Heterologous Production of Functional Nonribosomal Peptide Synthetases

Michael G. Thomas; Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706; thomas@bact.wisc.edu

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Introduction. Nonribosomal peptide synthetases (NRPSs) catalyze the assembly of a vast number of biologically active small molecules. For many years, the *in vitro* characterization of NRPSs from bacteria was hindered by the inability to obtain soluble or functional enzymes when they were overproduced in *Escherichia coli* or other heterologous hosts. Recent work by our group (1) and others (2, 3, 4) determined that in many cases, the solubility and functionality issues can be overcome by the co-production of the targeted NRPS with its associated MbtH-like protein (MLP). For example, fully functional enterobactin synthetase, EntF, requires the involvement of YbdZ, the MLP from *E. coli* (Fig. 1). This requirement for an appropriate MLP has been previously overlooked during biochemical and metabolic engineering studies of NRPSs.

Methods. Co-production of a targeted NRPS with its cognate MLP has enable us to dissect the enzymology of NRPSs that were previously recalcitrant to *in vitro* analysis. Additionally, the use of genetic selections and screens are being used to understand how MLPs influence NRPS enzymology and how these interactions can be manipulated.

Results. We used MLP/NRPS co-production in *E. coli* to obtain, for the first time, soluble and functional enzymes from the mycobactin biosynthesis pathway from *Mycobacterium tuberculosis*. This enabled us to decipher mycobactin biosynthesis and propose a new biosynthetic scheme (Fig. 2; 5). Using bacteria- and yeast-based genetic selections and screens we have been dissecting and evolving the interactions between cognate and noncognate MLP/NRPS pairs.

Conclusions. The identification of MLPs as integral components of NRPSs has provided a mechanism for characterizing biosynthesis pathways that were previously unobtainable due to enzyme insolubility and inactivity. Furthermore, understanding the role MLPs play in NRPS enzymology and how these interactions can be manipulated will be a key step in generating functional hybrid NRPSs for the assembly of "unnatural" natural products.

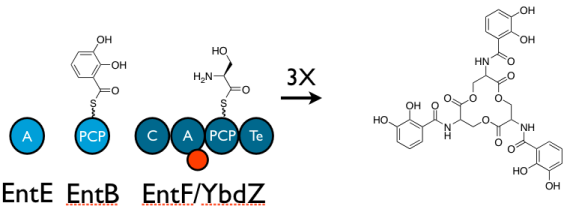

Fig.1 Schematic of enterobactin biosynthesis from *E. coli*. The MLP, YbdZ (red), complexes with the A domain of EntF (dark blue) to influence NRPS solubility and activity (1). A, adenylation domain; PCP, peptidyl carrier protein domain; C, condensation domain; Te, thioester domain.

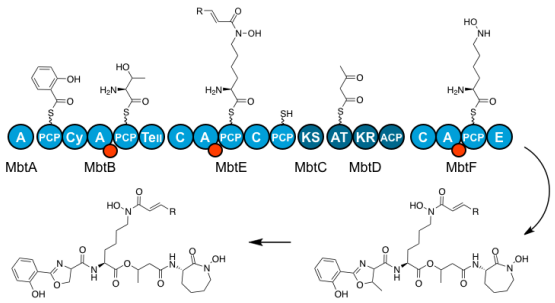

Fig.2 Schematic for a revised mycobactin biosynthesis pathway. The MLP, MbtH (red), complexes with three distinct A domains from MbtB, MbtE, and MbtF (light blue). Abbreviations are as in Fig. 1, with the addition of Cy, cyclization; TeII, type II thioesterase; KS, ketosynthase; AT, acyltransferase; KR, ketoreductase; ACP, acyl carrier protein; E, epimerase.

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