

The mechanisms of self-assembly and polymorphic switching of the bacterial flagellar filament

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Introduction

Bacterial flagellum is a helical filament by means of which bacteria swim. Each filament is rotated by the motor at its base, working as a screw that propels the cell, but it is not simply a rigid propeller. In wild-type strains of *Salmonella* and *Escherichia coli*, the filament is normally in a left-handed supercoiled form and several of them form a bundle behind the cell when bacteria swim. But, the filament switches its supercoiled form into a right-handed one upon quick reversal of the motor rotation. This makes the filament bundle fall apart quickly and smoothly, enabling the cell to tumble for a fraction of a second. Alternative repeat of the straight swimming and tumbling plays an essential role in the tactic behavior of bacteria.

The filament is a tubular structure formed by helical assembly of single protein, flagellin. The supercoiling of the filament is thought to involve regular arrays of two distinct subunit conformations and/or packing [1,2] in a filament structure, whose mechanism is interesting in terms of conformational distinctness and adaptability of flagellin. To understand the mechanisms of self-assembly and polymorphism of the filament, X-ray fibre diffraction and electron cryomicroscopy (EM) have been used to analyze the structures of various straight filaments. This report describes how these two methods have been combined in a complementary way to deduce the structures of the filaments, and the molecular mechanism of polymorphic supercoiling based on the deduced structure.

Materials and Methods

Two types of the straight filaments, which have distinct helical symmetries called L and R-types, were isolated from two mutant strains of *Salmonella typhimurium*. Electron cryomicrographs of frozen hydrated filaments were collected by using an electron microscope equipped with a field emission electron source and a specimen stage that can be cooled down to 1.5 K with liquid helium (JEOL JEM3000SFF). Helical image analysis was carried out for selected images of the filaments that show sharp layer lines in their Fourier transforms. After correcting for the contrast transfer function, 15 to 20 filament images were aligned to one another and averaged to produce a three dimensional density map [3]. For X-ray fibre diffraction, we developed a new method to orient liquid crystalline sols of filamentous assemblies of macromolecules [4]. The method involves sequential steps of liquid crystallization, slow centrifugation, and magnetic orientation. The flagellar filaments were well aligned with their angular distribution of 0.6 degree. X-ray fibre diffraction patterns from such well oriented sols allowed us to measure the layer-line spacings, helical symmetries, and layer-line amplitudes very accurately. These amplitude data were combined with phases from the EM analysis and the phases were refined by the solvent flattening procedure to obtain an electron density map [5].

Results and Discussion

In the density maps obtained by cryoEM analyses, the L and R-type filaments were found to have very similar structure as expected from direct comparison of their X-ray diffraction patterns. Both filaments have a densely packed core region out to a radius of 60 Å with a central channel 30 Å in diameter. The outer parts of the subunits are well separated from each other, and there are two domains of vertical and horizontal extension in each subunit. The outer radii of the filaments are 115 Å. The core region is formed by a concentric double-tubular structure (the inner and outer tubes) connected by radial spoke-like features. The structures of two straight filaments, the L- and R-type, which are thought to represent two states of the flagellin subunit that coexist in supercoiled filament structures, show only a small difference in the subunit packing and orientation, and

no appreciable differences in the overall subunit shapes were observed. These indicate that the structural changes involved in polymorphism are very small. The helical symmetries and repeat distances of the two types of the filaments were accurately determined by X-ray fibre diffraction. The intersubunit distances along the 11-stranded protofilaments were calculated from these structural parameters. They are 52.7 Å and 51.9 Å for the L- and R-type filament, respectively. The L-type is longer than the R-type by 0.8 Å, which quantitatively explains observed curvatures of various supercoils based on a two-state subunit model.

The electron density map at 9 Å resolution obtained for the R-type straight filament shows detailed molecular features of the flagellin subunit within the core of the filament (Figure 1). Many rod-like densities are observed in the inner and outer tubes and are aligned almost parallel to the filament axis with lateral distances of about 10 Å. These densities most likely represent α -helices that are predicted in the amino acid sequence of both terminal regions. The structures of straight filaments reconstituted from flagellin fragments that were produced by removing 30 to 40 residues from both termini clearly show removal of the inner tube portion but almost

