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Cilia

Methods and Protocols

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Preface

This volume is the result of an explosion of molecular-based research on cilia which began with the discovery of the universality of intraflagellar transport (IFT) and ciliary genomics/proteomics at the turn of the millennium and attracted new interest and new investigators into the field. The cilium is a cell organelle with nanometer substructure which can be studied with techniques at the cutting edge of molecular biology. We invited expert contributors associated with these techniques, their sometimes specialized instrumentation and their implications for ciliary biology, to join us in writing chapters for the volume. Unlike previous Methods volumes, this one is broadly based, covering motile, sensory, and primary cilia and it should be attractive to anyone interested in entering the field of ciliary biology using model organisms including flagellate algae (*Chlamydomonas*), ciliates (*Paramecium*), planaria, nematodes (*C. elegans*), insects (*Drosophila*), zebrafish and *Xenopus* or mammalian or human cells.

We appreciate the time and effort our contributors have given to making this volume highly useful to both seasoned and novice investigators. Similarly, we appreciate the efforts of John Walker in keeping our schedule mainly on track and our format mainly in order. If we have sometimes been delayed in communicating with you or in keeping to schedule, it has been largely due to the welcome arrival of Ella.

Support is acknowledged where appropriate in each chapter, but some of our overall collaboration resulting in our co-editing of this volume relied on a fellowship and support from the Lundbeck foundation.

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Introduction

Cilia are everywhere. By this we mean that most cells of all animal as well as many plant and protistan phyla bear cilia, defined as membrane bounded organelles based on a cylindrical nine doublet microtubule axonemal cytoskeleton arising from a centriole-like basal body. Cilia fall into different classes, which are defined by their axonemal arrangement and capacity to function as motile and/or sensory units [1]. Most types of cilia are assembled onto the basal body by intraflagellar transport (IFT), which is characterized by kinesin-2 and cytoplasmic dynein 2-mediated bidirectional trafficking of proteins along the axoneme from the ciliary base towards the tip and back [2]. Further, structural and functional barriers at the proximal region of the cilium, known as the ciliary transition zone, regulate ciliary formation, membrane composition, and function via the selective passage of proteins into and out of the ciliary compartment [3–5]. In many cases, the region between the ciliary membrane and the plasma membrane folds inwards to form a ciliary pocket that comprises an active site for exocytosis and clathrin-dependent endocytosis (CDE) of ciliary proteins [6–8].

Presumably, cilia arose during the evolution of the eukaryotic cell and were present in LECA, the last eukaryotic common ancestor, probably as motile cilia with a classic 9 + 2 microtubule-based axoneme, where dynein arms attached to the microtubule doublets hydrolyze ATP to power ciliary motility. The 9 + 2 axoneme is found in cilia as divergent as the comb plates of ctenophores, the sperm cell of *Gingko*, and the epithelium of the vertebrate respiratory tract. In certain organisms, such as *Chlamydomonas* and in male gametes, cilia are sometimes called flagella, but there are no consistent differences in structure, beat, number, or molecular biology, in the context of this book, between cilia and flagella. Although the term "flagella" is best reserved for the prokaryotic organelle, it persists for some important eukaryotic cells. Cells with motile 9 + 2 cilia may bear one or more cilia, even thousands per cell. Cilia are not found on prokaryotes; bacterial flagella and "stereocilia" of vertebrate hair cells of the ear are not cilia.

In addition to motile 9 + 2 cilia, major classes include primary and sensory cilia. Primary cilia are solitary organelles that emanate from the centrosomal mother centriole at the cell surface during growth arrest in most vertebrate cell types [1], whereas sensory cilia are modified as the receptive projections of sense organs or nerve cell dendrites [9–12]. Primary and sensory cilia usually are 9 + 0, missing the two central single microtubules and often axonemal dynein arms, which make them nonmotile. Depending on type, sensory cells may be multiciliated, although with a few, not thousands of, cilia. Where dynein arms are present, as in chordotonal organs of insects [13] or at the node in vertebrates [14], a form of motility is present.

