

1 **Classic Spotlight: Identifying the core of the flagellar motor**

2 **Judith P Armitage**

3 **Address for correspondence: Department of Biochemistry, University of Oxford,**
4 **South Parks Road, Oxford OX1 3QU, UK**

5 **Email: judith.armitage@bioch.ox.ac.uk**

6 **Tel: (+44) 1865 613293**

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10 When, in the 17th century, Antonie van Leewenhoek looked into a suspension of pepper
11 water he knew the tiny particles were alive because they were actively moving. The
12 description of these “animalcules” is almost certainly the first account of bacteria. However
13 because flagella are generally too thin to be resolved by light microscopy, it took about 300
14 years to understand how they move. The 1950s and 60s saw a flourishing of investigations
15 into the bacterial cell using electron microscopy. Both Dinah Abram (1) and Germaine
16 Cohen-Bazire (2) identified disc-like structures associated with the base of the hook region
17 of the filament in their 1965 and 1966 papers. The significance of these structures was
18 unclear, however, owing to the presence of large amounts of associated membrane and cell
19 wall. This changed in 1971, when Julius Adler and his PhD student Melvin DePamphilis
20 published three back-to-back papers (3,4,5) in Journal of Bacteriology, in an issue that also
21 included a complementary paper from the lab of Melvin Simon (6). These papers established
22 methods for the reliable extraction and concentration of bacterial flagella, allowing detailed
23 analysis of flagella and their commonly associated structures away from contaminating
24 membrane. The key was the use of lysozyme and EDTA to form spheroplasts, followed by
25 Triton X-100 lysis and CsCl gradient centrifugation. In the second paper they showed the
26 *Escherichia coli* hook was a fixed 47 nm long structure, distinct from the flagellar filament
27 and associated with a central feature of the basal body they called the rod. They showed
28 that four ~22-nm-diameter rings are mounted on the rod to form the 27 nm-long basal body,
29 and that the rod is likely hollow as judged by the penetration of stain. Rotational symmetry
30 analysis of isolated rings suggested a symmetry of around 16. In the following paper they
31 placed the rings into a membrane context by examining protoplasts with intact outer
32 membranes (confirmed by phage attachment). They were able to locate the L- and P-rings in
33 the outer membrane and peptidoglycan layer respectively, and features termed the M- and
34 S-rings (since found to be a single structure) were within, and just above, the cytoplasmic
35 membrane. Extension of the work to look at the Gram-positive *Bacillus subtilis* suggested a

similar overall rod-and-ring architecture, but without the L or P rings. These core features revealed in the DePamphilis & Adler micrographs are discernible today in the diagrams found in most bacteriology textbooks.

This set of back to back papers presented the first clear model of the organization of the regions of the bacterial flagellum relative to the cell wall and this structural analysis played a pivotal role in the subsequent work to develop models of the rotary motor critical to our current understanding of bacterial swimming and behaviour.

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