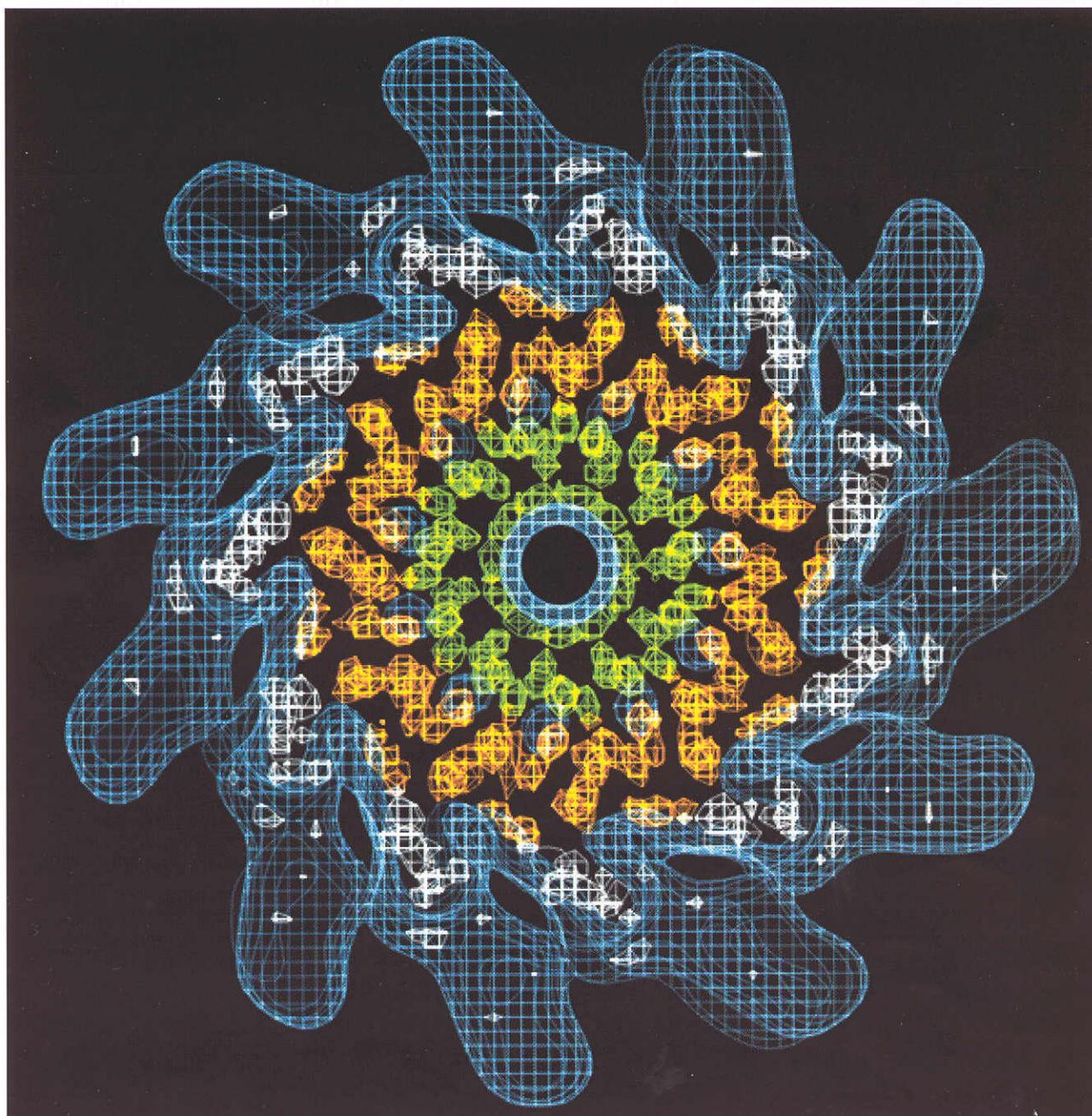


Figure 1: Electron density map of the R-type flagellar filament at 9 Å resolution. A cross section of a thickness of 50 Å is viewed down the axis. The smooth contours coloured white/blue represent the molecular envelope of the filament, and the detailed contours in green and brown colours are for the inner and outer tubes, respectively.

intact local subunit packing in the outer tube. This indicates that the intersubunit interactions in the outer tube are mainly responsible for filament formation and structural switching. The inner tube structure appears to be the base structure against which the subunit interactions in the outer-tube switch from one state to the other.

About 65 NH₂-terminal and 45 COOH-terminal residues are known to be in a flexible and disordered conformation in the monomeric form of flagellin in solution. Under physiological conditions, flagellin monomers alone do not form the filament; the structure of the distal end of the filament is required as a template on to which monomers assemble either in vivo or in vitro. Together with the location of the terminal regions in the filament structure, these can be interpreted such that the disordered terminal

regions play essential roles in regulating the self-assembly process, preventing spontaneous filament formation in the absence of the distal end structure of the filament.