A new era for primary and sensory cilia dawned with the discovery of IFT [15] and the subsequent link to polycystic kidney disease [16, 17]. These studies paved the way for understanding that primary cilia function as sensory organelles that detect and transmit extracellular cues to the cell. We now know that primary cilia coordinate a vast array of different signaling pathways to control specified cellular processes during development and in tissue homeostasis, such as those regulated by receptor tyrosine kinases [18], extracellular

matrix receptors [19], and transforming growth factor beta receptors [20] as well as by G-protein-coupled receptors of the A, B, and F classes [21], the latter including Smoothened in Hedgehog signaling [22]. Probably, the primary cilium functions as a hot spot for the balanced integration of multiple signaling pathways into higher order networks that dictate the biological output of pathway activity during development and in tissue homeostasis. Consequently, defects in formation and compartmentalization of primary cilia lead to defective cell signaling and abnormalities in cell cycle control, migration, polarization, and differentiation, often as a specific cluster of symptoms or syndromes termed ciliopathies [23–26].

Similarly, sense organs of multicellular organisms possess receptive motile or nonmotile cilia for vision, hearing, proprioception, and chemosensation leading to olfactory and mating responses. The persistence of cilia in recognizable form in most phyla suggests that the motile and sensory functions of cilia are often of evolutionary significance. Unicellular organisms rely on motile cilia to move through water, to walk on substrates, to sense favorable and unfavorable environments, to escape predation, to feed, to disperse, and to mate. Multicellular organisms use ciliated epithelia and motile sperm for similar purposes. In addition, motile cilia are used for osmoregulation and clearance in flame cells and kidneys [27], vertebrates use nodal cilia during development for left-right symmetry determination [28], and *Mytilus* uses certain tangled gill cilia as a kind of ciliary Velcro^{R} [29]. In conclusion, whether motile or not, all cilia are sensory in that they possess specific membrane receptors that respond to extracellular cues and transmit the information to control specified ciliary and cellular processes.

The variety and specialization of cilia make it imperative that for investigation of specific aspects of cilia molecular biology, preparative techniques be carefully developed and described. In this volume, a series of experts describes techniques for the study of fundamental aspects of the biology of cilia in a series of different systems. We cover methods to examine important aspects of ciliary biology in vertebrate cells and sense organs, particularly of fish, mouse, and man, in invertebrate cells and sense organs including those of *C. elegans* and *Drosophila*, and in protists such as *Chlamydomonas* and *Paramecium*.

We have asked contributors to write on advanced methodology, including super-resolution light microscopy. The techniques presented here, in combination with known ciliary genomics and proteomics, have made possible spectacular advances in our understanding. For motile cilia, advanced structural imaging and gene knockouts have brought us to the point where the basic mechanisms of motility and its cellular controls are known in considerable molecular detail and new information that holds promise in solving remaining problems is being generated at a rapid rate [30]. Nevertheless, very interesting problems concerning ciliary orientation, length control, and sensory function remain lightly explored in morphogenesis of multiciliated cells, both in protists and in metazoan (and metaphytan) organisms. Tracking techniques are being used to follow molecular paths into and along the axoneme. For sensory and primary cilia, super-resolution is defining the organization of the transition zone/ciliary necklace region responsible for molecular selection and transport within the cilium, facilitating signaling pathways and leading to an understanding of the development of ciliopathies. New trends consider integration and exchange of ciliary molecules with other cell organelles, including endosomes, autophagosomes, and the nucleus.

Many of the methods considered in the chapters that follow were necessary in developing these ideas and they can successfully contribute to continued exploration of this fast evolving field. In these chapters, detailed information is given on handling and examining ciliated systems and organisms. We invite the reader to use the methods described to join this effort.

Peter Satir
Søren T. Christensen

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