JOURNAL OF BACTERIOLOGY, Apr. 2005, p. 2890–2902 Vol. 187, No. 8
0021-9193/05/$08.00+0 doi:10.1128/JB.187.8.2890–2902.2005

Crystal Structure of the Flagellar Rotor Protein FliN from *Thermotoga maritima*†

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Received 14 October 2004/Accepted 4 January 2005

FliN is a component of the bacterial flagellum that is present at levels of more than 100 copies and forms the bulk of the C ring, a drum-shaped structure at the inner end of the basal body. FliN interacts with FliG and FliM to form the rotor-mounted switch complex that controls clockwise-counterclockwise switching of the motor. In addition to its functions in motor rotation and switching, FliN is thought to have a role in the export of proteins that form the exterior structures of the flagellum (the rod, hook, and filament). Here, we describe the crystal structure of most of the FliN protein of *Thermotoga maritima*. FliN is a tightly intertwined dimer composed mostly of β sheet. Several well-conserved hydrophobic residues form a nonpolar patch on the surface of the molecule. A mutation in the hydrophobic patch affected both flagellar assembly and switching, showing that this surface feature is important for FliN function. The association state of FliN in solution was studied by analytical ultracentrifugation, which provided clues to the higher-level organization of the protein. *T. maritima* FliN is primarily a dimer in solution, and *T. maritima* FliN and FliM together form a stable $FliM_1$-$FliN_4$ complex. *Escherichia coli* FliN forms a stable tetramer in solution. The arrangement of FliN subunits in the tetramer was modeled by reference to the crystal structure of tetrameric $HrcQB_C$, a related protein that functions in virulence factor secretion in *Pseudomonas syringae*. The modeled tetramer is elongated, with approximate dimensions of 110 by 40 by 35Å, and it has a large hydrophobic cleft formed from the hydrophobic patches on the dimers. On the basis of the present data and available electron microscopic images, we propose a model for the organization of FliN subunits in the C ring.

The bacterial flagellum is a complex assembly formed from about two dozen proteins having copy numbers ranging from a few to more than 10,000 (1, 6, 35, 41, 42). The FliN protein is a major component of the C ring, a drum-shaped structure at the bottom of the basal body that is approximately 45 nm in diameter and 15 nm high (19, 31, 68, 79, 81) (Fig. 1). FliN interacts with FliG and FliM to form the switch complex, which is essential for assembly of the flagellum and also for rotation and clockwise (CW)-counterclockwise (CCW) switching (27, 40, 59, 67, 71, 77, 78). The switch complex attaches to the MS ring, which is in the cytoplasmic membrane and is formed from a single protein, FliF (24, 72). The precise locations of FliG, FliM, and FliN in the switch complex are unknown, but FliG and FliM are likely to be near the upper (membrane-proximal) part of the C ring, because FliG binds to FliF (18, 21, 33, 68) and also makes multiple contacts with FliM (9, 44-46, 70). The C-terminal domain of FliM is independently stable and forms the binding site for FliN (46, 70).

The sequence of events in flagellar assembly has been deduced from studies of mutants arrested at various steps (28, 37, 42, 62, 63). The MS ring is formed first (Fig. 1). The proteins that form the rod, hook, and filament are actively transported to their destinations by way of a central channel through the structure (41, 51). Export is driven by an apparatus at the base of the flagellum, which is evolutionarily related to the type III secretion system used by pathogenic bacteria for the export of virulence factors (8, 36, 41). This export apparatus is centered within the MS ring and is formed from six membrane proteins and at least three cytosolic proteins (Fig. 1) (42, 47). It assembles at about the same time as the switch complex, but the sequence of events is not known exactly. The proteins to be exported arrive in a partially folded state in association with specialized chaperones (2, 4, 5, 20). Energy for transport comes from the hydrolysis of ATP, which is catalyzed by the protein FliI (14, 17) and is regulated by the protein FliH (48). Although the C ring appears to be well separated from the membrane-bound parts of the export apparatus (30), FliN has been implicated in flagellar export. Proteins with sequence similarity to FliN are found in the virulence factor export apparatus of various species, where they presumably carry out functions related to export rather than motility (42, 65). A temperature-sensitive FliN mutant was unable to regrow flagellar filaments after shearing at the restrictive temperature, indicating that there was a failure in the export of flagellin or the flagellar cap protein (75).

Other mutations in FliN have a range of effects that depend on the expression level of the protein. Certain *fliN* mutations prevent rotation while allowing flagellar assembly when the protein is expressed at normal levels (27) but allow both assembly and rotation when the protein is overexpressed (40). When wild-type FliN is underexpressed, flagella are still as-

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† Supplemental material for this article may be found at http://jb.asm.org/.

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