The observed difference of the local subunit lattice between the L and R-type and the axially aligned α -helices in the outer tube region indicates that the switching consists of two distinct mutual sliding movements of the subunits at the 11-stranded joints of the protofilaments: one of them, between subunits neighboring along the 6-start helix, is a sliding of 2.6 Å; the other, between subunits neighboring along the -5-start helix, is a sliding of 1.8 Å. These movements result in a shortening of the intersubunit distance along the strand joints by 0.8 Å upon switching from the L to the R-type lattice (Figure 2).

Switching of the outer-tube domain interactions

The 0-11 distance: L-type, 52.7 Å; R-type, 51.9 Å
 The red-blue distance: A_R-A_L , 1.8 Å; B_R-B_L , 2.6 Å

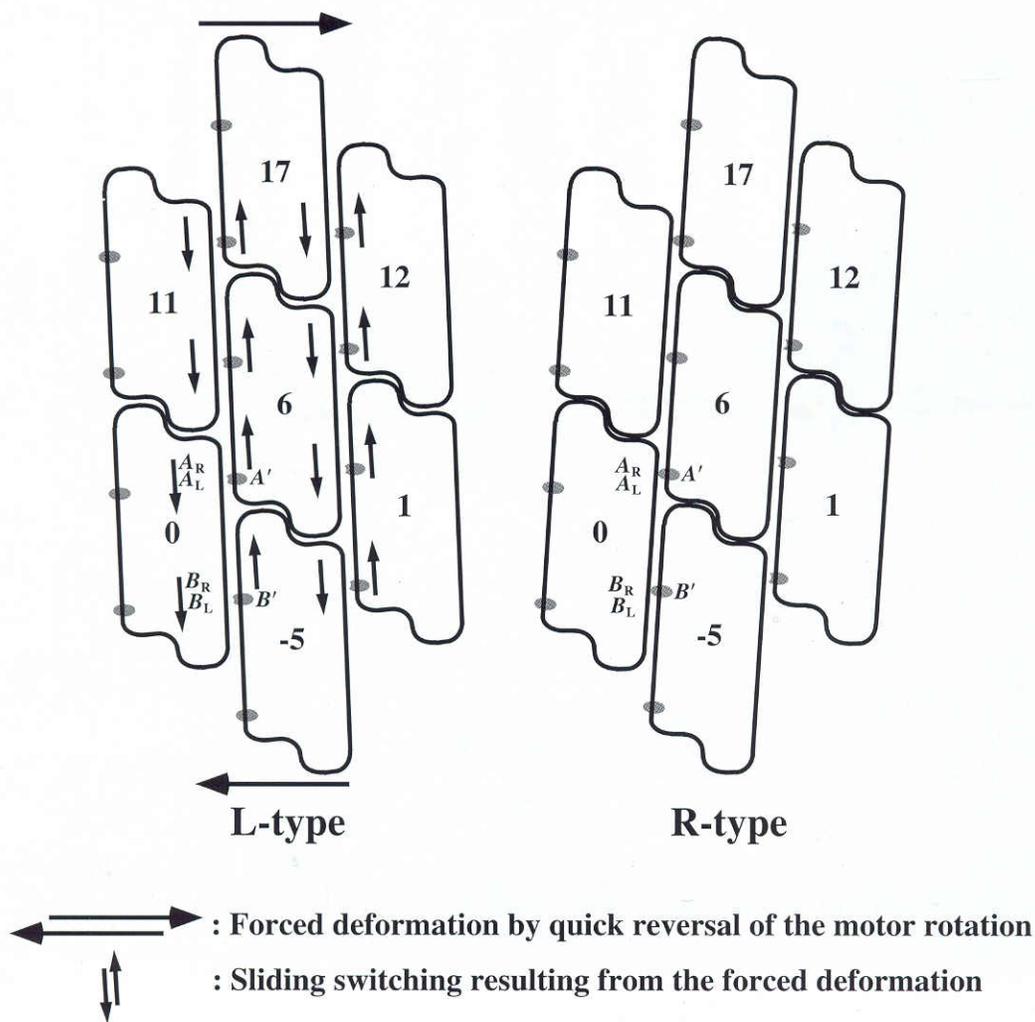


Figure 2: Model of switching at the strand joints explaining how the L- and R-type lattices are formed by the two distinct modes of subunit interactions.

A mutual coaxial disposition of the inner- and outer-tube lattices restrains the strand joint lattice to be twisted against the filament axis by a specific angle, resulting in a mismatch in the subunit interactions at a strand joint closing the tubular structure. Closure of the tube then requires a certain mixture of the two types of lattice, which in turn limits the curvature and twist of the filament to specific values associated with a specific type of supercoil. Thus, 10 distinct types of supercoil can be predicted using the lattice parameters of the L- and R-type straight filaments, and these predicted supercoils have curvatures and twists in good agreement with those of actual supercoiled filaments observed by dark field microscopy (Figure 3).

