

P067 Identification of the glycosyltransferase genes involved in *N*-linked glycosylation of the flagellin proteins in the archaeon *Methanococcus maripaludis*

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N-linked protein glycosylation involves the attachment of glycan to an Asn residue within the sequon Asn-Xaa-Ser/Thr. This process is found in all domains of life and requires glycosyltransferases to assemble individual activated sugars into the glycan, which is initially attached to a lipid carrier, prior to its transfer to protein by an oligosaccharyltransferase. Identification of genes encoding glycosyltransferases has been known for some time in eukaryotes and bacteria, while information in archaea is still fragmentary. *Methanococcus maripaludis* possesses flagellin proteins that contain only *N*-linked glycan, providing an excellent model for study. Using in-frame deletions, four glycosyltransferases involved in glycan assembly in *M. maripaludis* were identified. Immunoblotting with anti-flagellin antisera revealed that deletion of each glycosyltransferase resulted in a decrease in flagellin molecular weight due to the absence of one or more sugars in the attached glycan. Order of action for each glycosyltransferase was deduced since the molecular weight of the flagellins in the mutants decreased in a stepwise fashion compared to wild type flagellins. Complementation of the deleted gene returned flagellin molecular weight to, or near, wild type levels, depending on the glycosyltransferase. Electron microscopy showed that attachment of a minimum two-sugar glycan to the flagellins is required for their proper assembly into filaments although mini-swarm plate analysis revealed impaired motility in all mutants, even in those that were able to assemble flagella.