Overview of Structure and Function of Mammalian Cilia

Peter Satir[1] and Søren Tvorup Christensen[2]

[1]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461; email: satir@aecom.yu.edu

[2]Institute of Molecular Biology and Physiology, Department of Biochemistry, University of Copenhagen, DK-2100 Copenhagen OE, Denmark; email: stchristensen@aki.ku.dk

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

Annu. Rev. Physiol. 2007. 69:377–400

First published online as a Review in Advance on September 29, 2006

The *Annual Review of Physiology* is online at http://physiol.annualreviews.org

This article's doi: 10.1146/annurev.physiol.69.040705.141236

Copyright © 2007 by Annual Reviews. All rights reserved

0066-4278/07/0315-0377$20.00

Key Words

ciliary motility, primary ciliary dyskinesia, primary cilia, sensory organelles, signaling, tissue homeostasis, development

Abstract

Cilia are membrane-bounded, centriole-derived projections from the cell surface that contain a microtubule cytoskeleton, the ciliary axoneme, surrounded by a ciliary membrane. Axonemes in multiciliated cells of mammalian epithelia are 9 + 2, possess dynein arms, and are motile. In contrast, single nonmotile 9 + 0 primary cilia are found on epithelial cells, such as those of the kidney tubule, but also on nonepithelial cells, such as chondrocytes, fibroblasts, and neurons. The ciliary membranes of all cilia contain specific receptors and ion channel proteins that initiate signaling pathways controlling motility and/or linking mechanical or chemical stimuli, including sonic hedgehog and growth factors, to intracellular transduction cascades regulating differentiation, migration, and cell growth during development and in adulthood. Unique motile 9 + 0 cilia, found during development at the embryonic node, determine left-right asymmetry of the body.

INTRODUCTION

Cilia are membrane-bounded, centriole-derived, microtubule-containing projections from the cell surface. The microtubule cytoskeleton of the cilium, the ciliary axoneme, grows from and continues the ninefold symmetry of the centriole, which is nearly identical to, and often becomes, a ciliary basal body. Mammalian ciliary axonemes, like axonemes elsewhere in the animal kingdom, are formed with two major patterns: 9 + 2, in which the nine doublet microtubules surround a central pair of singlet microtubules, and 9 + 0, in which the central pair is missing (1, 2). Usually, 9 + 0 cilia are also missing the molecular motors, axonemal dyneins, which are responsible for ciliary movement; such cilia are therefore nonmotile. In contrast, 9 + 2 cilia are motile (**Figure 1**). In addition, whereas epithelial cells may possess several hundred 9 + 2 motile cilia, 9 + 0 cilia are usually solitary. Nonmotile 9 + 0 cilia form the basis for various specialized sensory structures, including chemosensitive or proprioceptive sensilla of invertebrates, such as the insect ear. The outer segments of the rods and cones of the eye in mammals are expanded 9 + 0 cilia. Olfactory cells have nonmotile multiple long chemoreceptive cilia that are 9 + 2 at their base but lose this organization distally.

Cilia: microtubule-based cell organelles extending from a basal body, a centriole, at the apical cell surface, containing 9 + 2 or 9 + 0 axonemes surrounded by a specialized ciliary membrane

Axoneme: the microtubule cytoskeleton of the cilium, consisting of a ring of nine doublet microtubules surrounding a central pair (9 + 2) or missing the central pair (9 + 0)

Dynein: an AAA-type ATPase that functions as a motor to move cargo along microtubules to their slow-polymerizing (−) end or assembled as inner and outer rows of projections along the axonemal doublets to move the doublet microtubules with respect to one another, thus powering ciliary movement

Primary cilia: single 9 + 0 nonmotile cilia found on many mammalian cells, including kidney epithelia, chondrocytes, fibroblasts, and neurons

One aspect that received only the attention of specialists until recently is the widespread distribution of 9 + 0 cilia among the cells of the body. Such cilia are now called primary cilia. Primary cilia are found on epithelial cells such as the kidney tubule, the bile duct, the endocrine pancreas, and the thyroid but also on nonepithelial cells such as chondrocytes, fibroblasts, smooth muscle cells, neurons, and Schwann cells. In addition, unique motile 9 + 0 cilia, bearing dynein arms, are found at the embryonic node during development. For an up-to-date listing of cell types with primary cilia, see the Primary Cilium Resource Site (**http://www.primary-cilium.co.uk/**).

The structure and distribution of both 9 + 2 and 9 + 0 cilia have been widely studied since the advent of biological electron microscopy in the middle of the twentieth century. However, except for those examining photoreceptor visual transduction and olfaction, functional studies were largely confined to motile cilia until recently. Many people considered primary cilia to be vestigial, but motile cilia—which occur, for example, on respiratory epithelium, along the female reproductive tract, and on ependymal cells lining the ventricles of the brain and which move mucus or fluid—were thought to be important for health. In particular, the mucociliary escalator of the respiratory tract seemed important for respiratory clearance and the prevention of bacterial colonization (3, 4), although some ciliary function can be superceded by muscle contraction, such as coughing. The mammalian sperm tail contains a motile 9 + 2 axoneme surrounded by a specialized set of thick fibers. Until the development of in vitro fertilization procedures, sperm motility was essential for fertility, and because male infertility brought many individuals to the clinic, the first true ciliary disease, now known as primary ciliary dyskinesia (PCD), was diagnosed there (5).

It is instructive to realize how much of our present understanding of mammalian cilia depends on work on model organisms, particularly the ciliated protistans *Tetrahymena* and *Chlamydomonas*, and more recently on the model nematode, *Caenorhabditis elegans*. In each case, recent advances in genomics, combined with mutant analysis, have deepened the understanding of mechanisms that are preserved in mammalian cilia. The ciliary proteome has been defined for *Tetrahymena* (6) and *Chlamydomonas* (7). Genes encoding specific ciliary proteins are absent in yeast. There are mouse and human (8) orthologs for virtually all the structurally or functionally characterized ciliary proteins.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

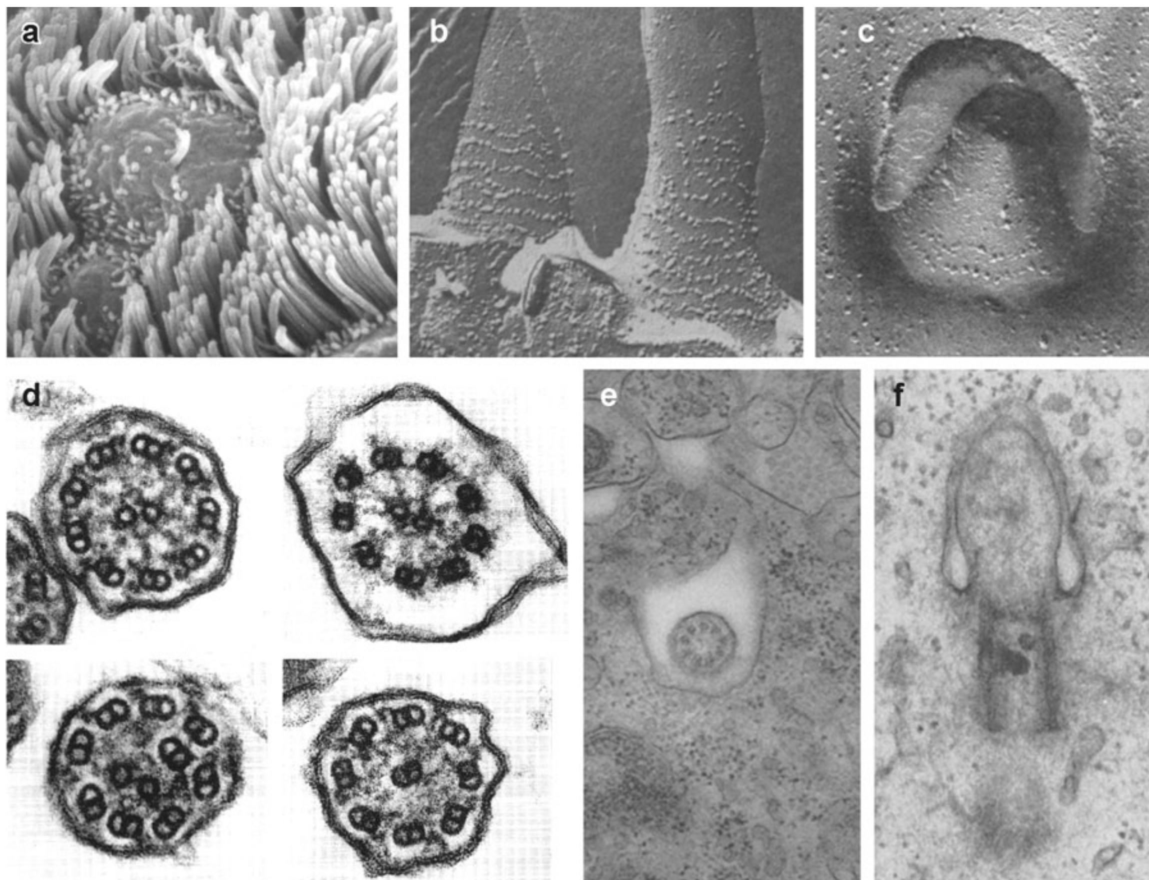

Figure 1

Images of cilia. (*a*) Scanning electron microscopy (SEM) image of oviduct cilia. The central cell shows a primary cilium. Surrounding cells are multiciliated with motile cilia (courtesy of E.R. Dirksen). (*b*) Freeze etch image of motile tracheal cilia showing the multistranded ciliary necklace (from Reference 12, courtesy of Marcel Dekker). (*c*) Freeze etch image of fibroblast primary cilium showing ciliary necklace (from Reference 41, courtesy of *Journal of Cell Biology*). (*d*) Cross sections of human cilia. (*Top left*) Normal motile 9 + 2 axoneme. Other images are of primary ciliary dyskinesia (PCD) cilia. In all images in *d*, ciliary diameter = 0.25 μm. From Reference 3, courtesy of J. Sturgess and *Handbook of Physiology*. (*e*) Cross section of 9 + 0 nonmotile fibroblast cilium (from Reference 64, courtesy of S. Sorokin and *Journal of Cell Biology*). (*f*) Initial step of ciliogenesis of a fibroblast primary cilium (from Reference 64, courtesy of S. Sorokin and *Journal of Cell Biology*).

Primary ciliary dyskinesia (PCD): a set of human diseases caused by mutations in ciliary proteins leading to immotile or discoordinated movement, resulting in impaired mucociliary clearance; often coupled to male infertility, hydrocephalus, otitis media, and situs inversus

MOTILE 9 + 2 CILIA

Axonemal Structure

The basic structure of 9 + 2 mammalian cilia was delineated by transmission electron microscopy; the description remains relatively current. The microtubules of the 9 + 2 axoneme polymerize from αβ tubulin heterodimers (9) with the fast polymerizing (+) end at the ciliary tip. Major structures that attach to the microtubules, the outer and inner dynein arms (ODAs and IDAs), the radial spokes, the central-pair projections, and so forth are defined protein complexes, some of whose subunits are AAA-type motors, EF hand proteins, protein kinases, A kinase–anchoring proteins (AKAPs), phosphatases, or proteins with other important

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

ODA: outer dynein arm

IDA: inner dynein arm

CBF: ciliary beat frequency

domains for function or assembly. The ODAs and IDAs are force-producing molecular motors that cause the doublet microtubules to slide with respect to one another (10). The doublet sliding is asynchronous with the progression of activity around the axoneme, yielding a helical beat (11). This beat is normally modified by interdoublet links and spoke–central-pair interactions to produce controlled bending, with an effective stroke and a recovery stroke. Maximum beat frequencies range up to approximately 100 Hz, although most reports of mammalian ciliary beat frequency are much lower, perhaps normally 10–20 Hz.

