Studies of the Two-Component Signal Transduction System RR/HK06 in *Streptococcus pneumoniae*

THE UNIVERSITY
OF ADELAIDE
AUSTRALIA

Alistair James Standish, B Biotech (Hons)

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Discipline of Microbiology and Immunology
School of Molecular and Biomedical Sciences
The University of Adelaide
Adelaide, S.A., Australia

ABSTRACT

Streptococcus pneumoniae (the pneumococcus) is a major human pathogen responsible for significant morbidity and mortality worldwide. Pneumococcal disease, which can include both invasive conditions such as pneumonia, bacteremia and meningitis, as well as less severe conditions such as otitis media, is almost invariably preceded by asymptomatic colonisation of the nasopharynx. To successfully adapt to the different ecological niches it encounters, the pneumococcus is likely to rely on the co-ordinated regulation of key virulence factors. As is the case for many other prokaryotes, this is likely to occur through two-component signal transduction systems (TCSTSs). TCSTSs comprise a histidine kinase (HK) and response regulator (RR). They respond to environmental stimuli, and regulate gene expression by interacting with the transcription machinery. Thirteen complete TCSTSs have been identified in *S. pneumoniae*, along with a lone RR. This study focused on one of these systems, designated RR/HK06.

In order to study this system, in-frame deletion mutants of *hk06* (D39Δ*hk06*) and *rr06* (D39Δ*rr06*) were constructed in *S. pneumoniae* D39. Western immunoblot and real time RT-PCR analysis showed that expression of the major virulence factor and protective antigen choline binding protein A (CbpA) was increased (approximately 5-fold) in D39Δ*hk06* but decreased (approximately 3-fold) in D39Δ*rr06*, compared to the wild-type D39. This suggested *cbpA* expression is regulated by RR/HK06. Furthermore, binding of RR06 to DNA upstream of the *cbpA* start codon was demonstrated by solid phase binding assays, confirming this regulation. Over-expression of the system showed that RR/HK06 activates expression of *cbpA* in D39. However, an in-frame deletion mutant in both *hk06* and *rr06* (D39Δ*rr/hk06*) produced similar levels of *cbpA* mRNA as the D39 wild-type.

Over-expression and mutation of *rr/hk06* in 3 other *S. pneumoniae* strains showed that RR/HK06 regulates the expression of *cbpA* across all 4 pneumococcal strains tested, albeit with some differences. Most RRs are active in the phosphorylated form, as illustrated by the fact that mutations in the cognate HK result in a reduction in regulated gene expression. Thus, the increased expression of *cbpA* in D39Δ*hk06* was unexpected and prompted further investigation. Amino acid substitutions in D39 HK06 led to the hypothesis that RR06 may activate *cbpA* expression in the non-phosphorylated form, as a substitution thought to specifically abrogate the phosphatase activity of HK06 led to levels of *cbpA* expression similar to that seen in the wild-type D39. However, further biochemical analysis is needed to confirm this.

Studies into the system's role in the virulence of *S. pneumoniae* showed that RR/HK06 is important for the ability of the pneumococcus to adhere to epithelial cells *in vitro* and to survive and proliferate in an *in vivo* model. Both D39Δ*hk06* and D39Δ*rr06* exhibited reduced adherence to human epithelial cells, even though D39Δ*hk06* showed increased levels of CbpA, a known pneumococcal adhesin. These findings clearly implicate additional RR/HK06-regulated factors in adherence to epithelial cells of human origin. *In vivo* experiments in mice showed that D39Δ*rr06* had an increased capacity to colonise the nasopharynx and cause disease compared to the parental strain, while D39Δ*hk06* was unable to persist in the lungs and blood. However, a strain deficient in CbpA showed no significant differences relative to the wild-type in its ability to colonise the nasopharynx or translocate to the lungs and blood. These data clearly indicated that other, as yet uncharacterized RR/HK06-regulated factors play a significant role in both colonisation and invasive disease, at least in the mouse model.

In order to identify other RR/HK06-regulated genes, microarray analysis was undertaken to investigate changes in gene expression when RR06 was over-expressed in both D39 and TIGR4 *S. pneumoniae*. *cbpA* and its co-transcribed upstream gene showed

substantial increases in expression when RR06 was over-expressed in both strains. However, there were no other similarities between the genes regulated by RR/HK06 in the two strains, suggesting that regulation varies between *S. pneumoniae* strains. In D39, RR06 over-expression decreased expression of numerous factors including the major virulence factor *pspA*, and another pneumococcal TCSTS *vncRS*. Further investigation of *pspA* regulation by RR/HK06 showed that the factor appeared to be regulated in a different manner to that seen for *cbpA*.