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Expression of a putative virulence protein of *Haemophilus influenzae* in *E. coli*

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Haemophilus influenzae, a small, gram-negative bacterium, is a commensal organism of the human upper respiratory system as well as an agent of diseases ranging from otitis media to meningitis. Nonencapsulated (nontypeable, or NTHi) strains of *H. influenzae* are generally associated with respiratory infections but not invasive disease (meningitis, septicemia). However, rare exceptions of virulent NTHi have been isolated from patients with invasive disease; these strains are of concern because they are resistant to the Hib vaccine, which is directed against encapsulated strains of serotype b. Virulent NTHi have been isolated from children with meningitis. A conjunctivitis-associated NTHi was also responsible for a lethal outbreak of septicemia (Brazilian purpuric fever) during the 1980's. We are studying one such virulent NTHi (R2866) which has a novel gene (*lav*) encoding a member of a widespread family of virulence-associated proteins. *Lav* is an autotransporter, an outer membrane protein which exports its effector amino-terminal domain (the "passenger") through a pore formed by its carboxyl-terminal beta-barrel domain. The expression of *lav* is translationally phase-variable, with ON or OFF phase determined by the number of GCAA repeats that follow the initiating methionine codon.

The function of an autotransporter is determined by its passenger domain. Passenger domains may function as toxins, adhesins, IgA proteases, or invasins. Except for adhesins, most of these functions require proteolytic cleavage and release of the passenger from the bacterial surface. Recent evidence from our work indicates that the *Lav* passenger is not cleaved but remains covalently bound at the surface. We are investigating the function of *Lav*, and testing the hypothesis that *Lav* is an adhesin. As a first step in investigating function, we have cloned *lav* into a low-copy number *E. coli* plasmid (pet29) and succeeded at expressing it in *E. coli*.