Mechanism of Motion

The ∼10–15-μm-long cilium obeys low Reynolds number hydrodynamics, for which viscous forces are paramount. In airway cilia, for example, the effective stroke is vertical, extends into the overlying mucus layer, and propels the mucus toward the pharynx, whereas in the recovery stroke much of the cilium is moving horizontally in the relatively stationary periciliary fluid layer beneath the mucus (12, 13). Freeze etch studies of mammalian sperm axonemes (14) reveal that the structural complexity of the ODAs and IDAs is similar to that in *Chlamydomonas*, except that the ODA of the mammalian sperm axonemes is two (and not three) headed. In general, because the molecular mechanism of motion is conserved from protists to humans, axonemal diameter, which dictates the relationship between doublet sliding and the amount of bending, must also be conserved; axonemal structure is likewise conserved, with little variation.

Recent studies of axonemal structure in PCD and other respiratory diseases reinforce these conclusions. Essentially, genetic defects in respiratory cilia structure that affect beat and transport efficiency lead to PCD (see also Reference 14a). The most prevalent structural defects result in missing ODAs, present in more than 60% of patients with classical PCD clinical profiles (15), sometimes combined with IDA defects. IDA defects are best characterized by computer-assisted image reinforcement (16). In all, arm defects account for approximately 90% of PCD cases when embryonic lethality is not considered. The genetic basis of the ODA defect is most commonly a mutation in *DNAH5*, which codes for one of the ODA heavy chains (17), or in *DNAH1*, coding for a human dynein intermediate chain (18). There is a strong correlation between the number of ODAs (but not of IDAs) present and ciliary beat frequency (CBF); PCD patients have significantly lower CBF (19). These findings are consistent with evidence that ODAs primarily control CBF by increasing or decreasing doublet sliding velocity in the axoneme without greatly affecting beat form, accomplished in many instances by changes in cAMP-dependent ODA light chain phosphorylation (20, 21). Control of CBF via local elevation of Ca^{2+} by mechanostimulation (22) may work partly through this mechanism. The cGMP pathway is also important in this process (see also Reference 22a).

IDAs primarily control parameters related to bend amplitude that affect beat form. This control operates at least in part via signaling kinases and phosphatases that phosphorylate or dephosphorylate radial spoke proteins that act on the velocity of IDA-limited doublet sliding (23). Therefore, although ODA activity controls the overall timing of the stroke cycle, IDA activity of specific doublets controls the amplitudes of the bends originating in the effective and recovery strokes. Radial spoke heads interact with central-pair projections to coordinate doublet sliding activity with bend direction and propagation. With defects in radial spokes, or of central-pair microtubules, IDA activity is not properly regulated, and ciliary beat is paralyzed or abnormal. Some PCD patients have axonemes with the normal complement and composition of dynein arms but with defects in the radial spokes or central-pair structures (**Figure 1**). Because many ciliary proteins that

affect normal beat or beat direction may not affect the known major axonemal structures, PCD may occur without obvious axonemal abnormalities.

Aspects of the central pair–radial spoke–dynein arm coordination mechanism have been uncovered by examination of central-pair orientation in *Chlamydomonas*, in which doublet activity is related to central-pair orientation (24) such that orientation is a passive response to bend formation and apparent rotation of the central pair is due to repositioning of the twisted central-pair structure by bend propagation (25). Through central-pair repositioning, different bends propagate along the axoneme at the same rate. This complexity probably is associated with the great potential for changes in the effective stroke direction known for this cell. In mammalian and many other metazoan cilia, the orientation of the effective stroke is fixed, and the orientation of the central pair is roughly perpendicular to that direction. Because all the ODAs, and probably all the IDAs, are unidirectional vectorial force producers, minus-end motors, the axoneme is effectively divided into two operational halves: One half, when active, is responsible for the generation of the effective stroke, whereas the other half generates the recovery stroke. Cilia and basal bodies have only a single enantiomorphic form, with doublet numbers running clockwise when the organelle is viewed from the base toward the tip, so that the two halves define the left and right sides of the cilium and perhaps eventually those of the body (26; see also below). Bend generation in the effective stroke is thought to involve dynein activity on doublets 9, 1, 2, 3, and 4, with doublets 5–6 at the leading edge of the stroke, whereas in the recovery stroke, with doublet 1 in the lead, activity switches to dyneins on doublets 5–6, 7, 8 (27). Ciliary arrest can occur at the switch points, and further beating, for example in respiratory cilia, often requires mechanical stimulation or a burst of cAMP synthesis. When groups of cilia become activated, they stimulate adjacent cilia and initiate metachronal waves that travel across the epithelium (27).

PCD is instructive, not only in demonstrating that the mechanisms of human ciliary motility and its genomic and proteomic control are consistent with what is known from model organisms, but also in delineating the physiological role that motile cilia play in the body. Clinical features of the disease are indications of processes in which ciliary motility is essential (28). Strong phenotypic markers of PCD are chronic rhinitis/sinusitis, otitis media and, as indicated above, male infertility. Female fertility is probably decreased. There is an increased incidence of hydrocephalus, as loss of motile cilia in brain ventricles alters choriod plexus epithelium function and reduces the flow of cerebrospinal fluid through the ventricles (29, 30). There is no correlation with smoking. Cystic fibrosis patients have motile cilia. The function of nodal cilia, the special category of motile $9+0$ cilia discussed further below, is important for the subset of PCD patients who exhibit reversal of left-right (LR) body asymmetry (situs inversus totalis), which, together with chronic rhinitis and infertility, was identified as Kartageners syndrome.

Left-right (LR) asymmetry: the condition of unpaired organs, such as the heart in the vertebrate body, whereby the organ position on one side of the body is determined; often reversed in animals with PCD

THE CILIARY MEMBRANE

Both $9+2$ and $9+0$ ciliary axonemes are surrounded by a ciliary membrane, which extends from and is continuous with the cell membrane but is selectively different from the cell membrane in overall composition. Aside from general descriptions of structure, especially by freeze fracture techniques, and information on lectin or cationic particle binding (31, 32), surprisingly little was known about the ciliary membrane until quite recently. Now, through cilia fractionation and proteomics, a picture of the membrane proteins of the cilium is emerging. In unicellular organisms, in which ciliary response pathways are necessary for survival, control of the $9+2$ motile cilium depends on specific receptor and channel proteins, including cyclic nucleotide

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

Ciliary necklace: a specialized region at the base of a cilium defined in freeze fracture electron microscopy; the probable assembly region for transport of cargos into the cilium

receptors, Ca^{2+} channels, and receptors involved in growth control pathways, that are localized to the ciliary membrane (33). In *Chlamydomonas*, membrane proteins in the proteome (7) include six ion pumps or channels, including a homolog of human polycystin 2; three predicted plasma membrane Ca^{2+}-ATPases (PMCAs); and four closely related proteins that have 8 to 12 transmembrane helixes and a PAS domain, a sensory motif involved in detecting diverse stimuli ranging from light or oxygen to redox state and small ligands (34). Evolutionary persistence of sensory function in 9 + 2 cilia of metazoans, and therefore of specific receptors and channels of the mammalian ciliary membrane, would be expected. Because the control of CBF in airway epithelial cells depends upon cyclic nucleotides (35), one might anticipate that, as is true for ciliates, both adenylyl and guanylyl cyclase (AC and GC, respectively) might be localized to the ciliary membrane. However, except in olfactory sensory cilia, for which an odorant-sensitive type 3 AC has been reported, and the adjacent respiratory epithelial cilia, for which lower levels of nonodorant-sensitive AC have been reported (36), there is little specific information as to whether these proteins are in ciliary membranes. There are more than 1000 different odorant receptors, each presumably localized to the ciliary membranes of one olfactory neuron.

Although information on airway ciliary membrane receptors remains sparse, the concept that all cilia have significant sensory function, heavily supported by the new work on primary cilia cited below, led Teilmann & Christensen to compare the distribution of certain receptor tyrosine kinases in primary versus motile cilia of the female reproductive tract. The angiopoietin receptors Tie-1 and Tie-2 localize to motile cilia of the infundibulum (**Figure 2**) and the ampulla of the oviduct (37). The ion channel TRPV4 and polycystins 1 and 2 also localize to ciliary membranes of 9 + 2 motile oviduct cilia (38). The ciliary level of polycystins increases upon ovulation, implying that the ciliary activity of these proteins is associated with the detection of physiochemical changes that establish the environment for oocyte transport, for priming of the ampulla for reception of the oocyte, and for the fertilization and transport of the fertilized oocyte to the uterus.

As we discuss below, polycystins are now commonly known to be present in ciliary membranes of 9 + 0 primary cilia. We predict that similar types of channels and receptors are present in the membranes of all mammalian motile cilia and that they play a role in epithelial homeostasis. Not all important specific channels are localized to the ciliary membrane, of course; for example, CFTR localizes to the apical region of ciliated airway cells but not to the cilia themselves (39).

THE CILIARY NECKLACE

Because specific proteins are localized to or concentrated in the ciliary membrane, as opposed to the rest of the cell membrane, several research groups have postulated that there is a selective barrier at the cilium entrance. This barrier occurs near or more likely in connection with the loading zone for cargo destined for intraciliary transport. The physical manifestation and mode of operation of the barrier are still uncertain, but selection has certain features resembling the passage of material from the cytoplasm into the nucleus, where the barrier is the nuclear envelope (40). One specialized feature of the barrier region that is found on all 9 + 2 and 9 + 0 mammalian and invertebrate cilia that have been studied by freeze fracture electron microscopy, but that is not universally found on sperm, is the ciliary necklace (41). The necklace consists of multiple strands of intramembrane particles that are especially prominent in airway and oviduct cilia (**Figure 1**). The necklace particles line the edges of a cup-like structure with a stem connecting to the center of each basal body doublet just at the transition zone below the origin of the central pair of microtubules and the axoneme. The necklace region

