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Cilia Orientation and the Fluid Mechanics of Development

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Summary

Motile cilia produce large-scale fluid flows critical for development and physiology. Defects in ciliary motility cause a range of disease symptoms including bronchiectasis, hydrocephalus, and situs inversus. However, it is not enough for cilia to be motile and generate a flow - the flow must be driven in the proper direction. Generation of properly directed coherent flow requires that the cilia are properly oriented relative to tissue axes. Genetic, molecular, and ultrastructural studies have begun to suggest pathways linking cilia orientation to planar cell polarity and other long-range positional cues, and also suggest that cilia-driven flow can itself play a causal role in orienting the cilia that create it. Errors in cilia orientation have been observed in human ciliary disease patients, suggesting that orientation defects may constitute a novel class of ciliopathies with a distinct etiology at the cell biological level.

Introduction

Cilia are key players in many human diseases (1). One major function of cilia is to propel fluid across the surface of an epithelial sheet. Cilia-driven flow is strongly directional and extends over long distances relative to a cell. Cilia-driven flow can move large quantities of fluid and can even serve as a long-range orientational signal.

But directional flow comes with a price: you must be able to tell direction. The strongest flow in the world won't do a lick of good if it flows the wrong way. For example, mis-oriented ciliary flow would tend to drive mucus into the lungs, rather than out the mouth, with potentially catastrophic results.

Cilia-driven fluid flows in development and physiology

Loss of ciliary flow in human immotile cilia diseases, such as primary ciliary dyskinesia (PCD), blocks mucus transport in the airways, leading to bronchiectasis and chronic sinusitis (1). Ciliated cells in the female reproductive tract, produce flow that transports the egg from the fallopian tube to the uterus. Loss of flow can misplace the egg, causing ectopic pregnancies. In the brain, ciliated ependymal cells produce long-range flow of cerebrospinal fluid (CSF) within the ventricles. In the mouse forebrain, this flow directs the long range migration of neuroblasts along the lateral walls of the olfactory bulb (2). Cilia orientation in ependymal cells is tightly coupled to the anterior-posterior neuraxis, reflecting the need to move CSF from sites of production to sites of reabsorption. Without this flow, CSF pressure builds up causing hydrocephalus (3)

Ciliary flow also has a dramatic role in early development, where it operates in the node during gastrulation to determine the outcome of left/right symmetry breaking. Node cells contain motile monocilia (4) which generate a leftward flow of extraembryonic fluid that determines the direction of symmetry breaking (4,5). Cilia driven flows play a conserved role in symmetry breaking in many different vertebrates (6-8). But how are the cilia oriented to determine leftward flow?

Degrees of freedom for ciliary orientation

Ciliary orientation is dictated by the basal body, a derivative of the centriole that docks underneath the plasma membrane and nucleates cilia formation (9). The basal body is a cylindrical array of nine parallel microtubule triplet “blades” whose plus-end corresponds to the end where the cilium emerges.

As illustrated in Figure 1, the basal body or cilium requires six numbers to describe its position and orientation. Three of the six are the Cartesian coordinates that specify the translational position of the basal body within the cell. Basal bodies are often found near one end of the cell with a positional bias determined by overall tissue polarity (10). Basal body orientation is further specified by two angles ϕ and θ to describe tilt and a third angle ψ to describe rotation about the long axis. The rotational orientation angle ψ determines the direction of ciliary beating.

Asymmetrical structures that project from the basal body may provide handles that the cytoskeleton can act upon. For example, the basal feet point towards the direction of flow (11,12), suggesting a pulling force exerted on the basal feet would tend to rotate the centrioles so as to align the ciliary beat in the direction of the pulling force. Consistent with this idea, myosin localizes to the basal feet during the “anchoring phase” when orientation of basal bodies is being established (13).

Establishing XYZ position

Motile cilia localize apically in epithelial cells, requiring positioning and docking of basal bodies along the apical surface. This is likely to be an active process. Pharmacological inhibition of actin prevents basal bodies from moving to the cell surface (14). Actin is associated with nascent basal bodies as they move towards the surface where it appears to assemble into long “comet tails” similar to those that drive *Listeria* movements which might drive basal body movement (15).

