

University of Maryland Baltimore Graduate School

Announcement of Doctoral Dissertation Defense*

Candidate: Stephen Becker

Date, Time, and Place: July 25th, 2007, 1:00pm, room 102 BRF

Dissertation Title:

Whole Genome Analysis of Gene Expression Changes During Competence Development in *Haemophilus influenzae*.

Dissertation Abstract:

In *H. influenzae*, natural DNA transformation is highly regulated and commences with cellular metabolic and structural changes that lead to the development of the "competent state." Competent cells bind, translocate and internalize naked DNA from their environment and process it in several ways, depending upon the complementarity of the transforming DNA sequences to resident DNA sequences. Some genes involved in the DNA transformation process have been identified previously by chemical and genetic methodologies and are found to be conserved in many bacteria regardless of transformability; however, only a rudimentary knowledge of the transformation process exists at the protein level.

To improve upon this, we prepared a whole genome microarray of *H. influenzae* strains KW20 (wild type), transformation mutants JG87 (*crp::mini-Tn10kan*), GBH6.6 (*tfoX::aad*), and a constitutively competent strain GBH6.6/pGB18cx. The expression profiles of these strains during the induction of competence for genetic transformation using chemically defined M-IV medium were characterized. We identified 32 known or hypothetical genes that were significantly up-regulated from 3 to 85-fold between the initiation of competence development (t_0) and its approximate mid-way point (t_{40}) in the wild type strain only. Distributed within this set of 32 genes were the previously identified competence-inducible genes *comA*, *comM*, *dprA*, *pilA* and *rec2*. To further substantiate our results, we selected the four-gene operon containing *pilA* and one new candidate gene from our list, HI1008, and analyzed their biological function by insertion mutagenesis using a polar *cat-kan* transcriptional fusion element and non-polar *kan* cassette from pUC18k. Strains carrying *orf1008::cat*, *pilA::cat*, *pilA::cat* with the GBH6.6 mutation, and *pilD::cat* fusions were analyzed for the production of chloramphenicol acetyl transferase (*cat*) activity during the induction of competence for transformation in MIV medium. The average change in *cat* activity over the course of MIV competence induction for each fusion directly corresponds with the level of gene activation during the microarray analysis. As also seen in the microarray, the introduction of the GBH6.6 *tfoX::aad* mutation eliminates *pilA::cat* MIV-induced expression. In addition, we found that insertional inactivation of the entire *pil* operon, or any gene individually, virtually eliminates DNA transformation ($<10^{-8}$), whereas trans complementation with a plasmid carrying either the whole operon or serial deletions from the 3' end, restores partial activity. Inactivation of HI1008 was also found to decrease DNA transformation 80% relative to wild-type. These results are consistent with the identification of HI1008 as a new transformation gene in the *H. influenzae* system. Further analysis of HI1008 and the remaining candidate genes should rapidly advance our understanding of this important biological process at the molecular level.

Additionally, to further improve our understanding of the function of conserved proteins involved in transformation, we looked at the ability of *dprA* (also called *smf*) from *E. coli* (*E. coli* has not been shown to be able to undergo natural transformation), to complement the *H. influenzae* *dprA* mutation in strain GBH37. Our results indicated that *E. coli* *smf* can complement the GBH37 mutant in trans, suggesting that conserved proteins involved in natural genetic transformation may have additional roles in the cell beyond transformation, or transformation may be more prevalent than currently demonstrable.

Dissertation Committee Chair (name and title):

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Dissertation Committee Members (names and titles):

Nicholas Carbonetti Associate Professor

James Kaper Professor and Chairperson

James Carney Assistant Professor

/Diana Oram, Assistant Professor

Lindsay Black Professor of Biochemistry

*The Open Presentation is open to the university community and invitees of the candidate. Any member of the Graduate Faculty may observe the Final Examination. Only committee members may vote. For more information, see [Procedures for Examination of the Doctoral Dissertation](#).