PREFACE

Cilia are found on almost every cell in the body and play innumerable roles in development and physiology, and yet it was not until the last days of the twentieth century that the importance of cilia began to be fully appreciated. For decades prior, they were thought of by most cell biologists (if, indeed, they were thought of at all) as vestigial remnants of some sort, playing no important biological role. How anyone could think an organelle unimportant when it is found on every cell in the body, and conserved throughout the eukaryotes, is a question best left to psychiatrists. In any case, the field of ciliary biology has seen explosive growth in the new millennium. A new field of inquiry requires new methods of exploration, and these volumes are designed to present the broadest possible range of methods currently in use for the study of ciliary biology.

One of the first things one has to do when studying a new structure is to determine all of the parts, and this cataloging of parts for cilia is still an ongoing undertaking. Consequently, the reader will notice that many of the chapters are devoted to methods for analyzing ciliary composition using biochemical, proteomic, or bioinformatic methods. However, understanding the parts list is a first step and not an end in itself. The volumes thus include protocols for studying how the biochemical components interact and work together during assembly and function of cilia.

Much of what we know about ciliary assembly and function has come from microscopy-based studies. For example, it was careful high-resolution light microscopy that first led to the discovery of intraflagellar transport (IFT). After that, the recognition that IFT proteins are encoded by genes involved in polycystic kidney disease was the spark that set off the ciliary disease explosion. Without the initial microscopy-based discovery, who knows how long it would have taken to get to this point by other methods? The methods for studying cilia under the microscope are still evolving. The implementation of highly sensitive optical microscopy and cryo-electron tomography have pushed the boundaries of cilia imaging, but that is not to say that more established methods have by any means outlived their usefulness. Hence, we have included a range of imaging methods.

The diversity of ciliary functions is staggering. Cilia not only generate flow of fluid during both development and normal physiology, they also act as sensors for mechanical stimuli and biochemical ligands. The diversity

of ciliary functions prompts the need for a diverse set of methodologies for studying these functions, and here we present an extensive set of protocols for studying the mechanical and sensory functions of cilia in different physiological contexts. Another consequence of the diversity of ciliary functions is the fact that different model organisms will have different advantages for studying different aspects of cilia. For example, it is hard to beat, or even come close to, the unicellular green alga Chlamydomonas for biochemical or genetic analysis of ciliary assembly and motile function. On the other hand, you cannot study cilia function in mucus clearance in algae, as they do not make mucus, nor can you study cilia function in hedgehog signaling as algae do not signal using this ligand. Thus, a growing range of standard and less-standard model organisms are being exploited to study cilia in developmental and physiological contexts. The chapters here include a set of protocols for studying cilia in a range of systems.

Finally, like everything else in the cell, the cilium is a complex system and cannot, ultimately, be understood one molecule at a time. A wide range of "omics" methods have been applied to cilia including proteomics, transcriptomics, comparative genomics, and bioinformatic sequence analysis. These types of systematic methods have of course been applied to other organelles as well, but what is interesting for cilia is that, since interest in cilia started to grow right at the time that these methods started coming online, a very large fraction of our knowledge about ciliary composition has come directly from systematic omics studies, unlike in other more heavily studied organelles where large-scale systematic methods have to a large extent merely extended what had already been known from decades of intensive biochemical and genetic work.

For a long time now, ciliary researchers have had to pull themselves up by their own bootstraps, inventing new methods every time a new question was posed. It is a sign of the growing maturity of the field that we can now compile a set of protocols that are in common enough use to be considered a standard toolbox. This collection of detailed protocols will serve as an entry point for newcomers to the field while, it is hoped, also being of use to those already in the field. I want to give thanks to all the authors who contributed to this pair of volumes. The present chapters have been written by the leaders in the field, who include among their number many of the pioneers who contributed to the explosion of interest in cilia and who are continuing to drive the field forward into unexplored territory. Their willingness to share their methods with the whole community does them a great credit, and we all benefit from their insights. Since cilia touch on virtually every

aspect of cell biology and development, I expect that more and more researchers will find the study of cilia unavoidable, and these chapters should greatly ease their way into this area of study.

Finally, I would like to acknowledge the influence of Joel Rosenbaum both on myself and on the whole field. Joel has done more than any single person to touch off the revolution in cilia and he continues to act as the cutting edge, behind whom the rest of us follow. Although he was too busy with new research to contribute his own chapter, his pervasive influence will be easily seen in the numerous chapters contributed by his former students and postdocs. I dedicate these volumes to him.

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