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Epitope tagging of a virulence protein in *Haemophilus influenzae*

Haemophilus influenzae, a small, gram negative bacterium, is a commensal organism of the human upper respiratory tract. Before the introduction of the Hib vaccine, encapsulated *H. influenzae* of serotype b was a major cause of childhood meningitis. Nonencapsulated *H. influenzae*, an important cause of respiratory illnesses, rarely causes invasive disease. However, unusually virulent NTHI strains have occasionally been isolated and raise the concern of vaccine-resistant meningitis. We are studying one such NTHI, strain R2866, isolated from a meningitis patient. R2866 and other unusually virulent NTHI contain a novel, phase variable gene termed *lav*, which is predicted to encode a member of the AIDA-I/VirG/PerT family of virulence-associated autotransporters. Autotransporters are outer membrane proteins with three domains, passenger, linker and beta-barrel. The "passenger" gives the protein its function, while the beta-barrel imbeds into the outer membrane and forms a pore through which the passenger domain exits from the cell. To understand the function and location of the *Lav* protein, we engineered an epitope tag within the carboxy terminal beta-domain. Structure of the beta-domain was predicted by homology to the *E. coli* AIDA-1 protein. We placed a FLAG tag at an external loop near the C-terminus. A tagged construct was used to replace the chromosomal *lav* gene by natural transformation of R2866. We intend using the FLAG-tagged R2866 to ask whether *Lav* is cleaved, to determine translational phase variation of *lav*, and to investigate whether *Lav* plays a role in adhesion to mammalian tissue.