Cooperative remodeling of the FlhA ring structure coordinates flagellar type III protein export with assembly

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The bacterial flagellum is a supramolecular motility machine consisting of basal body rings and a tubular axial structure. For construction of the axial structure beyond the cell membranes, flagellar axial proteins are translocated across the cytoplasmic membrane by a type III export apparatus at the flagellar base, diffuse down a 2 nm central channel inside the nascent structure and assemble at the distal end with the help of a capping structure. The flagellar type III export apparatus consists of a transmembrane export gate complex and a cytoplasmic ATPase ring complex and acts as a proton/protein antiporter to couple an inward-directed proton flow through the export gate with an outward-directed protein translocation. Since the export apparatus coordinates flagellar protein export with assembly in a highly organized and well-controlled manner, the protein export process involves a substantial number of checkpoints to ensure the correct order of export.

A transmembrane export gate protein FlhA is composed of an N-terminal transmembrane domain with eight transmembrane helices ($FlhA_{TM}$) and a relatively large C-terminal cytoplasmic domain ($FlhA_C$). FlhA forms a nonameric ring structure through interactions between $FlhA_C$ domains. The $FlhA_C$ ring structure is a docking platform for the ATPase complex, flagellar type III export chaperones and export substrates and plays an important role in the energy coupling mechanism of flagellar protein export. It has been reported that the $FlhA_C$ platform coordinates flagellar protein export with assembly. However, it remains unknown how. Here, we report direct observation of $FlhA_C$ ring formation on mica by high speed atomic force microscopy. $FlhA_C$ formed a homo-nonamer with the diameter of 10.1 ± 0.02 nm. The probability of $FlhA_C$ ring formation on mica was increased considerably with an increment in its protein concentrations, indicating that $FlhA_C$ forms the ring structure in a positively cooperative manner. Glu-351, Trp-354 and Asp-356 in a flexible linker ($FlhA_L$) connecting $FlhA_C$ to $FlhA_{TM}$ were critical for efficient $FlhA_C$ ring formation. The E351A/D356A and W354A mutations considerably affected filament assembly but not hook-basal body assembly. Therefore, we propose that cooperative remodeling of the $FlhA_C$ ring structure coordinates flagellar protein export with assembly by ordered export of flagellar axial proteins to parallel with their order of assembly.