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## Genetic analysis of bacterial motility

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## Abstract

The genes involved in the assembly and function of the polar flagellum of *Vibrio parahaemolyticus* have been analyzed systematically by mutagenesis, DNA sequencing and transcriptional studies.

## Significance and context

Many motile bacteria are propelled by the rotation of flagella. Mechanisms of motility and chemotaxis have been investigated in a variety of bacteria, but large-scale analyses of flagellar systems involving systematic genetic and phenotypic characterization of mutants, DNA sequencing and transcriptional studies are rare. The paradigms are the peritrichously flagellated bacteria *Escherichia coli* and *Salmonella typhimurium*, where a hierarchy of regulation has been elucidated for flagellar synthesis, and details of flagellar structure and function are known. In *E. coli* there are three classes of flagellar genes. Class I are the master control genes, probably linked to cell-cycle control. Class I genes turn on class II genes, which have a class-specific promoter type, and all these genes must be transcribed and the flagellum partially assembled before class III genes can be turned on. Class III genes have a different type of promoter from Class II. The bacterium *Caulobacter crescentus* has a single polar flagellum, synthesis of which has also been extensively studied. In this case, a clear link between cell division and flagellation was revealed.

*Vibrio parahaemolyticus* is distinct from other flagellated bacteria in that it possesses two flagellar systems: a polar system, expressed continuously, for movement in liquid environments and a lateral (peritrichous) system, expressed when the bacterium is on a solid surface or in a viscous environment. Genetic analysis has shown the two systems to be distinct - no structural components or components required for its assembly are shared - and in certain conditions two distinct flagellar organelles can be assembled. The different flagella are even driven by different energy sources - the proton-motive force (as is used in *E. coli*) for the lateral flagella and the sodium-motive force for the polar flagellum. The polar flagellum also acts as a mechanosensor: conditions inhibiting its rotation induce lateral flagella synthesis.

## Key results

A previously mutated strain of *V. parahaemolyticus* which does not synthesize lateral flagella, but which can still synthesize polar flagella, was mutagenized. Bacteria impaired in polar flagellar synthesis and/or function were selected by the lack of movement on semi-solid agar. Forty such mutants were selected and analyzed phenotypically. Genetic analyses revealed 60 genes potentially involved in the polar flagellar gene system, organized into five regions on the chromosome, in two very large gene clusters and three smaller ones. It is probable that in the larger clusters the genes are arranged in big operons. Transcriptional analysis revealed hierarchical organization similar to that seen in *E. coli*, with at least two distinct types of promoter associated with the gene clusters. The majority of the genes were homologs of previously sequenced genes in other flagellar systems. For example, the authors suggest that two genes, which are also found in other polar flagellated bacteria (but not *E. coli*), might act together to determine the selection of the flagellum site - at the pole. There were, however, three novel genes. The protein encoded by one of these had some homology to a protein involved in cell-cycle control. Theoretically, this could be a link between flagellar synthesis and cell division, as is seen in *C. crescentus*. Surprisingly, six copies of the flagellin genes, which encode the structural subunit of the flagella, were found. They are highly homologous to each other but not to the single copy of the lateral flagellin gene. Interestingly, some of the mutants created in the work were 'leaky', and occasionally, in some cells in a population, some motility was restored. The authors postulate that components of the lateral flagellar system can infrequently complement their polar flagellar equivalents, albeit ineffectively.

## Reporter's comments

This paper describes a large, detailed study which will be of great use to researchers of bacterial motility. Moreover, many strains of *Vibrio* and *Pseudomonas*, which also has a polar flagellar system, are pathogenic, with the flagella being important virulence factors. This study also paves the way for a complementary analysis of the lateral flagella system, allowing a direct comparison between the polar and lateral systems to determine whether any components are shared. The basic structure and mechanism of flagellar assembly are likely to be very similar. The major differences between the systems could be in the regulation of flagellar synthesis, interactions between the different motors and how the structure has been adapted to the different environmental conditions in which each system is functional. For example, why does the polar flagellum need six flagellin monomers, when the lateral flagellum has only one?

## Table of links

*Journal of Bacteriology*

## References

1. Kim Y-K, McCarter LL: Analysis of the polar flagellar gene system of *Vibrio parahaemolyticus*. J Bacteriol. 2000, 182: 3693-3784. 0021-9193