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

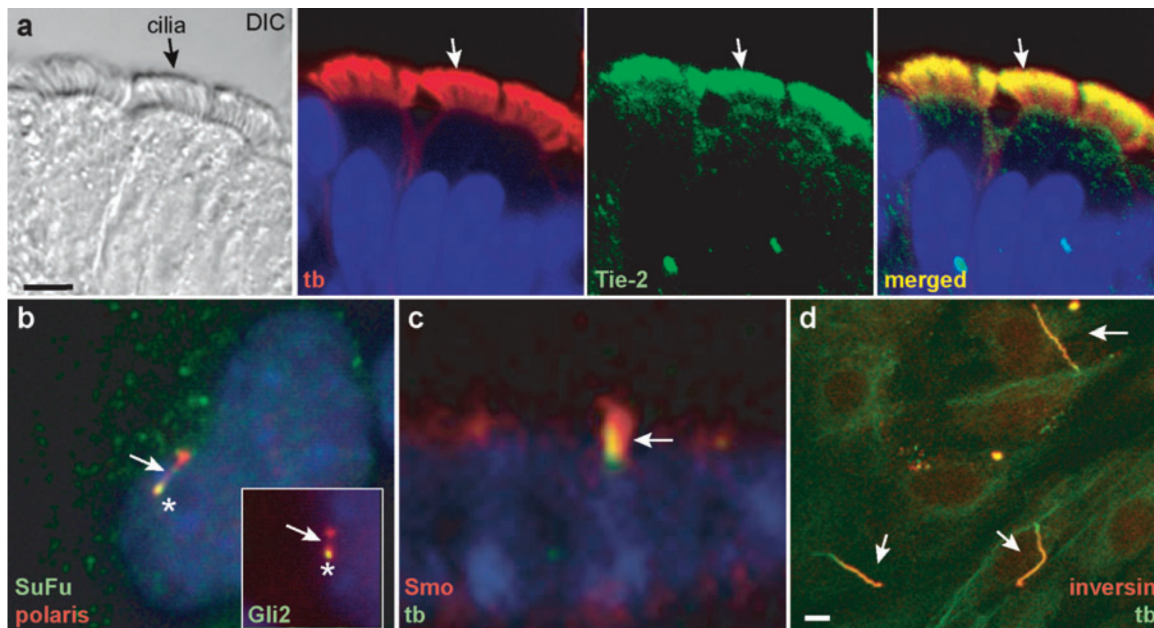

Figure 2

Receptors and signaling molecules localize to cilia (*arrows*). (*a*) Angiopoietin receptor Tie-2 localizes to motile cilia [anti-acetylated α-tubulin (tb)] of the mouse oviduct infundibulum. Nuclei are stained with DAPI (*blue*). Scale bar: 5 μm. From Reference 37 with permission. (*b*) Localization of SuFu (Supressor of Fused) and Gli2 transcription factors to the distal tip of the primary cilium (antipolaris) in mouse primary limb cell cultures (the *asterick* marks the tip of the cilium). From Reference 100 with permission. (*c*) Localization of Smoothened (Smo) to primary cilia (antiacetylated α-tubulin) in cultures of mouse embryonic fibroblasts. From Reference 93 with permission. (*d*) Localization of inversin to primary cilia (tb) of kidney epithelial cells. Similar to Reference 64 (courtesy of L. Eley & J. Goodship).

is readily identified in transmission electron microscopy (TEM) images by this characteristic cross-sectional appearance. Transport proteins have been localized to the necklace region near the repeating intersection of the cup and the membrane (42), which may imply that the cup-like regions are assembly sites for transport of membrane and axonemal cargos.

As befits a barrier, the membrane in the necklace region of airway cilia has a different composition in terms of, for example, lectin binding, anionic charge, and free-cholesterol distribution from the rest of the ciliary and cell membrane (31). Researchers have attempted to isolate and biochemically characterize the necklace region, especially from photoreceptor outer segments (43). A putative guanine nucleotide exchange factor (GEF), retinitis pigmentosa GTPase regulator (RPGR), and its interacting protein are localized to a necklace defining a cross section of the connecting cilium of the photoreceptor (44). RPGR isoforms are also found in the necklace region of motile cilia of the trachea (45). RPGR is mutated in patients with an X-linked phenotype that includes PCD, suggesting that this molecule is required in the development of virtually all mammalian cilia (46). The importance of the necklace in tracheal cilia is reemphasized by its disruption and disappearance upon infection with *Bordetella pertussis* and *Mycoplasma* after attachment of these bacteria to the cilium and prior to cell death. Moreover, when cilia are shed, as in the adult human cochlea, or when deciliation is induced by Ca^{2+} shock, the point of breakage and

GEF: guanine nucleotide exchange factor

membrane resealing occurs just above the ciliary necklace, and the necklace persists.

If the necklace region is the site of assembly and coupling of membrane protein cargos to the transport machinery, we may reasonably expect to find that membrane proteins targeted to this machinery had ciliary localization signals. Researchers have initiated several searches for localization signals, normally by truncation or site-directed mutagenesis of a known ciliary transmembrane protein, such as polycystin 2 (47). Published information is still fragmentary, but signaling for ciliary localization likely has several different components, depending on the protein species involved and how it is associated with the membrane and membrane scaffold proteins, such as GEFs.

Ciliogenesis: the processes of assembly and growth of cilia during cell differentiation

Intraflagellar transport (IFT): the process of transport of materials into and along the cilium for assembly of certain axonemal proteins and placement of receptor and channel proteins in the ciliary membrane

CILIARY MORPHOGENESIS

In multiciliated cells of the mammalian trachea and oviduct, ciliogenesis requires synthesis and assembly of multiple basal bodies and axonemal proteins, accompanied by an enormous increase in membrane area. Human tracheal development has four stages (48). Up to 11 weeks gestation, the trachea is covered by a columnar epithelium with primary cilia. At approximately 12 weeks, ciliogenesis begins with the appearance of fibrogranular masses in the cell cytoplasm. As Dirksen (49, 50) first described, these masses develop central elements, called condensation forms or deuterosomes, around which an explosive development of centrioles, which will become ciliary basal bodies, occurs. The mature centrioles move to the cell membrane, to which they attach, and the ciliary necklace develops as the axoneme begins to elongate (32). The necklace enlarges, and strands are added as the cilium grows. Axonemal dynein is synthesized and found in the cell cytoplasm before ciliogenesis is apparent (51). By 24 weeks, the ciliated border with 200–300 9 + 2 cilia is mature. In the oviduct, centriolar placement and ciliary growth are asynchronous. Cilia first grow at the cell periphery, giving the cell surface a daisy-like appearance: Longer cilia at the periphery encircle shorter cilia at the cell center (52). A distinct tip with an axonemal cap and a unique glycocalyx called the ciliary crown develops on the mature cilium (53), and growth ceases.

At least one transcription factor, hepatocyte nuclear factor 3/forkhead homolog 4 (HFH-4), is known to be involved in regulating the morphogenesis of motile cilia in multiciliated mammalian epithelia (54). In *HFH-4*-null mice, classic 9 + 2 motile cilia are absent in epithelial cells, but 9 + 0 cilia, including nodal cilia, are present, although nodal cilia are probably nonfunctional. Ultrastructural analysis of these animals showed that, in the epithelial cells missing motile cilia, centriole migration and apical docking are abnormal.

The axoneme grows and continuously turns over at its distal tip (55, 56). Axoneme growth and maintenance depend on transport of axonemal precursors from the cell body to the assembling tip. Rosenbaum and collaborators (40), through their work with *Chlamydomonas*, first envisioned and analyzed the molecular basis of the transport process. Historically, the 9 + 2 cilia of this organism were termed flagella, and therefore the transport process building the axoneme was termed intraflagellar transport (IFT). A transport complex consisting of IFT proteins can be visualized moving up and down the cilium, between the membrane and the growing axoneme. Axonemal components such as radial spoke proteins move as cargo, partially assembled, with these complexes and are deposited at the growing tip of the axoneme, where they dock into their proper positions (57). The complexes and their cargos are moved toward the tip by a kinesin-2 molecular motor. After depositing the cargo and picking up axonemal turnover products, the complexes are moved back toward the base by a special isoform of cytoplasmic dynein, dynein 2, also known as dynein 1b (40, 58a). The unloading/loading of cargo proteins and exchange of transport motors at the flagellar tip may occur by a three-step mechanism that may be regulated by

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

flagellar tip proteins (59). IFT protein complexes also participate in the movement of ciliary membrane proteins and signaling molecules (58b, 60). Presumably, vesicles derived from the Golgi apparatus with appropriate scaffold proteins fuse with the cell membrane near the ciliary necklace. This results in the addition of membrane around the elongating cilium and the delivery of peripheral membrane proteins, as well as of transmembrane channels and receptors such as polycystins and TRPV, to the transport apparatus for movement into the growing ciliary membrane. IFT orthologs are prominent in ciliary proteomes from all organisms studied (61), suggesting that this transport mechanism is ancient and that it has been retained for transport in both 9 + 2 and 9 + 0 cilia. IFT has been of great importance for understanding the function of mammalian 9 + 0 primary cilia as sensory organelles, as we discuss below. Hagiwara et al. (62) provide a more detailed review of ciliogenesis in mammalian cilia.

NONMOTILE PRIMARY 9 + 0 CILIA

Primary cilia, which are found on virtually every cell in the body, with some notable exceptions (63) retain the basic axonemal doublet structure as they grow from the basal body. However, they do not assemble the central microtubule complex or IDAs and ODAs, and therefore, like mutants of motile cilia lacking these structures, they do not actively beat. Other aspects of ciliogenesis are normal. There is a ciliary necklace and a ciliary membrane with receptors and channels, comparable with and sometimes identical to those found in motile cilia. Ciliogenesis is via transport mechanisms and IFT proteins completely orthologous to those in *Chlamydomonas*, although certain aspects of growth differ from those of multiciliated cells. In particular, there is no explosive growth of basal bodies from a fibrogranular mass. Instead, the single primary cilium usually originates when a Golgi-derived vesicle encapsulates the distal end of a mother centriole (**Figure 1**). The necklace region develops at the point of encapsulation. Fusion of additional vesicles liberates the growing cilium to the extracellular medium (64).

Although the motile function of 9 + 0 cilia has been lost, the sensory and signal transduction features necessary for survival in single-celled organisms have been retained and perhaps enhanced. There has been a persistent literature on primary cilia from the early 1960s, summarized in part by Wheatley (63, 65). These studies postulated, with some evidence, that the primary cilia could function as chemosensors, mechanosensors, or positional sensors and that their presence was coupled to and perhaps important for the cell cycle and cell differentiation (66). Nevertheless, for more than 30 years after their electron microscopy (EM) characterization, primary cilia were largely dismissed as insignificant or unimportant in leading textbooks of cell biology.

The Revelation of Primary Cilia Biology: Polycystic Kidney Disease

The rapid increase in our understanding of primary cilia biology was primarily due to the work of Pazour et al. (67), which relied on the development of *Tg737*^{orpk} mouse as a model for human autosomal-recessive polycystic kidney disease. Pazour et al. demonstrated that the mouse homolog of IFT protein 88 in *Chlamydomonas* is the mutated protein of the *Tg737* gene and that with this defect the primary cilia of the mouse kidney are abnormally short or absent.

Long primary cilia are seen by SEM on kidney tubule cells (68), and these can readily be visualized in light microscopy in kidney cell lines such as PtK1 or MDCK cells in confluence in tissue culture (69). These cilia can be bent by fluid flow (70) or mechanically. Praetorius & Spring (71, 72) showed that bending causes intracellular Ca^{2+} to increase and that removal of the cilium abolishes this flow-sensing response. The primary cilia of the

Polycystic kidney disease: a family of genetic diseases whereby the kidney tubule epithelium dedifferentiates and proliferates along the tubule to produce enlarged irregularly spaced expansions leading to loss of function; probably produced by defective signaling in primary cilia

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

kidney tubule act as mechanosensors that dose the cell with periodic increases in Ca^{2+}. Ca^{2+} is a well-known second-messenger molecule whose periodic influx influences specific gene activity. Mechanoregulation of Ca^{2+} is defective in the *Tg737*^{orpk} mouse kidney (73). A clear inference from these studies is that mechanotransduction via the primary cilium is necessary for continued normal function and cell differentiation of the kidney epithelium and that loss of the cilium leads to abnormal function, abnormal cell division, and polycystic kidney disease.

Many mutations that cause polycystic kidney disease are found not in genes that encode IFT proteins or the molecular motors involved in building the cilium. Rather, such mutations are found in genes that encode the polycystins, of which polycystin 1 is a transmembrane receptor and polycystin 2 is a Ca^{2+} channel. If the cilium is normal, but the polycystin is defective, just as if the polycystin is normal and the cilium defective, there will still be polycystic kidney disease; how is this possible? The answer is that the polycystins are proteins of the ciliary membrane (74, 75) and that the signal transduction pathway leading to Ca^{2+} influx must operate through the ciliary membrane to give a normal homeostatic response.

