Abstract

The archaeal flagella is a unique motility structure. Although it is functionally related to the bacterial flagellum, it is quite different structurally. Bacterial flagella have been well characterized at both the genetic and structural levels, but similar studies on the archaeal equivalent have lagged behind. In flagellated archaea, annotated flagellar genes fall within a single, co-transcribed operon (except for one encoding a preflagellin peptide FlaB). This operon contains the genes for the flagellar proteins (two or more genes designated as fla or flaJ), various other conserved proteins of unknown function (flaA, flaD, flaE, flaF, flaG and flaH), an ATPase (flaI) and a membrane protein (flaK). The requirement and potential of genes within the operon was investigated by deletion studies in the methanogen *Methanococcus maripaludis*. In-frame deletions were created in flagellar genes flaB1, flaD and flaE, as well as in a majority of the other genes within the operon. The operon of flaI was characterized for its ability to assemble flagella and achieve motility. With the exception of flaD, all mutants appeared non-motile by light microscopy and non-flagellated by electron microscopy. Further characterization of these mutants is ongoing to determine if any hook or anchoring structures are affected. At this time we are aware of the anchoring structure at the base of the flagellum, it is presumed that this is where many of the unknown genes play a role. The results to date represent the first experimental evidence that FlaC, FlaF, FlaG and FlaH are directly involved and critical for archaeal flagellar assembly.

Archaeal Flagella

Although similar in function to the bacterial flagellum, it has been well-established that the archaeal flagellum is a unique motility structure:

- archaeal flagellar filaments are generally thinner than bacterial flagella, with a central channel too small to allow flagellin subunits to pass through.
- archaeal flagellar filaments are composed of several different flagellin monomers, which are post-translationally modified in 2 ways:
  - removal of a short, signal peptide portion.
  - common, if not universally, post-translational modification with an N-linked glycan.
- the bulk of genes currently proven or suspected to be involved in archaeal flagellar function can be found on the chromosome of flagellated archaea. These genes include:
  - multiple flagellar genes (flaA-
  - a conserved protein of unknown function (flaM)
  - a nucleotidase (flaB)
  - a membrane protein (flaK)
  - a collection of proposed flagellar accessory genes (flaG-
- all of the mutants generated to date (flaB1, flaD, flaE, flaF, flaG, flaH, flaJ, flaK) have never been experimentally demonstrated to be involved in flagellation and, other than the major flagellar functions, the fla genes remain unknown.

In-frame Deletion Methodology

Recently, a method has been established to generate markerless, in-frame deletions in the methanogen *Methanococcus maripaludis* (1), based on a similar system in *Methanotherma acetivorans* (2). This method is based on a strain of *Methanococcus maripaludis* with a deletion of the type IV pilus operon (hypoxanthine phosphoribosyltransferase) gene designated Mm900. Because of this deletion, Mm900 is resistant to 8-azaguanine, while wild type *M. maripaludis* (S2 strain) is killed by this toxic agent. To generate a deletion:

- a 1 kb region upstream and downstream of the target gene are ligated together to maintain the reading frame of the operon, and then cloned into the vector pCP76Plasmid.
- the vector is introduced into Mm900 and excision (via homologous recombination) is used to select for a first recombination event.
- cells are then transferred into 8-azaguanine, selecting for a second recombination event that has removed the vector containing the target gene.
- the second recombination can either occur on the same side of the deletion as the first event or returning the chromosome to its wild type sequence or on the opposite side of the first event, creating a deletion.

- isolated colonies are then screened by PCR and deletions are confirmed by sequencing and Southern blot analysis.

flaB3 Flagella

To establish what effect the loss of flaB3 had on the flagella, whole flagella were detergent-extracted from wild type and flaB3, flaD, flaE mutant cells. The loss of flaB3 resulted in flagella with no apparent hook region (compare regions indicated by arrow). This added evidence additional to the current hypothesis that the FlaB3 protein makes up the hook region of the flagella.

Future work

- finish the operon deletion set by making deletions of flaD and flaE
- complement all mutants with the appropriate gene to ensure effects observed were due to the deleted gene alone.
- continue investigating the flagella anchoring structure and what effects, if any, the fla mutants have on its assembly and structure.

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References