Dissecting the mechanism of flagellar type-III secretion

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Several membrane proteins form the core of the type-III secretion apparatus in both the flagellum and injectisome. We have sought to define the specific functions of some of these proteins using a variety of mutational and biochemical approaches. Our results indicate that FlhA contains a set of conserved, functionally critical residues that likely form the site of energy transduction where the proton gradient is harnessed to drive transport. Among these is a functionally critical acidic residue, Asp158, that is the best candidate for a site of direct proton action. A mutation that mimics protonation of Asp158 (D158N) actuates a global conformational change extending to the other, cargo-engaging parts of the protein.

Biochemical labelling experiments and a number of other results indicate that FliP forms the cargo-conducting pore at the center of the apparatus. Transmembrane segments 3 and 4 of FliP appear likely to form the lining of the pore. A methionine-rich segment in the cytoplasm, between TM3 and TM4, is important for regulating the conductance of the pore. A mutation in this region increases the ability of FliP to conduct certain small molecules while sensitizing the cell to a number of toxic agents. An hypothesis for the transport mechanism incorporating these findings will be discussed. Type-III secretion is known to utilize both the proton gradient and ATP; a further means by which export might be energized will be discussed.