

Classic Spotlight(s): Putting things in order - matching genes to images of the flagellar motor

In a seminal series of papers in *J. Bacteriol.* in 1971, Julius Adler and others (1,2,3,4) placed the central structure of the bacterial flagellar motor into the context of the bacterial cell envelope, using negatively stained electron micrographs to place the different rings of the flagellar basal body in the inner membrane, the peptidoglycan layer and the outer membrane. However, extensive studies by groups led by Tetsuo Iino and Melvin Simon had shown that the synthesis of the flagella of *Salmonella typhimurium* (as it was then called) and *Escherichia coli* required more than 20 cistrons (genes) (5,6). At that time, only the genes encoding the filament and hook proteins had been identified. The identity of those 18 other genes, and whether the proteins they encoded were structural or regulatory, was unknown, as mutations in any of the individual genes led to a non-flagellated phenotype. In 1978 Suzuki et al (7) extended the approach of using osmotically shocked cells developed by the Adler and Simon groups to painstakingly search electron micrographs of membrane fractions for incomplete flagellar structures. They took 23 mutant strains, identified by lambda phage-mediated complementation, and named them in roughly the order in which they were identified: *flaA*I,II,III,B,C...U, etc. The nomenclature was standardized in 1988 when the workers in the field, then mainly studying *E. coli* and *Salmonella*, agreed to a unified nomenclature. Anyone with an historical bent who wants to know how totally confusing the gene names were and how helpful the agreement to devise a logical and unified nomenclature has been should read the Microbiol. Rev. paper (8).

The visualized structures were then labeled according to whether the images had structures that looked like filaments, hook-basal bodies, basal bodies, MS and P

27 rings, MS and rod, LP-rings with rods, MS and P rings with shorter rods, and so
28 forth. Sorting mutants into 9 structurally distinct categories allowed an assembly
29 pathway to be developed. Fourteen genes were shown to be required to produce the
30 first rod/MS ring structure (RIV). Each of the other gene products could be placed
31 into a logical assembly sequence, resulting finally in a complete flagellum. The
32 assembly sequence demonstrated that the flagellum develops from the inner to the
33 outer membrane. Therefore, proteins would need to be transported to their sites of
34 assembly in the different membranes, the periplasm, and the exterior of the cell.
35 Most obviously, many proteins would first have to be transported through the inner
36 membrane. It is difficult now to appreciate the laborious, manual work that went into
37 isolating and identifying the smallest parts of the motors, many which were at the
38 resolution limits of the microscopes then available. Once the parts were cataloged,
39 they had to be placed into categories and put into a rational order of assembly. This
40 Herculean labor provided the basis for our understanding of the flagellar regulon and
41 its control.

42 Almost 30 years later, the development of cryo-electron microscopy has allowed the
43 averaging of multiple images of unstained, isolated flagellar rotors. A particularly
44 important example is the 2006 J. Bact. paper of Thomas et al. (9), who used hook
45 basal bodies purified by methods adapted from those of Suzuki to obtain 3-D
46 reconstructions of flagellar rotors locked in the clockwise state. By sorting images
47 into groups displaying different symmetry (and also recognizing that different parts of
48 the structure have different symmetry), they obtained stunning images demonstrating
49 varied symmetry for both the MS-ring (24-26 fold) and the C-ring (32-36 fold). As the
50 authors state in the Introduction of their 2006 J. Bacteriol. paper "What is needed are
51 three-dimensional (3D) maps of the rotor into which one can fit atomic structures of

the components as they become known.” (8). Researchers are still fitting their structures into the 3D rotor structure described in this paper. Although there is as yet no complete high-resolution structure of the flagellum, structures are known for several components and researchers are presently engaged in efforts to fit these structures into the 3D rotor structure described in this paper. Although many details remain uncertain, the docking models indicate that the MS ring is linked to the C-ring through FliG, allowing predictions of the sites of interaction with the stator units (stator units are not isolated in any purified motor specimens or visible in cryo EM pictures of the flagella of enteric species.) This was one of the first cryo-EM structures of a large membrane complex and continues to provide a critically important framework to guide current attempts in current attempts to piece together the flagellum, the most complex structure and prodigious machine known to exist in a bacterial cell.

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