
Preface

Many motile bacteria can swim in liquid environments and move on semi-solid surface by rotating flagella. The bacterial flagellum is a supramolecular assembly composed of 30 different proteins and consists of at least three parts: the basal body, the hook, and the filament. The basal body is embedded within the cell membranes and acts as a bidirectional rotary motor. The energy for motor rotation is supplied by cation influx driven by an electrochemical potential difference of specific ions, such as H^+ and Na^+ , across the cytoplasmic membrane, i.e., the ion motive force. The hook and filament extend outwards in the cell exterior. The filament works as a helical propeller to produce thrust. The hook connects the filament with the basal body and functions as a universal joint to smoothly transmit torque produced by the motor to the helical filament. *Escherichia coli* and *Salmonella enterica* produce several flagella per cell. The *E. coli* and *S. enterica* flagellar motor can operate in either counterclockwise (CCW, viewed from filament to motor) or clockwise (CW) direction. When most of the motors rotate in the CCW direction, the filaments form a bundle and propel the cell smoothly. When one or more motors spin in the CW direction, the bundle is disrupted and hence the cell tumbles and changes the swimming direction. *E. coli* and *S. enterica* can move towards more favorable conditions and escape from undesirable ones for their survival by sensing temporal variations of environmental stimuli such as chemical attractants and repellents, temperature, and pH via methyl-accepting chemotaxis proteins (MCP). MCPs are transmembrane proteins with a large cytoplasmic domain involved in interactions with a histidine kinase CheA and an adaptor protein CheW. The MCPs control CheA autophosphorylation. Phosphorylated CheA transfers its phosphate group to a response regulator CheY, and then phosphorylated CheY (CheY-P) binds to the cytoplasmic face of the flagellar motor, letting the motor spin in the CW direction.

In this volume we have brought together a set of cutting-edge research protocols to study the structure and dynamics of the bacterial flagellum using bacterial genetics, molecular biology, biochemistry, structural biology, biophysics, cell biology, and molecular dynamics simulation. Our aim is to provide a pathway to the investigation of the bacterial flagellum derived from various bacterial species through techniques that can be applied. The protocols are generally applicable to other supramolecular motility machinery, such as gliding machinery of bacteria. Since the principal goal of the book is to provide researchers with a comprehensive account of the practical steps of each protocol, the Methods section contains detailed step-by-step descriptions of every protocol. The Notes section complements the Methods to get the hang of each experiment based on the authors' experiences and to figure out the best way to solve any problem and difficulty that might arise during the experiment.

Flagellar assembly begins with the basal body, followed by the hook and finally the filament. The flagellar transcriptional hierarchy is coupled to the assembly process. A remarkable feature is that the flagellar type III protein export apparatus, which is required for flagellar assembly beyond the cellular membranes, coordinates flagellar gene expression with assembly. *E. coli* and *S. enterica* are model organisms that have provided detailed insights into the structure, assembly, and function of the bacterial flagellum. Chapters in

Part I describe flagellar type III protein export (Chapters 1 and 2), assembly (Chapter 3), and gene regulation (Chapters 4 and 5) in *S. enterica*.

The flagellar motor consists of a rotor and a dozen stators. The rotor consists of several rings called the C, M-S, and P-L rings, and a drive shaft. The stator is composed of two transmembrane proteins, commonly referred to as MotA and MotB in the H⁺-driven motor of *E. coli* and *S. enterica* and PomA and PomB in the Na⁺-driven motor of marine *Vibrio*. The stator acts as an ion channel to couple the ion flow through the channel with torque generation by electrostatic interactions of MotA or PomA with a rotor protein FliG. The stator is anchored to the peptidoglycan layer through the C-terminal periplasmic domain of MotB (MotB_C) or PomB (PomB_C). MotB_C and PomB_C coordinate stator assembly around the rotor with ion channel formation, thereby suppressing undesirable ion flow through the channel when the stator is not installed into the motor. Chapters in Part II describe how to isolate the flagella from the bacterial cell bodies (Chapter 6) and how to carry out high-resolution structural and functional analyses of the flagellar motor (Chapters 7, 8, 9, and 11). In silico modeling of the MotAB proton channel complex (Chapter 10) is included in Part II.

Torque is produced by electrostatic interactions of MotA or PomA with a rotor protein FliG. Ion translocation through the ion channel is coupled with cyclic conformational changes of MotA or PomA for torque generation. CheY-P binds to FliM and FliN in the C ring, resulting in switching of flagellar motor rotation from the CCW to CW directions without changing the direction of the ion flow. Direct observations of flagellar motor rotation by nanophotometry with high spatial and temporal resolutions have revealed that the elementary process of torque generation by stator-rotor interactions is symmetric in CCW and CW rotation. Single molecule imaging techniques have shown that both stator and rotor are highly dynamic structures, thereby showing rapid exchanges between localized and freely diffusing forms even during motor rotation. Chapters in Part III describe how to measure flagellar motor rotation over a wide range of external load (Chapters 12, 13, and 14), how to measure ion motive force across the cytoplasmic membrane (Chapters 15), and how to measure the dynamic properties of the flagellar motor proteins by fluorescence microscopy with single molecule precision (Chapters 16 and 17).

Intact flagellar motor structures derived from different bacteria species have been visualized. Most components of the core structure of the basal body and their organization are well conserved among bacteria species. Recently, novel and divergent structures with different symmetries have been observed to surround the conserved core structure in different species. Chapters in Part IV describe the structure and function of Spirochetal (Chapters 18 and 19), *Vibrio* (Chapters 20 and 21), *Rhodobacter* (Chapter 22), *Shewanella* (Chapter 23), Alkaliphilic *Bacillus* (Chapter 24), and *Magnetococcus* flagellar motors (Chapter 25).

All the contributors are leading researchers in the bacterial flagellar field, and we would like to acknowledge them for providing their comprehensive protocols and techniques for this volume. We would like to thank Dr. John Walker, the Editor-in-Chief of the Methods in Molecular Biology series, for giving us a great opportunity to edit this volume and his continuous support and encouragement.

We hope you all have lots of fun with and benefit from this volume of Methods in Molecular Biology.

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The Bacterial Flagellum

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