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Point of VIEW

Ciliogenesis, Ciliary Function, and Selective Isolation

Raymond E. Stephens*

Department of Physiology and Biophysics, Boston University School of Medicine, Boston, Massachusetts 02118

ABSTRACT In addition to their classic role in cell motility, certain cilia have sensory or signaling functions. In sea urchin embryos, short motile cilia randomly propel the early embryo, while a group of long, immotile cilia appear later, coincident with directional swimming and localized within a region that gives rise to the larval nervous system. Motile cilia can be selectively removed by treatment with a novel derivative of dillapiol, leaving the putative sensory cilia for comparative investigation and a gently deciliated embryo ready for studies of regeneration signaling.

*Corresponding author,
rstephen@bu.edu.

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Ciliogenesis, the *de novo* formation of cilia, occurs in most organisms except bacteria, fungi, and most higher plants. The uniquely conserved "9 + 2" cross section arises by outgrowth from a "9 + 0" basal body template that originates from a centriole of the mitotic apparatus. Mitosis and ciliogenesis are sequentially coupled events in early development, with the mitotic apparatus reutilizing basal bodies from resorbed cilia as centrioles (*1*). Terminally differentiated cells can generate multiple cilia either from direct replication of basal bodies or by formation of unique generative complexes (*2*). Cilia function mainly to propel cells through fluids or to propel fluids over cells. Most recently, however, studies of the innate transport functions within certain specialized cilia have led to important new insights into signaling pathways, ranging from chemoreception and mating in simple organisms to polarity and patterning in vertebrate development (*3*). Work by Semenova *et al.* in this issue (p 95), using a novel small molecule to selectively deciliate sea urchin embryos, opens a new avenue for investigating the sensory function and structural determinants of early embryonic cilia and may help to unravel the mechanism of deciliation and provide new information on regeneration signaling (*4*).

Cilia and flagella grow and turn over from their distal tips, adding and removing components that are conveyed along the 9 + 2 axoneme (*5*). This overall process of intraflagellar transport (IFT) is mediated by kinesin-II (*6*) for tipward (anterograde) transport and cytoplasmic dynein for baseward (retrograde) transport, corresponding to the polarity of the axonemal microtubule tracks upon which the motors move (*7*). These molecular motors associate with complexes of ciliary building blocks to form particles that they convey bidirectionally between the cytoplasm and axoneme, ushering them through a functional diffusion barrier formed by constriction of the plasma membrane at the basal plate and necklace region (Figure 1, panel a). The final length of the cilium may result from equilibrium between these oppositely directed transport modes (*8*), and more recent evidence suggests that a basal, periodic gating mechanism may further regulate IFT-particle entry (*9*).

When cilia are retracted at the onset of mitosis, KAP, the non-ATPase subunit of the IFT anterograde motor kinesin-II, redistributes to the nucleus before nuclear membrane breakdown and only returns to the cilium as it is being rebuilt (*10*). This important result suggests that KAP, whose primary sequence reveals potential nuclear localization and export signals, may link the mitotic and ciliogenic cycles. Furthermore, IFT is intimately involved in conveying signaling molecules in a variety of sensory cilia (*3*), again demonstrating functional communication with both the cytoplasm and nucleus.

The sea urchin embryo has long provided an ideal system for studies of early development, with early stages paralleling those in humans. Eggs are obtainable in enormous quantity and develop synchronously in

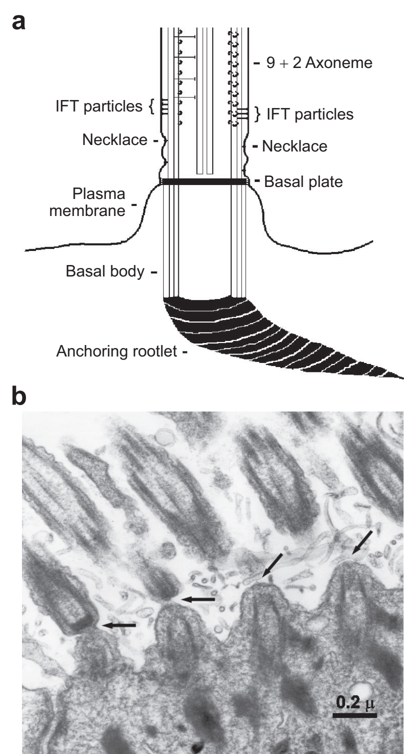

Figure 1. Ciliary basal structures and the process of deciliation. a) Schematic diagram of the axoneme-basal plate-basal body complex at the plasma membrane, encircled by a necklace of membrane particle—microtubule attachments. IFT particles transport ciliary building blocks from the cytoplasm, through the basal region, and up to the tip, where they assemble. Turnover likely takes place by the opposite route. b) Hypertonic deciliation of multiple cilia from a mussel gill epithelial cell. After excision of the basal plate from the basal body, the plasma membrane constricts and seals as the cilia are freed (arrows, left to right sequence).

mass culture after *in vitro* fertilization. At the blastula stage, just before hatching, each cell generates one cilium, producing a uniformly ciliated, swimming embryo. The cells continue to divide, typically producing motile cilia of consistent length. Later, before the enlarging hollow ball of cells invaginates at one pole (the *vegetal*), very long, immotile cilia grow at the opposite pole (the *animal*). Development of this "apical tuft" coincides with the acquisition of directional swimming and, thus, the tuft is thought to have a sensory function. In fact, specific cells of the apical plate later develop into the larval nervous system (*11*).

