Preface

Most intracellular movement in eukaryotic cells is conducted by three classes of molecular motors: the myosins, the dyneins, and the kinesins, operating on two types of polymers: actin filaments and microtubules. Because of the accessibility of muscle fibers, study of molecular motors began with the kinetic and microscopic analyses of the myosin II–actin interaction five decades ago. Likewise, the ease of purification of cilia and flagella from various organisms facilitated the isolation and characterization of axonemal dynein. Biochemical techniques were also used to identify and characterize kinesin-1, responsible for axonal transport, in the mid-1980s. However, with the introduction of molecular techniques, shortly after the discovery of kinesin-1 and its deposit into the database of motor domain sequences, came a virtual explosion in the number of genes encoding proteins with motor domains similar to kinesin and myosin. Molecular analysis of dynein genes has revealed genetic diversity in this group of motor genes as well. Together, these three motor classes power muscle contraction, vesicle transport, flagellar and ciliary beating, signal transduction, chromosome segregation, and numerous other essential forms of cellular motility. Another group of molecular motors that also harnesses the energy released by ATP hydrolysis to power movement are the rotary motors typified by the F_1-ATPase, and methods to study this interesting class of motors is included in this volume.

The protocols described in *Molecular Motor Protocols* are necessarily diverse, reflecting the varied cellular functions of motor proteins, and range from the basic protein purification and enzymatic assays to more demanding techniques, including the development of in vitro motility systems, structural analysis, and reverse chemical genetics. A majority of the protocols in this volume concern an emerging focus in the motor field: identification and characterization of protein–protein interactions important for motor function. Each experimental procedure provides step-by-step instructions to the investigator to ensure success and includes a detailed *Notes* section, a hallmark feature of the *Methods in Molecular Biology* series, based on the hands-on experience of the authors. The *Notes* provide detailed advice that allows even the nonspecialist to master the techniques and troubleshoot difficulties. In addition, advanced protocols offer cutting-edge methods to the experienced motor investigator. The protocols in this book are grouped loosely according to methodology and can be applied to the study of proteins of different motor classes. The first four chapters describe protein purification from natural and recombinant sources and

biochemical assays of polymer binding, ATPase activity, depolymerase activity, and phosphorylation status. The interrelationships of protein subunits within the motor protein are particularly important for the structurally complex dynein motor, and methods to investigate these interactions are described in Chapters 5 and 6. Cargo-binding properties of individual motors are essential for their cellular function, and methods to identify targeting sequences and candidate cargo molecules and/or receptors are discussed in Chapters 7 and 8, with ultrastructural localization of motor proteins presented in Chapter 9. Reconstitution of motility in vitro has been an important technique in the analysis of all motor classes, and three different systems—endocytic recycling using isolated liver vesicles, purification of specialized junctional complexes from the testis, and single molecule observation of the rotation of F_1-ATPase—are described in Chapters 9 through 11. Functional analysis of motor proteins is increasingly being driven by investigation of their structure at the molecular level. This volume includes three chapters describing different approaches to structural analysis: FRET, X-ray cystallography, and cryo-EM (Chapters 13, 14, and 15). The power of structural information to dissect motor function is clearly demonstrated in the final chapter (Chapter 16), which describes a reverse chemical-genetic approach to the study of unconventional myosins.

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