Actin may also be involved in anchoring basal bodies once they reach the surface. Basal bodies normally associate apically with a web-like actin network, and experimental treatments that eliminate this network, including mutation in the transcription factor FoxJ1 or prevention of RhoA activation, also prevent basal body docking (16,17).

Polarized actin-based processes are often oriented by the Par3-Par6-atypical PKC complex (18). In polarized epithelia, this Par complex acts as a mark for the apical surface, which is where the basal bodies migrate to assemble cilia. Possible involvement of the Par complex in basal body positioning is suggested by the fact that basal body proteins can directly interact with Par complex proteins (19). Furthermore, the Par6-interacting protein CRB3 is required for ciliogenesis (20) and other members of the same protein family can influence ciliary length and motility (21).

The planar cell polarity (PCP) pathway, which is a system to propagate orientational information across a tissue (22) also appears to be involved in translational position of cilia (23). PCP genes *inturned* and *fuzzy* are associated with apical localization of basal bodies in ciliated cells (24). Knock down of either gene disrupts the formation of the apical web-like

actin network and ciliogenesis, as does interfering with function of dsh or frizzled, core components of the PCP pathway (25). A major challenge is to dissect out the role of these processes in positioning of basal bodies, from a likely one in the downstream process by which basal bodies acquire their orientation.

Establishing Rotational Orientation—Rotational orientation of basal bodies dictates the direction of fluid flow (26). Rotational orientation of basal bodies in metazoan tissues must therefore be accurately aligned with the overall tissue polarity and must be consistent with the orientation of basal bodies in neighboring cells in the same tissue, in order to ensure that the cilia all beat in the same direction. If cilia in different cells were oriented in different directions, coherent long-range flow would not occur (Figure 2). Coordination of basal body orientation along the two-dimensional surface of an epithelium is referred to as planar polarity and the need to do this was faced early in evolution, given the wide-spread use of cilia-based motility in unicellular organisms and their early multicellular ciliated ancestors. For example, in the colonial alga *Volvox*, each cell in the colony orients both of their basal bodies according to their (A-P) position along the axis of the sphere (27) to allow the colony to swim in a defined direction.

Rotational orientation in ciliated cells appears to take place after basal body docking onto the surface, suggesting some mechanism must exist to rotate basal bodies into the correct orientation (12,28). During initial ciliogenesis early in development, prior to the onset of ciliary motility, basal bodies are rotationally disoriented, only becoming aligned later on when the cilia start moving (Fig. 3).

A variety of experiments emphasize the fact that ciliated epithelia acquire planar polarity developmentally prior to ciliated cell differentiation, and this polarity is subsequently used to orient basal bodies at later stages. In the early 1900's, for example, embryologists determine when the direction of flow produced by the ciliated skin of amphibians is specified during development (29,30). By inverting grafts of developing skin at different stages, embryologists showed this direction becomes fixed soon after completion of gastrulation and prior to ciliated cell differentiation. Similar results were obtained with other ciliated epithelia including oviduct and trachea (31). Thus, rotational orientation becomes set after some defined developmental stage, presumably by a signal propagated along polarized tissues and capable of orienting the cilia (Fig. 3).

The obvious candidate for such a signal is the planar cell polarity (PCP) pathway. A key feature of PCP signaling is that its activity in one cell profoundly affects the polarity of its neighbor. As a consequence, local interactions involving PCP signaling can propagate through an epithelium and establish order in terms of planar orientation, although how this process is cued spatially at a global level remains unclear. Several tantalizing lines of evidence linking basal bodies and non-core components of the PCP pathway have been recently uncovered in vertebrates as reviewed extensively elsewhere (32), but it should be kept in mind that the evidence thus far has not directly dealt with the issue of basal body orientation.

Flow-induced Rotational Orientation: One surprising feature of ciliary orientation is the fact that, just as orientation can determine flow, it now appears that in some cases flow can determine orientation, resulting in a fluid dynamics-mediated self-organizing system. As described above, cilia acquire their orientation gradually, becoming more refined and precise in their direction as they begin to beat and produce flow (Fig. 3). Blocking flow seems to prevent this refinement, suggesting that cilia motility plays a role in orientation. A more dramatic finding is that unpolarized cilia can be entrained to orient along an axis by exposing them to an external flow that mirrors the flow they normally produce (33). Thus, these results suggest a positive feedback model that explains how orientation and flow become optimized.