Moreover, polycystin 1 evidently functions as a G protein–coupled receptor that can activate growth control pathways via standard signal transduction cascades (76). Its extracellular domain can interact with carbohydrate or protein moieties. Although its physiological ligand is still unspecified, polycystin 1 may be a chemosensor as well as a mechanosensor. In addition to Ca^{2+} as a signal, receptor phosphorylation cascades and receptor- or mechanically induced regulated intramembrane proteolysis (77) may link ciliary membrane events to the cell cycle.

These features are consistent for all primary cilia studied. Like the motile cilia from which it is evolutionarily derived, the primary cilium incorporates specific receptors and channels into its ciliary membrane. Although some of the specific receptors may be cell type dependent, others, including polycystin 1 and 2, are more widely found. The primary cilium can respond either to mechanical stimuli or to defined ligands. The signal pathway is initiated in the cilium before transmission to the rest of the cell. Normal signaling leads either to homeostasis, that is, maintenance of the differentiated tissue state, or to controlled division and differentiation. In its homeostatic functions, the primary cilium has been compared to a cellular cybernetic probe (78; see also below) or a cellular global positioning device (79). Abnormal signaling can be due to faulty ciliogenesis or to misplacement or mutation of ciliary membrane proteins. This leads to abnormal patterns of tissue growth and cell division and to a variety of human pathologies.

PRIMARY CILIA OF CONNECTIVE TISSUES

In contrast to epithelial cells, primary cilia of connective tissues are embedded in the extracellular matrix. Consequently, these cilia may interact with matrix components and respond to tension on the matrix or to growth factors to control tissue homeostasis and cell cycling. Many of the ideas now applied to epithelial cell primary cilia were originally derived from studies of primary cilia of chondrocytes and fibroblasts.

The Chondrocyte Primary Cilium

Poole and coworkers (78, 80, 81) established that the position, projection, and orientation of the primary cilium in chondrocytes are influenced by the structural organization and mechanical properties of the extracellular matrix. This led to the suggestion that primary cilia in connective tissue cells transduce mechanical, physiochemical, and osmotic stimuli through ciliary receptors and ion channels to control tissue development and to construct a mechanically robust skeletal system. Indeed, *Tg737*^{orpk} mice with defective primary

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

cilia display limb/skull patterning defects, including polydactyly and brachydactyly. Jensen et al. (82) used double-tilt electron tomography to demonstrate that the chondrocyte primary cilium makes physical contact with matrix components such as collagen fibers and proteoglycans, probably via specific extracellular matrix (ECM) receptors, including integrins and NG2 in the chondrocyte cilium (83). This reinforces the idea that mechanical stimuli in cartilage are transmitted through the primary cilium. Integrins potentiating chemosensory, fibronectin-induced Ca^{2+} signaling are also found on primary cilia of MDCK cells (84), although the precise function of this signal pathway is unknown.

The Fibroblast Primary Cilium

Similarly, Albrecht-Buehler (85) used cultures of fibroblasts to illustrate a unique relationship between the orientation of the primary cilium and the direction of cell migration, in which the cilium is aligned with the intended direction of migration. Fibroblast primary cilia could sense the substrate as cells move and relay information from the extracellular milieu to the centrosomal region. In connective tissue, fibroblasts, like chondrocytes, are embedded in a series of protein fibers, some of which may attach and pull on the primary cilium, producing a mechanical stress. Fibroblasts in tissues are most often in a state of growth arrest, but if stimulated by growth factors, they may enter the cell cycle for proliferation and migrate throughout the matrix wherever they are needed, such as in wound healing and tissue reorganization.

As in most cells, the expression of primary cilia in cultured fibroblasts is closely regulated during the cell cycle (86), indicating a role of the cilium in growth control. Primary cilia grow when the cells become confluent and enter G_0. Tucker and coworkers (87, 88) found that stimulation with platelet-derived growth factor (PDGF) and administration of Ca^{2+} ionophores induce calcium fluxes as well as resorption of the primary cilium, which is an early event in the transition from growth arrest to cell proliferation in 3T3 fibroblast cultures. Tucker et al. concluded that signaling produced shortening of the cilia, which was required for normal growth factor–mediated cell cycle entrance.

PDGF: platelet-derived growth factor

PDGF may act directly through receptors in the primary cilium of fibroblasts (89), implying a direct role of the connective tissue primary cilium in communicating both mechanical and physiochemical information that controls migration, cell survival, and growth control in tissue homeostasis. In 1996 Lih et al. (90) demonstrated that PDGF receptor alpha (PDGFRα) is encoded by a growth-arrest-specific gene, such that the receptor is upregulated during growth arrest in cultures of NIH3T3 fibroblasts, which may facilitate the exiting of cells from growth arrest upon mitogenic stimulation by PDGF. The homodimer of PDGFRα, PDGFR$\alpha\alpha$, is specifically activated by PDGF-AA, which regulates several physiological and pathophysiological processes in a variety of tissues. Schneider et al. (89) (**Figure 3**) demonstrated that PDGFRα is targeted to the primary cilium during growth arrest in NIH3T3 cells and primary cultures of mouse embryonic fibroblasts. When PDGF-AA is added, ciliary PDGFR$\alpha\alpha$ is phosphorylated, followed by activation of the mitogenic Mek1/2-Erk1/2 pathway, which also operates in the cilium. Quiescent fibroblasts derived from *Tg737*^{orpk} mutants fail to upregulate PDGFRα, to form normal primary cilia, and to activate Mek1/2-Erk1/2, and these fibroblasts fail to re-enter the cell cycle after stimulation with PDGF-AA. In contrast, PDGF-BB-mediated signaling through PDGFRβ in the plasma membrane is not affected in *Tg737*^{orpk} mutants, indicating a unique function of the primary cilium in balancing the signals required to regulate cell cycle entrance and to maintain tissue homeostasis. Mutations in PDGFRα are known to play a role in the generation of a series of human cancers, supporting the conclusion that perturbation of the growth control pathway from PDGFRα-enriched primary

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

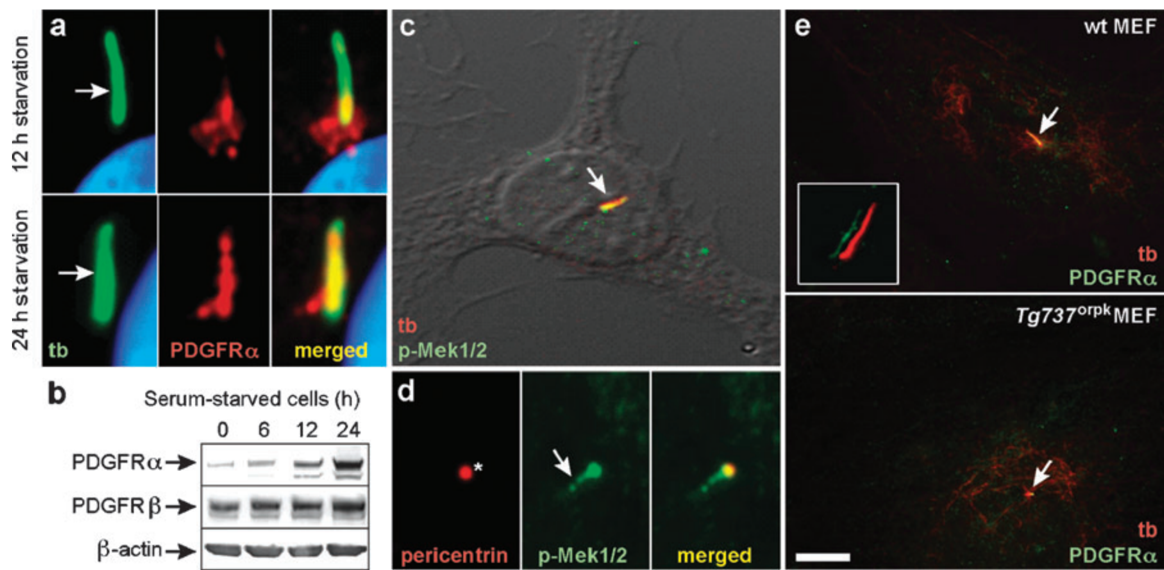

Figure 3

Growth factor–mediated signaling in fibroblast primary cilia (*arrows*). (*a*, *b*) PDGFRα is specifically upregulated and moves into the primary cilium by 24 h of serum starvation of NIH3T3 cells. (*c*, *d*) Mek1/2 is activated by phosphorylation in the cilium and at the ciliary basal body after ligand stimulation of PDGFRαα. (*e*) Comparison between wild-type (wt) and *Tg737*^{orpk} mouse embryonic fibroblasts shows that primary cilia are deficient in the mutant. Anti-acetylated α-tubulin (tb) identifies the primary cilium and pericentrin spots of the basal body. Scale bar: 10 μm. From Reference 89 with permission.

cilia may be important in the onset or prevention of oncogenesis. Furthermore, ciliary PDGFRα may continuously signal a mechanical stress on the connective tissue, known to activate PDGFRα in cultured aorta smooth muscle cells, which have primary cilia. Wheatley and coworkers (91) demonstrated the absence of primary cilia in early passages of cultured fibroblasts from patients with Werner syndrome and Mulvihill-Smith progeria–like syndrome.

These observations are consistent with the idea that the cilium has a sensory function in developmental processes and early adulthood, and to this end, it is tempting to speculate that there may be other signaling systems in the fibroblast primary cilium, such as inversin and components of the Wnt and Hh signaling pathways (92, 93; see also below), polycystin, or integrin, which may act in concert with PDGFRα to regulate fibroblast function in embryonic patterning and adult tissue homeostasis. The taurine transporter, TauT, which is expressed and localized to primary cilia of NIH3T3 cells (94, 95), may further contribute to this complex of signaling systems in the fibroblast primary cilium. Taurine helps to regulate cell volume control, ion channel activity, and calcium homeostasis in developing and adult mammalian tissues (96) and may thus modulate Ca^{2+}-dependent signaling in the primary cilium. **Figure 4** is primarily based on an extrapolation from those studies that suggest that in the absence of PDGF the fibroblast primary cilium acts essentially as a mechanotransducer, producing a rise in intracellular Ca^{2+}, which acts as in the kidney to maintain the G_0 state of the cell, but that in the presence of ligand, the cilium acts as a chemoreceptor, generating signals that induce normal cell cycling, for example, in wound healing.

