Requirement of conserved folding catalysts in the functionality of several essential proteins, metabolic pathways and key virulence factors

Underlying mechanism of protein folding and response to protein misfolding share common pathways that are vital to all organisms. Protein folding defects are associated with many human diseases that include neurodegenerative disorders, such as Alzheimer's and polyglutamine diseases. Thus, understanding the cellular processes that regulate protein aggregation will help in development of new treatments. *In vivo* protein folding often requires the action of molecular chaperones and protein folding catalysts. Protein folding catalysts accelerate rate-limiting steps in this process and belong to two families: protein disulfide isomerases and *cis/trans* peptidyl prolyl isomerases (PPIs). PPIs are universally conserved, present in all cellular compartments and belong to three distinct families: cyclophilins, FKBPs and parvulins. Model bacteria, like *Escherichia coli*, contains 6 members of PPIs in the cytoplasm. However, there is a significant lack of knowledge about *in vivo* function of most of the cytoplasmic PPIs. Our major goal is to unravel mechanism of function of cytoplasmic PPIs and identification of their substrates. Thus, we have constructed deletion sets of all PPIs encoding genes and purified all PPIs. Using pull-down experiments, some of the substrates were identified. Importantly, we found that substrate client proteins include several essential proteins, transcriptional factors involved in the regulation of stress response, essential steps in fatty acid biosynthesis, assembly/function of Fe-S containing proteins and cell division process. In this project, we investigate if these substrates require the PPIase activity of a specific PPI. Thus, several single amino acid substitutions in SlpA, Cyp18, Par10 and FklB were constructed. Experiments are planned to express these mutant proteins and perform pull-down experiments to substantiate *in vivo* substrates of PPIs. Substrates will be identified by 2-D gels analysis. Based on these studies, binding affinities of substrate-PPI and specific peptides of a substrate with its partner PPI will be measured using Isothermal Titration Calorimetry. In parallel, strains lacking all 6 PPIs were found to accumulate several proteins as aggregates and examined by fluorescence imaging and 2-D gel electrophoresis. We will now analyze complete spectrum of proteins that tend to aggregate under non-permissive growth conditions, when 6 PPIs are absent using 2-D gels.

To further understand the structure and function of specific PPIs, strains were constructed that contain a FLAG epitope appended to the C-terminal end of the wild-type *ppi* gene and their derivatives with single amino acid substitutions in the putative active site on the chromosome. We will use them to identify critical amino acid residues that are essential for PPIs function. The FLAG-epitope allows us to perform immunoprecipitations using the anti-FLAG specific antibody, followed by analysis of trapped proteins by 2-D gel electrophoresis. Furthermore, peptide arrays from different substrates will be used to identify specificity of recognition by PPIs and which amino acid of client proteins are required for their interaction. The identification of substrate-PPI complexes have been a major bottleneck in understanding function of these folding catalysts. Thus, we plan to use a proximity-dependent biotinylation technique to mitigate these problems and capture transitory interacting proteins.

As *E. coli* PPIs and their counterparts in humans exhibit similarities at several levels, study of such immunophilin family of proteins is fundamental and important, as they are natural targets of search for new inhibitors that can have application in treatment of various diseases. Furthermore, in several pathogenic bacteria PPIs are immunogenic proteins that can possibly modulate the host immune response and enhance persistence of the pathogen within the host by subverting host cell-generated stresses.