of IFT trains may cause defects in ciliary entry, a recent report suggests that not all proteins require IFT for trafficking into cilia [88].

In summary, while the question of 'why signalling inside cilia' remains a fascinating one to many cell biologists, the tools and information currently available are not sufficient to properly test the models of ciliary signalling. Considering the rapid progress witnessed in the field in the past 5 years and the influx of researchers into the fertile area of research on ciliary signalling, it is likely that transformative advances will be made to test the scaffold and compartment models in the next few years.

Acknowledgements. I thank David K Breslow and Nancy Zhang for critical reading of the manuscript and the entire Nachury laboratory for stimulating discussion. I apologize to my colleagues, whose work was not cited owing to space restriction.

References


http://rstb.royalsocietypublishing.org/ on May 26, 2017