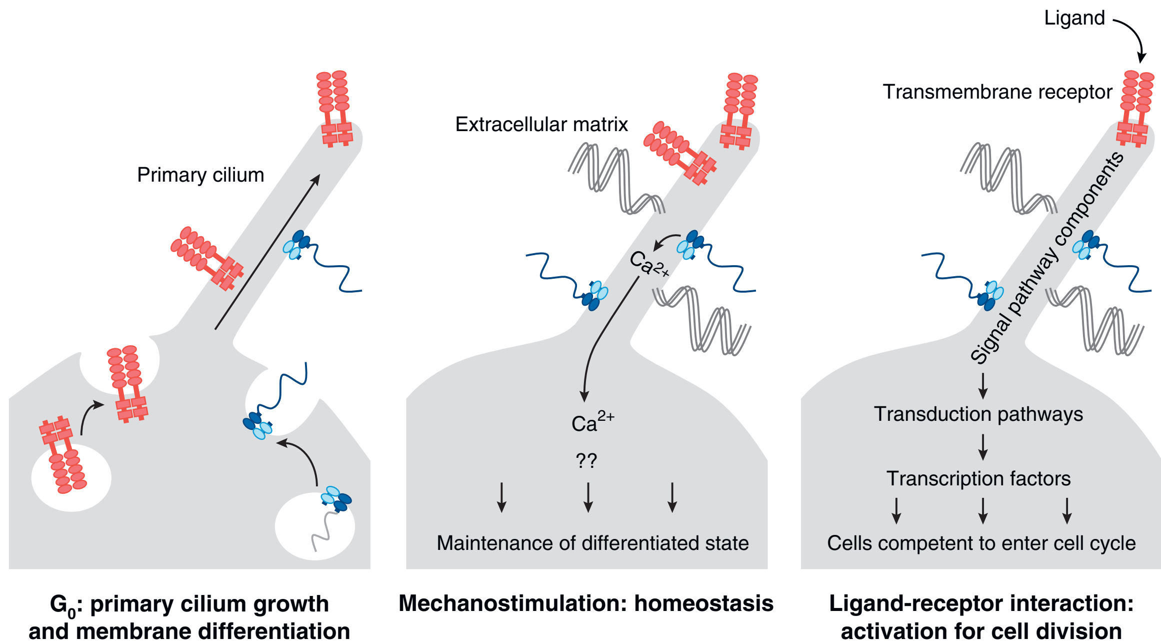

Figure 4

Pathways of membrane differentiation and response in primary cilia. (*Left panel*) In growth arrest (G_0), as the primary cilium grows, specific channels and receptors are concentrated in the ciliary membrane. (*Middle panel*) Channels such as polycystin 2 respond to mechanostimulation by an influx of Ca^{2+} necessary for maintenance of the differentiated cell. (*Right panel*) Receptors such as PDGFRαα respond to specific extracellular ligands by initiating signal transduction pathways that control cell division.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

CILIARY HEDGEHOG AND WNT SIGNALING IN DEVELOPMENT

Hedgehog (Hh) and Wnt signaling pathways highly regulate the orchestration of embryonic development and patterning in a range of tissues that direct the growth and spatial plan of the early embryo. Increasing evidence points to a strong relationship between abnormal Hh and/or Wnt signaling and mammalian disorders and pathologies that are caused by defects in cilia, including Bardet Biedl syndrome, Kartageners syndrome, heterotaxia, pulmonary dysfunction, hydrocephalus, holoprosencephaly, infertility, polydactyly, polycystic kidney disease, cancer, and retinal degeneration. Hh operates via primary cilia, and some Wnt signaling pathways require cilia for their function. Protein components participating in either signaling system are coupled to IFT proteins and ciliary assembly and may specifically be regulated in the primary cilia (93, 97–101). These findings open up a whole new realm of ciliary functions in development and tissue homeostasis for exploration.

Cilia and Hh Signaling

Important elements of vertebrate Hh signaling include a family of secreted Hh proteins that, upon binding to the transmembrane patch protein (Ptc), abolish the inhibitory effect of Ptc on the seven-transmembrane receptor smoothened (Smo). This allows Smo to transduce a signal via glioma (Gli) transcription factors to the nucleus for the expression of Hh target genes (102) that control an array of responses at different times and in different cell types (103, 104). In the mouse there are three homologs of Hh proteins: Sonic Hh (Shh), Indian Hh (Ihh), and Desert Hh (Dhh);

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

these play an essential role in embryonic development, such as in LR asymmetry (105), in limb development, and in neurogenesis. Gli proteins lie downstream in the control of the diverse functions of the Hh pathway. In the mouse Gli1 and Gli2 proteins generally act as activators, and Gli3 as both activator (Gli3A) and repressor (Gli3R) of the Hh pathway, and Smo may control the activation of Gli proteins as well as the proteolytic events that generate the Gli3 repressor (104).

Hh signaling is tightly coupled to the maintenance and function of primary cilia in various mammalian cell types. Three mouse IFT complex B proteins—*Tg737*/polaris as well as the anterograde IFT motor protein subunit Kif3a and the retrograde IFT motor protein subunit Dnchc2—are all necessary for Shh signaling at a step between Smo and Gli proteins, such that loss of IFT causes both neural tube and limb patterning defects at the level of Gli3 processing (100, 104, 106, 107). Haycraft et al. (100) more recently demonstrated that all three full-length Gli transcription factors as well as SuFu (suppressor of fused and repressor of the Hh pathway) colocalize to the distal tip of primary cilia in mouse primary limb bud cells (**Figure 2**). Importantly, loss of polaris in $Tg737^{\Delta 2-3\beta-Gal}$ mutants leads to a series of changes in Hh signaling, such as loss of *Ptc* and *Gli1* expression in response to Shh in the limb bud and disruption of Gli2 and full-length Gli3 function in primary limb bud cells; these result in severe polydactyly (108) similar to that following Gli3 loss (109). Haycraft et al. (100) further showed that partial loss of polaris function in $Tg737^{orpk}$ exacerbates the phenotype of *Gli3*-heterozygous mutants. Thus, it is increasingly evident that Hh signaling regulates embryonic development via the primary cilium, in which Gli regulation and processing occur in the cilium, and that loss of cilia results in inefficient Gli3 processing, leading to embryonic and patterning defects. Aberrant activation of Gli oncogenes may also cause cell transformation and tumorigenetic processes by directing target genes in growth control, cell survival, and metastasis (102), suggesting that dysfunctional Hh signaling in primary cilium also plays a major role in cancer.

Hh signaling follows the general rule whereby the primary signaling receptor must be localized to the ciliary membrane to permit normal cell development and signaling begins in responding proteins localized in the cilium itself, but perhaps there is amplification and feedback involved. Corbit et al. (93) demonstrated that whereas Smo localizes to the primary cilium (**Figure 2**), this localization becomes amplified in Shh signaling. In contrast, the Smo antagonist cyclopamine inhibits ciliary localization. Activation of the Hh pathway by Shh markedly upregulates ciliary Smo localization in cultures of MDCK cells, inner medullary collecting duct cells, and mouse embryonic fibroblasts. As discussed above, common motifs for ciliary localization of transmembrane proteins are to be expected, but the exact type of motif may vary, depending on how the transmembrane protein is coupled to the transport machinery at the ciliary necklace. Hh signaling depends upon a specific ciliary localization motif, comprising a conserved hydrophobic and basic residue similar to that of other ciliary seven-transmembrane receptors, including ODR-10 and STR-1 in olfactory cilia in *C. elegans* (110) and somatostatin receptor 3 and serotonin receptor 6 in primary cilia of the mammalian CNS (111, 112). Smo localizes to nodal cilia in early and late headfold stages as well as in the two-, three-, and five-somite stages, supporting a critical role for ciliary Shh signaling in later embryonic development.

Wnt Signaling and the Role of Inversin

The gene *Invs* encodes a protein named inversin that localizes to primary cilia in kidney epithelial cells (113) (**Figure 2**), to nodal cilia, to fibroblast cilia in cell cultures, and to the pituitary gland (92). Inversin is the protein that is mutated in nephronophthisis type 2 (114), and inversin in kidney cells binds

anaphase-promoting complex 2 (Apc2), indicating that the primary cilium serves to regulate the cell cycle and that the cilium plays a role in aberrant cell proliferation, which is a hallmark of the cystic process (113). Similarly, inversin contributes to LR determination and is essential for the generation of normal nodal flow (92). A phenotypically similar (iv^-) mouse is defective in nodal cilia LR dynein (115).

Wnt signaling is generally divided into two separate signal transduction pathways known as the canonical and the noncanonical Wnt pathways, which lead to two different end points. The canonical pathway operates through β-catenin, whereas the noncanonical pathway, also known as the planar cell polarity (PCP) pathway, acts through the membrane protein, Van gogh–like 2 (Vangl2). Inversin functions as a molecular switch between the two pathways (97) by targeting the Wnt pathway protein dishevelled for degradation; inversin inhibits the canonical pathway and activates the noncanonical pathway. Fluid flow increases the level of inversin in the cilia. Supporting the conclusion that the canonical Wnt signal transduction pathways are downregulated by the primary cilium, conditional inactivation of KIF3A of kinesin-2 that affects ciliogenesis in kidney tubule cells leads to increased expression of β-catenin and PKD as well as to cell proliferation (116). Similarly, Cano et al. (117) showed that in the *Tg737* ^{orpk} mouse there is an increase in β-catenin expression and localization to dilated ducts in pancreas and increased expression levels of Wnt signaling transcription factors (117). Finally, Ross et al. (118) showed that Vangl2 localizes to cilia and ciliary basal bodies in primary cilia of collecting duct cells as well as in human respiratory epithelial cells. In kidney cells Vangl2 genetically interacts with Bardet Biedl Syndrome (BBS) genes, indicating that cilia are intrinsically involved in PCP processes. PCP proteins in turn feedback into ciliogenesis pathways necessary for Hh signaling (119); in this way the Wnt and Hh pathways interact.

Is polycystin-mediated Ca^{2+} signaling in primary cilia also integrated with Wnt signaling? A Wnt-Ca^{2+} pathway has been implicated as a third Wnt signaling cascade; this pathway is thought to influence both the PCP and canonical Wnt pathways, thereby regulating cell adhesion and cell movements during gastrulation (120). Upon activation the Wnt-Ca^{2+} pathway increases intracellular levels of Ca^{2+}. It is therefore tempting to speculate that this Wnt pathway may interplay with those signaling systems that control Ca^{2+}-mediated processes in mammalian cilia, such as those regulated by polycystins in both primary and motile cilia. Indeed, we may have seen only the tip of the iceberg as to the plethora of ciliary functions associated with Wnt as well as Hh signaling.

NODAL CILIA

The correlation of human ciliary disease with randomization of LR asymmetry manifested as situs inversus led Afzelius (5) to conclude that embryonic ciliary function was necessary for proper LR positioning of organs, such as the heart, within the body. Unexpectedly, such cilia are present at the site of gastrulation, the embryonic node (121). Nodal cilia, like primary cilia, number one per cell with $9 + 0$ axonemes, but nodal cilia possess dynein arms with LR dynein (115, 122) and are motile, generating a leftward flow across the node (124). When the mouse intraciliary transport motor KIF3B was disrupted by gene targeting, the node lacked cilia, and LR asymmetry was randomized (123). This led to the hypothesis that nodal flow generated by the cilia was critical for the development of LR asymmetry. Further evidence for the nodal flow hypothesis was provided by examining mutant mice with paralyzed nodal cilia and from experimentally reversing flow, thus reversing LR asymmetry (124).

To produce nodal flow, the cilia must have an asymmetrical component to their movement. Rotation is always clockwise when one looks along the cilium base to tip, suggesting

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

that doublet activity moves around the axoneme clockwise from doublet 9 to 1 to 2 and so on. Just as in $9 + 2$ cilia discussed above, for which the effective stroke of the cilium always corresponds to certain doublet activity, the doublets that produce effective flow are a constant half-subset of the axoneme. In this way, the basic asymmetry of the ciliary axoneme determines the basic LR asymmetry of the body. The effective force is enhanced by a posterior tilt of the cilium (11) and by additional mechanisms (125).

