

Stony Brook University The Graduate School

Doctoral Defense Announcement

Protein-Protein Interactions in the FAS-II Pathway of *M. tuberculosis*

By

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There is a growing need for the development of novel chemotherapeutics to combat the spread of tuberculosis (TB), a disease that infects one third of the world's population. The fatty acid biosynthesis (FAS-II) pathway of *Mycobacterium tuberculosis* (MTB) is a validated target for drug discovery and performs an essential role by producing the long chain fatty acid (C50) precursors required for the biosynthesis of mycolic acids, essential components of the mycobacterial cell wall. The mycolic acids give structure to the cell wall and aid in pathogenesis by protecting the bacterium against the harsh environment within the macrophage. Several current anti-TB drugs inhibit cell wall biosynthesis including the front-line drug isoniazid (INH), which likely has several targets within the cell including one or more components of the FAS-II pathway. Drug resistance to INH is a severe problem and a major focus of my research has focused on probing the molecular basis for the action of this drug.

INH is known to inhibit InhA, the FAS-II NADH-dependent enoyl reductase, via formation of an adduct with NADH. Significantly, InhA mutations identified in INH-resistant clinical isolates retain their ability to both bind and be inhibited by the drug adduct. In order to clarify the mechanism of drug action and resistance, chemical cross-linking, MALDI and analytical ultracentrifugation have been used to examine how enzyme inhibition modulates the oligomerization and conformation of InhA.

We have hypothesized that enzymes in the FAS-II pathway form a noncovalent multienzyme complex and that interactions within this complex play a critical role in controlling the inhibition of fatty acid biosynthesis by INH. I have used two hybrid methods coupled with pull-down experiments to identify and characterize protein-protein interactions involving components of the FAS-II pathway. Using the bacterial 2-hybrid system I identified a novel interaction between the FAS-II β -keto-acyl synthase, KasA and PpsB, a protein involved in the synthesis of a virulence factor. Significantly, this interaction provides a novel link between fatty acid and polyketide biosynthesis, and could provide an important mechanism for increasing the diversity of lipid components in the mycobacterial cell wall.

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