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BACTERIAL PHYSIOLOGY

Vibrio uptake apparatus

Natural transformation (also known as competence) in bacteria involves the uptake of DNA from the environment and its integration into the recipient genome, and is thus an important mode of horizontal gene transfer. Although the regulatory networks involved are likely to differ in the wide range of Gram-negative and Gram-positive species that are naturally transformable, it is thought that the basic mechanism of DNA uptake is conserved. This mechanism involves a competence-associated type IV pilus (Tfp), a DNA receptor protein (ComEA), a transmembrane channel (ComEC) and numerous associated proteins. Writing in *Proceedings of the National Academy of Sciences USA*, Patrick Seitz and Melanie Blokesch now report the first visualization and detailed characterization of the DNA-uptake apparatus that is involved in natural transformation in *Vibrio cholerae*.

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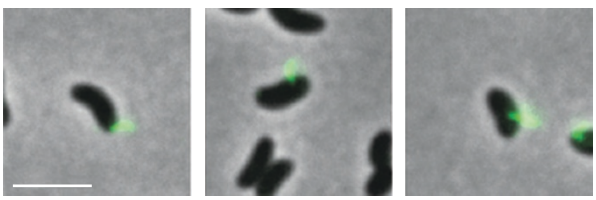

The major pilin, PilA, was visualized by immunofluorescence microscopy of competence-induced *Vibrio cholerae* cells. The pili (which were mostly present as a single copy per cell) did not necessarily emanate from the polar region. The images shown are merged images from the fluorescence and the phase contrast channels. Scale bar represents 2 μm. Images courtesy of M. Blokesch, Swiss Federal Institute of Technology in Lausanne, Switzerland.

To define the minimum competence regulon in *V. cholerae*, the authors identified genes that were reproducibly upregulated when competence was induced and then assessed the conservation of this gene set in other naturally transformable species, including other *Vibrio* species. This strategy identified 19 upregulated genes, including genes that encode components of a putative Tfp, as well as *comEA* and *comEC* genes. To determine their involvement in natural transformation, each gene was individually deleted from a wild-type strain of *V. cholerae*. The transformability of strains that lacked Tfp-related genes was impaired and, in each case, the defect could be complemented by a plasmid that expressed the deleted gene. In addition, transferred DNA could not be detected in the periplasm of strains that lacked putative components of the Tfp, which indicates that there was no DNA transport across the outer membrane.

These data suggest that the minimum regulon encodes components of the Tfp and that the Tfp could be involved in the transport of DNA across the outer membrane. To visualize the Tfp, the authors replaced the gene that encodes the putative major pilin gene, *pilA*, with a *Strep*-tag II-tagged version and, using immunofluorescence microscopy, observed competence-induced pili of various lengths. In contrast to *Bacillus subtilis*, in which the DNA-uptake machinery is confined to the cell poles, the competence pili in *V. cholerae* were observed at several cellular locations and were mostly present as one pilus per cell. The authors also examined the localization of other competence proteins using translational fusion constructs and found that PilQ (which is the putative outer-membrane secretin) colocalizes with the pilus in the majority of piliated cells, and that PilB (which is the putative ATPase involved in pilus elongation) is required for Tfp formation in *V. cholerae*.

The authors combine these new data with previous data on the regulatory network to propose a detailed model for the mechanism of DNA uptake by *V. cholerae* during transformation. They propose that uptake involves at least two main steps: first, Tfp-dependent translocation across the outer membrane, and then Tfp-independent shuttling of DNA through the periplasm and across the inner membrane.

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