Exactly how nodal flow induces LR asymmetry is still uncertain, but it seems likely that, in common with the primary cilia discussed above, induction involves a mechanosensory mechanism involving polycystins, leading to Ca^{2+} influx at the left side of the node, and a chemosensory pathway involving Hh and perhaps additional signaling pathways. McGrath et al. (126) found that LR dynein was present only on a centrally located set of nodal cilia, which are presumably those generating flow, whereas polycystin 2 was found on all nodal cilia. These investigators suggest that the cilia around the periphery of the node are nonmotile primary cilia that sense the flow and respond to initiate asymmetric Ca^{2+} signaling. Such signaling may act further via a Wnt signaling pathway (127). Tanaka et al. (128) propose a second mechanism involving FGF-induced release of membrane-bounded vesicles termed nodal vesicular particles (NVPs) that seem morphologically similar to lung surfactant. NVPs carry Shh and retinoic acid; they are transported leftward to impinge on the primary cilia of the left wall, initiating Shh and other transduction pathways. In support of this hypothesis, Tanaka et al. (128) show that FGF receptor inhibition at the node inhibits the leftward Ca^{2+} influx observed by McGrath et al. (126). The Ca^{2+} signals may be restored by Shh, retinoic acid, and Ihh. Motile $9 + 2$ cilia in other tissues may function similarly to form a gradient of signal molecules in the local cellular environment to control tissue homeostasis and function, such as in the oviduct, which is subjected to dramatic changes in the physiochemical milieu during the estrous cycle. Indeed, Sawamoto et al. (129) showed that neuroblast migration from the subventricular zone to the olfactory bulb in the adult brain parallels cerebrospinal fluid flow generated by beating ependymal cilia in which the relocation of cells may be guided by the formation of a gradient of signal molecules along the path of migration.

In summary, nodal cilia are an interesting and important intermediate between $9 + 2$ motile cilia and $9 + 0$ primary cilia in the body, relying on features of each for their function. Additional perspectives on these features are found in Tabin & Vogan (130), Hirokawa et al. (131), and new reviews on ciliary signaling (132, 133). The interrelationships between signaling pathways in nodal cilia that lead to the determination of LR asymmetry parallel the complexity of signaling in primary cilia of other tissues with different physiological endpoints and probably in motile $9 + 2$ cilia as well. Exactly how general the features of ciliary signaling are and whether important variations will prove to be tissue-specific, especially for complex tissues such as respiratory epithelium, remain to be determined.

SUMMARY POINTS

1. Mammalian cilia occur in two major patterns: motile $9 + 2$ cilia and usually nonmotile $9 + 0$ primary cilia.
2. Both motile and primary cilia have an axoneme surrounded by a ciliary membrane that selectively incorporates specific receptors and ion channels.

3. All cilia have sensory function, in part as mechanotransducers and in part in chemoreception.
4. Motile cilia transduce messengers such as cAMP and Ca^{2+} into increased beat frequency or changes in beat form for efficient mucociliary transport.
5. Primary cilia function as cellular positioning systems with hedgehog and Wnt signaling pathways running through them, as well as in growth control through ciliary PDGFRαα signaling.
6. Mutations in ciliary proteins lead to ciliopathies such as primary ciliary dyskinesia, polycystic kidney disease, retinitis pigmentosa, and hydrocephalus.
7. Motile 9 + 0 nodal cilia create a leftward flow of morphogens that is critical for left-right asymmetry development in the body.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

ACKNOWLEDGMENTS

We gratefully acknowledge Charles F. Guerra for help with figures and diagrams and Dr. Lotte B. Pedersen for valuable comments on the IFT section. We apologize to those whose work is not described in this review owing to restricted space. This work was supported in part by The Carlsberg Foundation, Fonden af 1870 and The Danish National Science Research Council grant 57462 (S.T.C.) and in part by NIDDK grants DK41918 and DK41296 (P.S.).

LITERATURE CITED

1. Porter KR. 1957. The submicroscopic morphology of protoplasm. *Harvey Lect*. 51:175–228
2. Satir P. 2005. Tour of organelles through the electron microscope: a reprinting of Keith Porter's classic Harvey Lecture with a new introduction. *Anat. Rec. A* 287:1184–85
3. Satir P, Dirksen ER. 1985. Function-structure correlations in cilia from mammalian respiratory tract. In *Handbook of Physiology-Respiratory System*, ed. AP Fishman, AB Fisher, 1:473–94. Bethesda, MD: Am. Physiol. Soc.
4. Satir P, Sleigh MA. 1990. The physiology of cilia and mucociliary interactions. *Annu. Rev. Physiol*. 52:137–55
5. Afzelius BA. 1976. A human syndrome caused by immotile cilia. *Science* 193:317–19
6. Smith JC, Northey JG, Garg J, Pearlman RE, Siu KW. 2005. Robust method for proteome analysis by MS/MS using an entire translated genome: demonstration on the ciliome of *Tetrahymena thermophila*. *J. Proteome Res*. 4:909–19
7. Pazour GJ, Agrin N, Leszyk J, Witman GB. 2005. Proteomic analysis of a eukaryotic cilium. *J. Cell Biol*. 170:103–13
8. Ostrowski LE, Blackburn K, Radde KM, Moyer MB, Schlatzer DM, et al. A proteomic analysis of human cilia: identification of novel components. *Mol. Cell Proteomics* 1:451–65
9. Nogales E, Whittaker M, Milligan RA, Downing KH. 1999. High-resolution model of the microtubule. *Cell* 96:79–88
10. Satir P. 1997. Cilia and related microtubular arrays in the eukaryotic cell. In *Handbook of Physiology*, ed. JF Hoffman, JD Jamieson, pp. 787–817. New York: Oxford Univ. Press

11. Okada Y, Takeda S, Tanaka Y, Belmonte JC, Hirokawa N. 2005. Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* 121:633–44
12. Sanderson MJ, Dirksen ER, Satir P. 1990. Electron microscopy of respiratory tract cilia. In *Electron Microscopy of the Lung*, ed. DE Schaufnagel, pp 47–69. New York: Marcel Dekker

12. A useful review of ultrastructural aspects of mammalian respiratory tract cilia.

13. Boucher RC. 2004. New concepts of pathogenesis of cystic fibrosis lung disease. *Eur. Respir. J.* 23:146–58
14. Vernon GG, Woolley DM. 2002. Microtubule displacements at the tips of living flagella. *Cell Motil. Cytoskel.* 52:151–60
14a. Zariwala MA, Knowles MR, Omran H. 2007. Genetic defects in ciliary structure and function. *Annu. Rev. Physiol.* 69:423–50
15. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, et al. 2004. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am. J. Respir. Crit. Care Med.* 169:459–67
16. Escudier E, Couprie M, Duriez B, Roudot-Thoraval F, Millepied MC, et al. 2002. Computer-assisted analysis helps detect inner dynein arm abnormalities. *Am. J. Respir. Crit. Care Med.* 166:1257–62
17. Olbrich H, Haffner K, Kispert A, Volkel A, Volz A, et al. 2002. Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry. *Nat. Genet.* 30:143–44
18. Pennarum G, Escudier E, Chapelin C, Bridoux AM, Cacheux V, et al. 1999. Loss-of-function mutations in a human gene related to *Chlamydomonas* IC78 result in primary ciliary dyskinesia. *Am. J. Hum. Genet.* 65:1508–19
19. de Iongh RU, Rutland J. 1995. Ciliary defects in healthy subjects, bronchiectasis, and primary ciliary dyskinesia. *Am. J. Respir. Crit. Care Med.* 151:1559–67
20. Hamasaki T, Nielson JH, Satir P. 1998. Regulation of outer arm dynein activity via light chain phosphorylation. In *Cilia, Mucus and Mucociliary Interactions*, ed. GL Baum, Z Priel, Y Roth, N Liron, E Ostfeld, pp. 21–25. New York: Marcel Dekker
21. Christensen ST, Guerra C, Wada Y, Valentin T, Angeletti RH, et al. 2001. A regulatory light chain of ciliary outer arm dynein in *Tetrahymena thermophila*. *J. Biol. Chem.* 276:20048–54
22. Sanderson MJ, Lansley AB, Evans JH. 2001. The regulation of airway ciliary beat frequency by intracellular calcium. In *Cilia and Mucus*, ed. M Salathe, pp. 39–57. New York: Marcel Dekker
22a. Salathe M. 2007. Regulation of mammalian ciliary beating. *Annu. Rev. Physiol.* 69:401–22
23. Habermacher G, Sale WS. 1997. Regulation of flagellar dynein by phosphorylation of a 138-kD inner arm dynein intermediate chain. *J. Cell Biol.* 136:167–76
24. Wargo MJ, Smith EF. 2003. Asymmetry of the central apparatus defines the location of active microtubule sliding in *Chlamydomonas* flagella. *Proc. Natl. Acad. Sci. USA* 100:137–42
25. Mitchell DR, Nakatsugawa M. 2004. Bend propagation drives central pair rotation in *Chlamydomonas reinhardtii* flagella. *J. Cell Biol.* 166:709–15
26. Afzelius BA. 1999. Asymmetry of cilia and of mice and men. *Int. J. Dev. Biol.* 43:283–86
27. Sanderson MJ, Sleigh MA. 1981. Ciliary activity of cultured rabbit tracheal epithelium: beat pattern and metachrony. *J. Cell Sci.* 47:331–47
28. Afzelius BA. 2004. Cilia-related diseases. *J. Pathol.* 204:470–77
29. Banizs B, Pike MM, Millican CL, Ferguson WB, Komlosi P, et al. 2005. Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development* 132:5329–39

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

30. Ibanez-Tallon I, Pagenstecher A, Fliegauf M, Olbrich H, Kispert A, et al. 2004. Dysfunction of axonemal dynein heavy chain Mdnah5 inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation. *Hum. Mol. Genet.* 13:2133–41
31. Tuomanen E. 1990. The surface of mammalian respiratory cilia. In *Ciliary and Flagellar Membranes*, ed. RA Bloodgood, pp. 363–88. New York: Plenum
32. Chailley B, Boisvieux-Ulrich E, Sandoz D. 1990. Structure and assembly of the oviduct ciliary membrane. In *Ciliary and Flagellar Membranes*, ed. RA Bloodgood, pp. 337–62. New York: Plenum
33. Satir P, Guerra C. 2003. Control of ciliary motility: a unifying hypothesis. *Europ. J. Protistol.* 39:410–15
34. Taylor BL, Zhulin IB. 1999. PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol. Mol. Biol. Rev.* 63:479–506
35. Wyatt TA, Forget MA, Adams JM, Sisson JH. 2005. Both cAMP and cGMP are required for maximal ciliary beat stimulation in a cell-free model of bovine ciliary axonemes. *Am. J. Physiol. Lung Cell Mol. Physiol.* 288:L546–51
36. Lazard D, Barak Y, Lancet D. 1989. Bovine olfactory cilia preparation: thiol-modulated odorant-sensitive adenylyl cyclase. *Biochim. Biophys. Acta* 1013:68–72
37. Teilmann SC, Christensen ST. 2005. Localization of the angiopoietin receptors Tie-1 and Tie-2 on the primary cilia in the female reproductive organs. *Cell Biol. Int.* 29:340–46
38. Teilmann SC, Byskov AG, Pedersen PA, Wheatley DN, Pazour GJ, Christensen ST. 2005. Localization of transient receptor potential ion channels in primary and motile cilia of the female murine reproductive organs. *Mol. Reprod. Dev.* 71:444–52
39. Kreda SM, Mall M, Mengos A, Rochelle L, Yankaskas J, et al. 2005. Characterization of wild-type and deltaF508 cystic fibrosis transmembrane regulator in human respiratory epithelia. *Mol. Biol. Cell* 16:2154–67
40. Rosenbaum JL, Witman GB. 2002. Intraflagellar transport. *Nat. Rev. Mol. Cell Biol.* 3:813–25
41. Gilula NB, Satir P. 1972. The ciliary necklace. A ciliary membrane specialization. *J. Cell Biol.* 53:494–509
42. Deane JA, Cole DG, Seeley ES, Diener DR, Rosenbaum JL. 2001. Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. *Curr. Biol.* 11:1586–90
43. Horst CJ, Johnson LV, Besharse JC. 1990. Transmembrane assemblage of the photoreceptor connecting cilium and motile cilium transition zone contain a common immunologic epitope. *Cell Motil. Cytoskel.* 17:329–44
44. Hong DH, Yue G, Adamian M, Li T. 2001. Retinitis pigmentosa GTPase regulator (RPGRr)-interacting protein is stably associated with the photoreceptor ciliary axoneme and anchors RPGR to the connecting cilium. *J. Biol. Chem.* 276:12091–99
45. Hong DH, Pawlyk B, Sokolov M, Strissel KJ, Yang J, et al. 2003. RPGR isoforms in photoreceptor connecting cilia and the transitional zone of motile cilia. *Invest. Ophthalmol. Vis. Sci.* 44:2413–21
46. Moore A, Escudier E, Roger G, Tamalet A, Pelosse B, et al. 2006. RPGR is mutated in patients with a complex X-linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa. *J. Med. Genet.* 43:326–33
47. Geng L, Okuhara D, Yu Z, Tian X, Cai Y, et al. 2006. Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *J. Cell Sci.* 119:1383–95
48. Gaillard DA, Lallement AV, Petit AF, Puchelle ES. 1989. In vivo ciliogenesis in human fetal tracheal epithelium. *Am. J. Anat.* 185:415–28

