The Emb Proteins in *Mycobacterium smegmatis*

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Introduction

Numerous attempts have been made to develop improved strategies for the prevention and treatment of tuberculosis. The cell wall of *Mycobacterium tuberculosis*, the causative agent of this global disease, has been the subject of numerous studies. Mycobacterial D-arabinans are complex molecules, predominantly found in the two major polysaccharides, arabinogalactan (AG) and lipopolysaccharide (LAM). Whereas AG is a key molecule involved in immunopathogenesis, AG is attached to mycolic acids contributing to the integrity of the cell wall. Structural studies of LAM show the arabinan attached to a mannosic core which extends from a phosphatidylglycerol mannosic anchor at the reducing end. The terminal end of the arabinan of AG consists of a branched Ara44, and that of LAM consists of a linear Ara66 (Fig 1). It has been shown that the C-terminal of EmbC acts as the substrate in which arabinan is added to the remaining 430 amino acids. The N-terminal of EmbC recognizes the LAM precursor(LM) and the C-terminal of EmbC acts as the catalytic site for the synthesis of the complex arabinan of LAM.

Hypothesis

The N-terminal of EmbC recognizes the LAM precursor(LM) and the C-terminal of EmbC acts as the catalytic site for the synthesis of the complex arabinan of LAM.

Results continued

**Summary and Conclusion**

The results of SDS-PAGE and Western Blot show no detectable arabinosylation of LM to form LAM. It is observed that the first eight transmembrane domains cannot complement the LAM defect in ∆embC. The 23-1/3 hybrid contains 668 amino acids from EmbC, and the 1/3 hybrid contains 570 amino acids. This 98 amino acid difference may be where the mannos recognition site resides, allowing LAM biosynthesis to occur. Future efforts will be made to establish the site within these 98 amino acids where catalytic activity begins. Also, work is in progress to create a new hybrid containing 7/8embC-2/3embB (∼815 amino acids from EmbC; 247 amino acids from EmbB). This construct contains all 13 transmembrane domains of the EmbC protein, so it is expected that full-length LAM will be produced when complementing ∆embC mutants.

**Future Work**

- Purification of LM from the 1/3 hybrid using HPLC and comparative analysis to WT LM
- Creation of hybrid focusing on the N-terminal region between amino acid 570 and amino acid 668 to determine recognition of LM to form LAM occurs
- Work to understand the mechanism of the C-terminal of EmbC, and how this enables the synthesis of the complex arabinan found in LAM.