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## **Investigation of the Influence of Phase Variable Restriction-Modification Systems on Resistance of *Haemophilus influenzae* to Infection by Bacteriophage**

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*H. influenzae* is a commensal of the human respiratory tract with the potential to cause diseases such as otitis media and meningitis. Many surface structures of this species are subject to stochastic, reversible ON and OFF switches in gene expression (Moxon *et al.* 2007; Bayliss, 2009). This process, termed phase variation, is mediated by mutations or changes in the length of tandem DNA repeat tracts (also termed microsatellites). These changes in repeat number cause switches in gene expression by altering the reading frame or the relative positions of promoter elements. Intriguingly two of the restriction-modification (R-M) systems of *H. influenzae* strain Rd are subject to phase variation. One is a type I R-M system called HindI and consists of three proteins:- HsdM, HsdS and HsdR. Changes in a pentanucleotide repeat located within the reading frame of the *hsdM* gene cause ON/OFF switches in expression of restriction and methylation activity (Zaleski *et al.*, 2005). Phase variation of this system alters susceptibility to infection by HP1, an *H. influenzae* specific bacteriophage. The other system is a type III R-M system and has a tetranucleotide repeat tract within the Mod gene (De Bolle *et al.*, 2000). Phase variation of this latter system alters expression of several other genes in the genome suggesting the phase variable R-M systems are a stochastic mechanism for adaptation to environmental challenge (Srikhanta *et al.* 2005). An alternate view is that phase variable R-M systems are a population-based mechanism for preventing the development of resistance to restriction among bacteriophages (Bayliss *et al.*, 2006).

The importance of bacteriophages is being increasingly realized and they have been shown to play key roles in bacterial evolution (Clokie *et al.*, 2011). Establishing a genetic basis for these roles can often be challenging but this well characterised system gives us an ideal opportunity to decipher how phages may control bacterial population dynamics.

Switching OFF of *HindI* renders *H. influenzae* strain Rd susceptibility to infection by HP1. However replication of the bacteriophage in an ON phase variant of *HindI* results in methylation of the phage genome and insensitivity to restriction by this RM system (Zaleski *et al.*, 2005). We speculate that phase variation of *HindI* prevents development of phage resistance and reduces spread of the phage through *H. influenzae* populations. Resistance to phage infection is also mediated by phase variation of the *lic2A* gene, which encodes a glycosyltransferase involved in biosynthesis of the receptor for this phage (Zaleski *et al.*, 2005). The specific aims of this project are:- (i) to investigate the diversity and phase variation rates of the R-M systems present in different *H. influenzae* strains; (ii) to develop a protocol for investigating the spread of bacteriophage through a bacterial population; (iii) to investigate whether alterations in phase variation rate of R-M systems change the susceptibility of bacterial populations to spread of bacteriophage; (iv) to investigate whether the presence of multiple phase variable R-M systems increases resistance to bacteriophage spread; (vi) development of computer models for predicting the spread of phage through a phase variable bacterial population.

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