31. A unique treatment of respiratory cilary membrane properties, including bacterial attachment.

38. The first images comparing ciliary membrane TRP channel distribution on functional mammalian motile cilia and related primary cilia.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

49. Dirksen ER, Crocker TT. 1965. Centriole replication in differentiating ciliated cells of mammalian respiratory epithelium: an electron microscopic study. *J. Microsc.* 5:629–56
50. Dirksen ER. 1991. Centriole and basal body formation during ciliogenesis revisited. *Biol. Cell* 72:31–38
51. Carson JL, Reed W, Lucier T, Brighton L, Gambling TM, et al. 2002. Axonemal dynein expression in human fetal tracheal epithelium. *Am. J. Physiol. Lung Cell Mol. Physiol.* 282:L421–30
52. Dirksen ER. 1974. Ciliogenesis in the mouse oviduct. A scanning electron microscope study. *J. Cell Biol.* 62:899–904
53. Portman RW, LeCluyse EL, Dentler WL. 1987. Development of microtubule capping structures in ciliated epithelial cells. *J. Cell Sci.* 87:85–94
54. Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD. 2000. Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. *Am. J. Respir. Cell Mol. Biol.* 23:45–51
55. Johnson KA, Rosenbaum JL. 1992. Polarity of flagellar assembly in Chlamydomonas. *J. Cell Biol.* 119:1605–11
56. Marshall WF, Rosenbaum JL. 2001. Intraflagellar transport balances continuous turnover of outer doublet microtubules: implications for flagellar length control. *J. Cell Biol.* 155:405–14
57. Qin H, Diener DR, Geimer S, Cole DG, Rosenbaum JL. 2004. Intraflagellar transport (IFT) cargo: IFT transports flagellar precursors to the tip and turnover products to the cell body. *J. Cell Biol.* 19:255–66
58a. Pan J, Snell WJ. 2005. *Chlamydomonas* shortens its flagella by activating axonemal disassembly, stimulating IFT particle trafficking, and blocking anterograde cargo loading. *Dev. Cell* 9:431–38
58b. Qin H, Burnette D, Bae YK, Forscher P, Barr MM, Rosenbaum JL. 2005. Intraflagellar transport is required for the vectorial movement of TRPV channels in the ciliary membrane. *Curr. Biol.* 15:1695–99
59. Pedersen LB, Geimer S, Rosenbaum JL. 2006. Dissecting the molecular mechanisms of intraflagellar transport in *Chlamydomonas*. *Curr. Biol.* 16:450–59
60. Pan J, Snell WJ. 2002. Kinesin-II is required for flagellar sensory transduction during fertilization in *Chlamydomonas*. *Mol. Biol. Cell* 13:1417–26
61. Fliegauf M, Omran H. 2006. Novel tools to unravel molecular mechanisms in cilia-related disorders. *Trends Genet.* 22:241–45
62. Hagiwara H, Ohwada N, Takata K. 2004. Cell biology of normal and abnormal ciliogenesis in the ciliated epithelium. *Int. Rev. Cytol.* 234:101–41
63. Wheatley DN. 1982. *The Centriole: A Central Enigma of Cell Biology*. Amsterdam: Elsevier. 232 pp.
64. Sorokin S. 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* 15:363–77
65. Wheatley DN. 2005. Landmarks in the first hundred years of primary (9 + 0) cilium research. *Cell Biol. Int.* 29:333–39
66. Fonte VG, Searls RL, Hilfer SR. 1971. The relationship of cilia with cell division and differentiation. *J. Cell Biol.* 49:226–29
67. Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, et al. 2000. *Chlamydomonas IFT88* and its mouse homologue, polycystic kidney disease gene *tg737*, are required for assembly of cilia and flagella. *J. Cell Biol.* 151:709–18
68. Andrews PM. 1975. Scanning electron microscopy of human and rhesus monkey kidneys. *Lab. Invest.* 32:510–18

67. The key paper first demonstrating the role of ciliary proteins in polycystic kidney disease.

69. Roth KE, Rieder CL, Bowser SS. 1988. Flexible-substratum technique for viewing cells from the side: some in vivo properties of primary (9 + 0) cilia in cultured kidney epithelia. *J. Cell Sci*. 89:457–66
70. Schwartz EA, Leonard ML, Bizios R, Bowser SS. 1997. Analysis and modeling of the primary cilium bending response to fluid shear. *Am. J. Physiol*. 272:F132–38
71. Praetorius HR, Spring KA. 2001. Bending the MDCK cell primary cilium increases intracellular calcium. *J. Membr. Biol*. 184:71–79
72. Praetorius HR, Spring KA. 2003. Removal of the MDCK cell primary cilium abolishes flow sensing. *J. Membr. Biol*. 191:69–76
73. Liu W, Murcia NS, Duan Y, Weinbaum S, Yoder BK, et al. 2005. Mechanoregulation of intracellular Ca^{2+} concentration is attenuated in collecting duct of monocilium-impaired *orpk* mice. *Am. J. Physiol. Renal Physiol.* 289:F978–88
74. Pazour GJ, San Agustin JT, Follit JA, Rosenbaum JL, Witman GB. 2002. Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in *orpk* mice with polycystic kidney disease. *Curr. Biol*. 12:R378–80
75. Yoder BK, Hou X, Guay-Woodford LM. 2002. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are colocalized in renal cilia. *J. Am. Soc. Nephrol*. 13:2508–16
76. Parnell SC, Magenheimer BS, Maser RL, Zien CA, Frischauf AM, Calvet JP. 2002. Polycystin-1 activation of c-Jun N-terminal kinase and AP-1 is mediated by heterotrimeric G proteins. *J. Biol. Chem*. 277:19566–72
77. Chauvet V, Tian X, Husson H, Grimm DH, Wang T, et al. 2004. Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. *J. Clin. Invest.* 114:1433–43
78. Poole CA, Flint CA, Beaumont BW. 1985. Analysis of the morphology and function of primary cilia in connective tissues: a cellular cybernetic probe? *Cell Motil.* 5:175–93
79. Benzing T, Walz G. 2006. Cilium-generated signaling: a cellular GPS? *Curr. Opin. Nephrol. Hypertens*. 15:245–49
80. Poole CA, Jensen CG, Snyder JA, Gray CG, Hermanutz VL, Wheatley DN. 1997. Confocal analysis of primary cilia structure and colocalization with the Golgi apparatus in chondrocytes and aortic smooth muscle cells. *Cell Biol. Int*. 21:483–94
81. Poole CA, Zhang ZJ, Ross JM. 2001. The differential distribution of acetylated and detyrosinated alpha-tubulin in the microtubular cytoskeleton and primary cilia of hyaline cartilage chondrocytes. *J. Anat*. 199:393–405
82. Jensen CG, Poole CA, McGlashan SR, Marko M, Issa ZI, et al. 2004. Ultrastructural, tomographic and confocal imaging of the chondrocyte primary cilium in situ. *Cell Biol. Int.* 28:101–10
83. McGlashan SR, Jensen CG, Poole CA. 2006. Localization of extracellular matrix receptors on the chondrocyte primary cilium. *J. Histochem. Cytochem.* 54(9):1005–14
84. Praetorius HA, Praetorius J, Nielsen S, Frokiaer J, Spring KR. 2004. β1-integrins in the primary cilium of MDCK cells potentiate fibronectin-induced Ca^{2+} signaling. *Am. J. Physiol. Renal Physiol.* 287:F969–78
85. Albrecht-Buehler G. 1977. Phagokinetic tracks of 3T3 cells: parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. *Cell* 12:333–39
86. Wheatley DN. 1971. Cilia in cell-cultured fibroblasts. 3. Relationship between mitotic activity and cilium frequency in mouse 3T6 fibroblasts. *J. Anat*. 110:367–82

78. One of the first papers to introduce the enormous potential of the primary cilium as a chemical and physical sensory device.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

87. Tucker RW, Pardee AB, Fujiwara K. 1979. Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells. *Cell* 17:527–35
88. Tucker RW, Scher CD, Stiles CD. 1979. Centriole deciliation association with the early response of 3T3 cells to growth factors but not to SV40. *Cell* 18:1065–72
89. **Schneider L, Clement CA, Teilmann SC, Pazour GJ, Hoffmann EK, et al. 2005. PDGFRαα signaling is regulated through the primary cilium in fibroblasts. *Curr. Biol.* 15:1861–66**
90. Lih CJ, Cohen SN, Wang C, Lin-Chao S. 1996. The platelet-derived growth factor α-receptor is encoded by a growth-arrest-specific (gas) gene. *Proc. Natl. Acad. Sci. USA* 93:4617–22
91. de Silva DC, Wheatley DN, Herriot R, Brown T, Stevenson DA, et al. 1997. Mulvihill-Smith progeria-like syndrome: a further report with delineation of phenotype, immunologic deficits, and novel observation of fibroblast abnormalities. *Am. J. Med. Genet.* 69:56–64
92. Watanabe D, Saijoh Y, Nonaka S, Sasaki G, Ikawa Y, et al. 2003. The left-right determinant Inversin is a component of node monocilia and other 9 + 0 cilia. *Development* 130:1725–34
93. **Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. 2005. Vertebrate Smoothened functions at the primary cilium. *Nature* 437:1018–21**
94. Voss JW, Pedersen SF, Christensen ST, Lambert IH. 2004. Regulation of the expression and subcellular localization of the taurine transporter TauT in mouse NIH3T3 fibroblasts. *Eur. J. Biochem.* 271:4646–58
95. Christensen ST, Voss JW, Teilmann SC, Lambert IH. 2005. High expression of the taurine transporter TauT in primary cilia of NIH3T3 fibroblasts. *Cell Biol. Int.* 29:347–51
96. Lambert IH. 2004. Regulation of the cellular content of the organic osmolyte taurine in mammalian cells. *Neurochem. Res.* 29:27–63
97. **Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, et al. 2005. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. *Nat. Genet.* 37:537–43**
98. Germino GG. 2005. Linking cilia to Wnts. *Nat. Genet.* 37:455–57
99. Pan J, Wang Q, Snell WJ. 2005. Cilium-generated signaling and cilia-related disorders. *Lab. Invest.* 85:452–63
100. **Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, Yoder BK. 2005. Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet.* 1:e53**
101. Huangfu D, Anderson KV. 2006. Signaling from Smo to Ci/Gli: conservation and divergence of Hedgehog pathways from *Drosophila* to vertebrates. *Development* 133:3–14
102. Kasper M, Regl G, Frischauf AM, Aberger F. 2006. GLI transcription factors: mediators of oncogenic Hedgehog signaling. *Eur. J. Canc.* 42:437–45
103. Kalderon D. 2005. The mechanism of hedgehog signal transduction. *Biochem. Soc. Trans.* 33:1509–12
104. Huangfu D, Anderson KV. 2005. Cilia and Hedgehog responsiveness in the mouse. *Proc. Natl. Acad. Sci. USA* 102:11325–30
105. Zhang XM, Ramalho-Santos M, McMahon AP. 2001. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R symmetry by the mouse node. *Cell* 106:781–92

89. Strong evidence that the receptor tyrosine kinase PDGFRαα needs to be localized to primary cilia to function in growth control.