How might cilia use flow as an orientation cue? One possibility is that ciliated cells themselves sense and respond to flow much the same way that endothelial cells respond to blood flow (34). However, endothelial cells only respond to a shear stress an order of magnitude higher than that produced by ciliated epithelium. In addition, immotile cilia do not seem to reorient in response to an external flow (33), suggesting flow is acting not on the cell but rather directly on the cilia. Sensory cilia sense flow using mechanical sensors in the form of the TRP channels, which flux calcium, and members of the TRP family are expressed in motile cilia (35), suggesting that here too, mechanical stress could be translated into chemical signals that ultimately change basal body orientation. However, it is equally possible that flow-driven orientation is purely mechanical in nature. This view is based on the idea that hydrodynamic forces placed on the axoneme influences dynein engagement, and therefore, shape the power stroke (36,37). If the cilia encounter a counteracting flow during beating, one can imagine that the shape of the power stroke would change, and this change would translate through the base of the axoneme to the basal bodies. Distinguishing between these various models is a formidable problem in biophysics and cell biology but is key in determining how ciliary flow is self-organized

Establishing Tilt: The only case known so far in which tilt plays a key role is generation of leftward flow in the node. Nodal cilia beat in a roughly circular beating pattern, and if the cilia was untilted this should circulate fluid around the node rather than flow it across the node. Both theoretical (38) and experimental fluid models (39) show that rotation about a tilted axis can lead to linear flow, with posterior tilt producing leftward flow.

How does tilting of node cilia arise? Node cells have an almost hemispherical surface, with the cilia projecting from the posterior end of the cells due to a posterior bias in basal body position (39). This posterior position coupled with the convex shape of the cell surface causes the cilium to emerge with an overall posterior tilt relative to the plane of the node, just as observed. Mechanisms similar to those described above (i.e. PCP signaling and flow) may also coordinately position the cilia posteriorly in node cells.

Ciliary orientation defects - a new class of ciliary disease?: Randomization of rotational orientation of basal bodies and cilia has been observed in individual instances of primary ciliary dyskinesia (40,41). In one systematic study, ciliary rotational disorientation in PCD patients was always accompanied by immotility or dynein deficiency (42). This result suggests ciliary disorientation is a byproduct of abnormal motility, rather than a cause, however other studies have found examples of ciliary rotational mis-orientation in cilia that were otherwise ultrastructurally normal (43). At this point, the most conservative conclusion would be that the importance of rotational orientation defects as a sub-class of PCD remains to be fully explored.

Case studies in human patients have been reported in which the tilt of the cilia appears to be abnormal (44), and other studies have noticed misplaced cilia inside the cytoplasm suggesting translational mis-positioning (45). However in none of these cases is the underlying molecular genetic defect known.

Conclusions

Generation of coherent ciliary flows requires precise orientation of basal bodies according to multiple positional cues including the PAR pathway, the planar cell polarity pathway, and cilia-generated flow itself. Defects in ciliary orientation may represent a sub-class of ciliary disease.

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Figure 1. Degrees of freedom of ciliary orientation. Cilia orientation is specified by three coordinates of position (X,Y, and Z), two angles of tilt (θ and ϕ) defined relative to the plane of the epithelium, and one angle of rotation (ψ) about the long axis of the cilium.

Figure 2.

Ciliary orientation must be aligned across multiple cells in a tissue in order to produce coherent overall flow of fluid. (A) when cilia are aligned, locally produced flows (red arrows) can sum to produce a coherent flow. (B) when cilia are randomly aligned, local flows run in different directions leading to overall chaotic motion of the fluid that resembles turbulence.

Figure 3.

Cilia orientation is acquired gradually via several processes. When cilia first form, the orientation of the basal body (arrows) is very imprecise (top diagram). Tissue patterning perhaps involving PCP signaling helps to align the orientation of cilia along a common tissue axis. Ciliary flow is then used as a self-organizing cue to refine orientation further, thus producing laminar, directed flow.