Haemophilus influenzae metabolic requirements during lung infection

Most healthy adults (~75%) carry the bacterium *Haemophilus influenzae* in their nasopharynx without any complications. Nevertheless, *H. influenzae* can spread to other parts of the respiratory tract—the lungs or middle ear—resulting in illness and sometimes mortality. *H. influenzae* is an obligate human pathogen; therefore, the human host provides many nutrients *H. influenzae* requires for survival. However, little is known of the metabolism of *H. influenzae* during lung infection. The goal of this study was to answer the following questions: 1) Which nutrients are available for *H. influenzae* to consume in the lung? 2) Which nutrients are unavailable to *H. influenzae* in the lung, potentially because of nutritional immunity (host nutrient sequestration)? To tackle these questions, we used the whole-genome approach, transposon insertion site sequencing (Tn-seq), to determine the fitness of thousands of mutants simultaneously. We subjected the *H. influenzae* Rd transposon mutant library, which contains over 70,000 mutants, to an infection (murine lungs) and a defined metabolic environment (*in vitro* defined media). Almost half (~48%, 79/166 genes) of the genes *H. influenzae* requires during lung infection are also required during growth in defined media. Therefore, *H. influenzae*'s genetic requirements for lung infection are heavily related to metabolism. Based on the nutrients the defined media lacks, the genes required for growth without those nutrients, and the genes required in lung infection, we predict that the bacterium's environment in the lung lacks or the host sequesters many nutrients including purines and purine precursors, tryptophan and tryptophan precursors, valine, alanine, methionine, serine and zinc. Future studies will focus on further characterizing lung nutrient biosynthetic genes. Overall, these data provide a more complete view of the nutritional environment of the lung during *H. influenzae* infection and potential therapeutic targets.