The twisting force produced at the base of the filament by quick reversal of the high speed motor rotation is converted by the strand lattice of the filament into a shear force at strand joints, and the shear force turns some of the L-state strand joints into the R-state, which results in a macroscopic

switching of the supercoiled filament from a left handed one called normal to a right handed one called curly. This switching in the helical handedness makes the bundle of filaments fall apart and this is how the reversal of the motor rotation makes the cell tumble.

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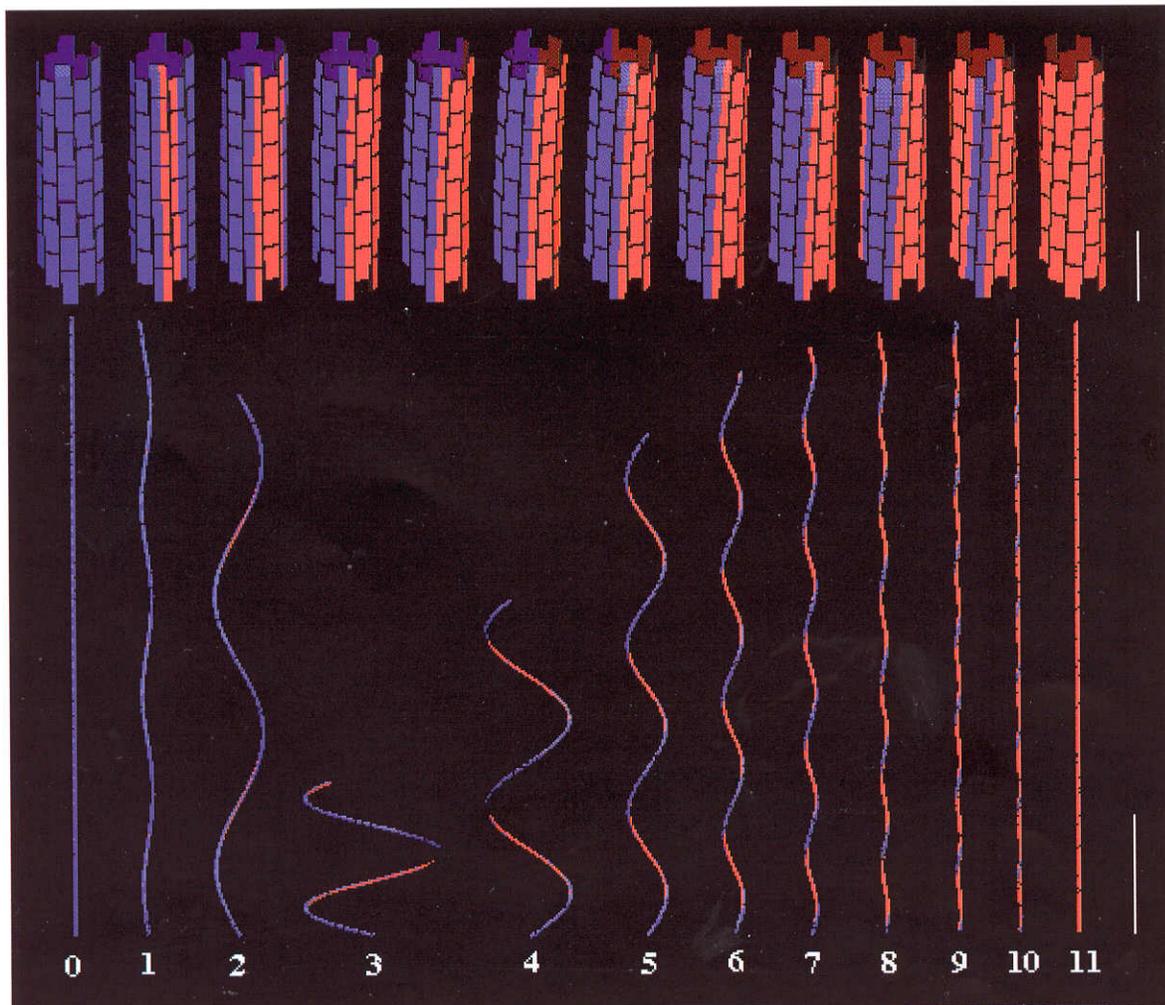


Figure 3: Supercoils predicted from the lattice parameters of the L- and R-type filaments obtained by X-ray fibre diffraction. The upper panel shows subunit lattices of a short filament segment and the lower panel shows the overall morphology of supercoils. The L- and R-type strand joints are coloured blue and red, respectively. The numbers at the bottom indicate the number of the R-type strand joints.