Other than being immotile and of unusual length, apical cilia are structurally indistinguishable from the somatic cilia that propel the embryo. When the embryo is experimentally deciliated by brief hypertonic shock, all of the cilia regrow with identical kinetics. However, the initially motile apical cilia simply continue to grow further, gradually losing motility, with both types asymptotically approaching their final, characteristic lengths (*12*). Conventional deciliation has not provided a means for separately isolating these two cilia types, so we currently know nothing about any biochemical differences that may reflect a sensory function or explain the lack of motility. Now, with the ability to selectively release motile cilia (*4*), followed by conventional deciliation to release the remaining apical tuft cilia, it should be relatively easy to evaluate apical tuft *versus* somatic cilia and their membranes for relevant channels, receptors, or distinct structural components, as has been done recently for mouse photoreceptor and rat olfactory cilia by proteomic analysis (*13, 14*).

It must be pointed out that simply "animalizing" the embryo by chemical treatment (*e.g.*, Zn ions) to produce a predominantly long cilia phenotype may not address the broader issue of sensory function. An expanded but unpatterned apical plate will develop in the absence of vegetal signaling (*11*), suggesting that experimental animalization is an oversimplification. Furthermore, such procedures may lead to overriding "shock" responses that could affect normal developmental pathways (see below).

The new differential release procedure (*4*) could lead to a better evaluation of apical cilia length determination. For example, the transcription factor SpNF2.1 uniquely defines the apical region (*15*). The amount of tektin A1, one of three helical proteins that copolymerize with tubulin to form outer doublet microtubules (*16*), is strictly metered to limit the assembly of a specific, maximum length of cilium from large pools of most other precursors at ciliogenesis or after deciliation (reviewed in ref 17). "Knockdown" of SpNF2.1, which serves as an upstream activator of the tektin gene, results in apical depletion of tektin A1 mRNA and, consequently, produces apical cilia of approximately normal length (*18*). By selectively removing somatic cilia, it should now be possible to quantify tektin dynamics specifically in the remaining apical cilia to confirm that a proportionate increase in tektin synthesis in the apical cells can bring about a proportionate increase in ciliary length. Alternatively, or in addition, components of IFT may be up-regulated in apical *versus* somatic cilia, resulting in greater distal transport and consequent growth. Differential isolation and protein analysis should reveal this.

Deciliation/deflagellation occurs by breakage of the 9 + 2 axoneme beneath the basal plate (*19*). The process is generally calcium-dependent and likely to be mediated by the microtubule severing protein katanin (*20*). The sequence of deciliation is illustrated in Figure 1, panel b, for a multiciliated mussel gill cell. Deciliation in sea urchin embryos is typically accomplished by hypertonic shock (*21*). The procedure is hardly gentle. Hypertonic deciliation results in an unusual shock response wherein heat shock protein 70 (Hsp70) family proteins remain unaffected, bulk protein synthesis is decreased, and an Hsp40 cognate is upregulated (*22, 23*). Hypertonicity alone will not trigger this response; cilia must be physically lost. Although overall protein synthesis is decreased, deciliation replays the developmental subroutine that results in the synthesis and assembly of the required ciliary proteins, while other aspects of development continue unabated (*17, 23*). We cur-

rently know almost nothing about the signaling mechanisms that regulate this ciliary gene re-expression, although an intriguing report has shown that deciliation both induces thermoresistance and activates the p38 mitogen-activated protein kinase pathway (*24*). Our lack of knowledge is complicated by concurrent shock responses and other potentially irrelevant consequences of global calcium release through the plasma membrane and/or from intracellular stores. The availability of a novel deciliating agent, the *p*-methoxyphenyl isoxazoline derivative of dillapiol, functioning reversibly at micromolar levels, may now clear the way for evaluating regeneration signaling processes more objectively.

The molecular mechanism of this derivative in causing deciliation is unclear at this point, although it is fair to say that significant binding to tubulin is unlikely because mitosis is not disrupted and already-assembled ciliary microtubules are well-known to be stable to tubulin-binding drugs. Semenova and coworkers (*4*) were initially influenced by the fact that the combretastatins function by destabilizing microtubules, correlating with their well-documented antimitotic/antiproliferative effects. Having an A-ring configuration similar to combretastatin A-2 but lacking the B-ring, apiol and dillapiol require quite high concentrations to affect sea urchin embryos with regard to mitosis, cleavage, and early development. These compounds mainly affect calcite spicule growth at the pluteus stage, suggesting interference with large-scale calcium mobilization or deposition. Introduction of an isoxazoline moiety to maintain subsequent proper biaryl orientation allowed Semenova and coworkers (*4*) to test a wide variety of substituents in place of combretastatin A-2's B-ring, resulting in efficacy at nearly 2 orders of magnitude lower concentration than the parent molecules. Two of these compounds, the *p*-methoxyphenyl derivatives of apiol and dilapiol isoxazolines, which differ only in the reciprocal positions of –H and –OMe on the aromatic A ring, stood out in their ability to immobilize embryos through ciliary loss without affecting development. Considering the probable role of calcium in the deciliation process (*20*) and the parent compounds' action on spicule growth, it is imperative to investigate the action of the derivatives with respect to calcium mobilization, perhaps using late-gastrula-stage embryos, where calcite spicule deposition is initiated and easily observed under polarized light. It may well be that these compounds trigger ciliary excision by selective activation of a basally localized calcium-dependent event or calcium store, with motile cilia somehow being the most susceptible.

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