93. The first evidence that Smo needs to be localized to the primary cilium for regulation of the Hh pathway and correct morphogenesis.

97. The first evidence that ciliary inversin acts as a flow-regulated molecular switch between canonical and noncanonical Wnt pathways in developmental processes.

100. Strong evidence for a direct role of the primary cilium in Gli processing and Shh signal transduction in limb bud development.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

106. Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. 2003. Hedgehog signaling in the mouse requires intraflagellar transport proteins. *Nature* 426:83–87
107. Liu A, Wang B, Niswander LA. 2005. Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors. *Development* 132:3103–11
108. Zhang Q, Murcia NS, Chittenden LR, Richards WG, Michaud EJ, et al. 2003. Loss of the Tg737 protein results in skeletal patterning defects. *Dev. Dyn.* 227:78–90
109. Mo R, Freer AM, Zinyk DL, Crackower MA, Michaud J, et al. 1997. Specific and redundant functions of Gli2 and Gli3 zinc finger genes in skeletal patterning and development. *Development* 124:113–23
110. Dwyer ND, Adler CE, Crump JG, L'Etoile ND, Bargmann CI. 2001. Polarized dendritic transport and the AP-1 mu1 clathrin adaptor UNC-101 localize odorant receptors to olfactory cilia. *Neuron* 31:277–87
111. Handel M, Schulz S, Stanarius A, Schreff M, Erdtmann-Vourliotis M, et al. 1999. Selective targeting of somatostatin receptor 3 to neuronal cilia. *Neuroscience* 89:909–26
112. Brailov I, Bancila M, Brisorgueil MJ, Miquel MC, Hamon M, Verge D. 2000. Localization of 5-HT(6) receptors at the plasma membrane of neuronal cilia in the rat brain. *Neuron* 31:277–87
113. Morgan D, Eley L, Sayer J, Strachan T, Yates LM, et al. 2002. Expression analyses and interaction with the anaphase promoting complex protein Apc2 suggest a role for inversin in primary cilia and involvement in the cell cycle. *Hum. Mol. Genet.* 11:3345–50
114. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, et al. 2003. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat. Genet.* 34:413–20
115. Supp DM, Witte DP, Potter SS, Brueckner M. 1997. Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. *Nature* 389:963–96
116. Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, et al. 2003. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc. Natl. Acad. Sci. USA* 100:5286–91
117. Cano DA, Murcia NS, Pazour GJ, Hebrok M. 2004. *orpk* mouse model of polycystic kidney disease reveals essential role of primary cilia in pancreatic tissue organization. *Development* 131:3457–67
118. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, et al. 2005. Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat. Genet.* 37:1135–40
119. Park TJ, Haigo SL, Wallingford JB. 2006. Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat. Genet.* 38:303–11
120. Habas R, Dawid IB. 2005. Dishevelled and Wnt signaling: is the nucleus the final frontier? *J. Biol.* 4:2
121. Sulik K, Dehart DB, Iangaki T, Carson JL, Vrablic T, et al. 1994. Morphogenesis of the murine node and notochordal plate. *Dev. Dyn.* 201:260–78
122. Supp DM, Brueckner M, Kuehn MR, Witte DP, Lowe LA, et al. 1999. Targeted deletion of the ATP binding domain of left-right dynein confirms its role in specifying development of left-right asymmetries. *Development* 126:5495–504
123. Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, et al. 1998. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* 95:829–37

106. The first paper to show that the IFT machinery has an essential and vertebrate-specific role in Hh signal transduction.

107. Strong evidence that IFT proteins are required for proteolytic processing and transcriptional activities of Gli proteins as well as for Hh ligand–induced signaling cascade.

117. Evidence for a role of primary cilia in Wnt signaling.

118. Evidence that primary cilia are required for PCP processes.

119. Strong evidence for a central role for PCP signaling in development of cilia and Hh signaling.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

124. Nonaka S, Shiratori H, Saijoh Y, Hamada H. 2002. Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature* 418:96–99
125. Buceta J, Ibanes M, Rasskin-Gutman D, Okada Y, Hirokawa N, Izpisua-Belmonte JC. 2005. Nodal cilia dynamics and the specification of the left/right axis in early vertebrate embryo development. *Biophys. J.* 89:2199–209
126. **McGrath J, Somlo S, Makova S, Tian X, Brueckner M. 2003. Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell* 114:61–73**
127. Nakaya MA, Biris K, Tsukiyama T, Jaime S, Rawls JA, Yamaguchi TP. 2005. *Wnt3a* links left-right determination with segmentation and anteroposterior axis elongation. *Development* 132:5425–36
128. **Tanaka Y, Okada Y, Hirokawa N. 2005. FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. *Nature* 435:172–77**
129. Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, et al. 2006. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 311:629–32
130. Tabin CJ, Vogan KJ. 2003. A two-cilia model for vertebrate left-right axis specification. *Genes Dev*. 17:1–6
131. Hirokawa N, Tanaka Y, Okada Y, Takeda S. 2006. Nodal flow and the generation of left-right asymmetry. *Cell* 125:33–45
132. Davis EE, Brueckner M, Katsanis N. 2006. The emerging complexity of the vertebrate cilium: new functional roles for an ancient organelle. *Devel. Cell* 11:9–19
133. Michaud EJ, Yoder BK. 2006. The primary cilium in cell signalling and cancer. *Cancer Res*. 66:6463–67

126. Evidence for the role of primary cilia as well as motile nodal cilia in left-right asymmetry determination.

128. A new model implicating released morphogens and ciliary chemoreception in left-right determination.

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

Annual Review of Physiology

Volume 69, 2007

Contents

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.

Frontispiece
Clay M. Armstrong xx

PERSPECTIVES, *David L. Garbers, Editor*

Life Among the Axons
Clay M. Armstrong 1

CARDIOVASCULAR PHYSIOLOGY, *Jeffrey Robbins, Section Editor*

Mitochondrial Ion Channels
Brian O'Rourke 19

Preconditioning: The Mitochondrial Connection
Elizabeth Murphy and Charles Steenbergen 51

CELL PHYSIOLOGY, *David E. Clapham, Section Editor*

Iron Homeostasis
Nancy C. Andrews and Paul J. Schmidt 69

Transporters as Channels
Louis J. DeFelice and Tapasree Goswami 87

ECOLOGICAL, EVOLUTIONARY, AND COMPARATIVE PHYSIOLOGY, *Martin E. Feder, Section Editor*

Hypoxia Tolerance in Mammals and Birds: From the Wilderness to the Clinic
Jan-Marino Ramirez, Lars P. Folkow, and Arnoldus S. Blix 113

Hypoxia Tolerance in Reptiles, Amphibians, and Fishes: Life with Variable Oxygen Availability
Philip E. Bickler and Leslie T. Buck 145

ENDOCRINOLOGY, *Kathryn B. Horwitz, Section Editor*

Integration of Rapid Signaling Events with Steroid Hormone Receptor Action in Breast and Prostate Cancer
Carol A. Lange, Daniel Gioeli, Stephen R. Hammes, and Paul C. Marker 171

Nuclear Receptor Structure: Implications for Function
David L. Bain, Aaron F. Heneghan, Keith D. Connaghan-Jones, and Michael T. Miura 201

GASTROINTESTINAL PHYSIOLOGY, *John Williams, Section Editor*

Regulation of Intestinal Cholesterol Absorption
David Q.-H. Wang 221

Why Does Pancreatic Overstimulation Cause Pancreatitis?
Ashok K. Saluja, Markus M. Lerch, Phoebe A. Phillips, and Vikas Dudeja 249

NEUROPHYSIOLOGY, *Richard Aldrich, Section Editor*

Timing and Computation in Inner Retinal Circuitry
Stephen A. Baccus 271

Understanding Circuit Dynamics Using the Stomatogastric Nervous System of Lobsters and Crabs
Eve Marder and Dirk Bucher 291

RENAL AND ELECTROLYTE PHYSIOLOGY, *Gerhard H. Giebisch, Section Editor*

Molecular Mechanisms of Renal Ammonia Transport
I. David Weiner and L. Lee Hamm 317

Phosphatonins and the Regulation of Phosphate Homeostasis
Theresa Berndt and Rajiv Kumar 341

Specificity and Regulation of Renal Sulfate Transporters
Daniel Markovich and Peter S. Aronson 361

RESPIRATORY PHYSIOLOGY, *Richard C. Boucher, Jr., Section Editor*

Overview of Structure and Function of Mammalian Cilia
Peter Satir and Søren Tvorup Christensen 377

Regulation of Mammalian Ciliary Beating
Matthias Salathe 401

Genetic Defects in Ciliary Structure and Function
Maimoona A. Zariwala, Michael R. Knowles, and Heymut Omran 423

SPECIAL TOPIC, β-ARRESTINS, *Robert J. Lefkowitz, Special Topic Editor*

Regulation of Receptor Trafficking by GRKs and Arrestins
Catherine A.C. Moore, Shawn K. Milano, and Jeffrey L. Benovic 451

β-Arrestins and Cell Signaling
Scott M. DeWire, Seungkirl Ahn, Robert J. Lefkowitz, and Sudha K. Shenoy 483

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org
by National Institute of Health Library on 11/28/07. For personal use only.

Physiological Roles of G Protein–Coupled Receptor Kinases and Arrestins
Richard T. Premont and Raul R. Gainetdinov 511

Stop That Cell! β-Arrestin-Dependent Chemotaxis: A Tale of Localized Actin Assembly and Receptor Desensitization
Kathryn A. DeFea 535

Regulation of Receptor Tyrosine Kinase Signaling by GRKs and β-Arrestins
Christopher J. Hupfeld and Jerrold M. Olefsky 561

Indexes

Cumulative Index of Contributing Authors, Volumes 65–69 579

Cumulative Index of Chapter Titles, Volumes 65–69 582

Errata

An online log of corrections to *Annual Review of Physiology* chapters (if any, 1997 to the present) may be found at http://physiol.annualreviews.org/errata.shtml

Annu. Rev. Physiol. 2007.69:377-400. Downloaded from arjournals.annualreviews.org by National Institute of Health Library on 11/28/07. For personal use only.