EXPRESSION OF *Mycobacterium tuberculosis* APA PROTEIN IN *Streptomyces coelicolor* REVEALS THAT PPM AND PMT, BUT NOT LNT, ARE REQUIRED FOR GLYCOSYLATION, AND THAT M. TUBERCULOSIS PPM, BUT NOT PMT, IS ACTIVE WHEN HETEROLOGOUSLY EXPRESSED.

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Introduction. Actinomycetes encode a protein mannosylation system homologous to that of yeast, consisting of a polyprenyl-phosphate mannose synthase (Ppm) and a protein mannosyl transferase (Pmt). The gene encoding Ppm activity is often associated with the gene for lipoprotein *N*-acyltransferase (Lnt) activity (1). The secreted *M. tuberculosis* Apa protein antigen can be recognized and glycosylated when expressed in *Streptomyces* (2), implying that mycobacteria en streptomycetes share functionality in their protein glycosylation systems.
In this work we analyze whether the *S. coelicolor* components of the glycosylation sysem are required to glycosylate Apa, and whether the mycobacterial proteins involved in protein glycosylation are functional when expressed in *Streptomyces.*

Methods. *S. coelicolor* mutants affected in the genes encoding homologues of the *M. tuberculosis* protein mannosylation system (Ppm, Pmt and Lnt) were obtained by PCR-targeting (3). They were complemented by either the *S. coelicolor* or the *M. tuberculosis* genes encoding components of the protein mannosylation system, cloned in integrative vectors. Mannosylation of the Apa protein, detected by Western-blot using concanavalin A, and infection by bacteriophage ϕC31 were used as evidence of a functional glycosylation system. The bacterial two-hybrid system was used to determine whether *S. coelicolor* Ppm and Lnt proteins interact, as in mycobacteria.

Results. Both Ppm and Pmt proteins were required for mannosylation of *M. tuberculosis* Apa by *S. coelicolor*, since strains carrying targeted in-frame deletions of the genes encoding Ppm and Pmt (SCO1423 and SCO3154, respectively) were incapable of Apa glycosylation; as expected, they could not be infected by bacteriophage ϕC31. The genes encoding *M. tuberculosis* Ppm and Pmt (Rv2051c and Rv1002c, respectively) were cloned and expressed in *S. coelicolor* under the control of the P_{tipA} promoter. Ppm of *M. tuberculosis* complemented the *S. coelicolor* Ppm mutant; complementation was also achieved by expressing only the Ppm domain (D2) of the compound *M. tuberculosis* Ppm protein. Deletion of the SCO1014 gene, which encodes the *S. coelicolor* homolog of the N-terminal domain (D1) of *M. tuberculosis* Ppm and is a putative lipoprotein *N*-acyl transferase (Lnt), had no effect on glycosylation of Apa. In addition deletion of SCO1014 had no effect on the ability of phage ϕC31 to infect *S. coelicolor*. Cloning the SCO1014 and SCO1423 genes in a bacterial two hybrid system revealed that these two proteins do not interact *in vivo*, in contrast to their mycobacterial homologs. The *S. coelicolor* Pmt mutant could not be complemented by expressing *M. tuberculosis* Pmt, even though the protein was expressed and localized to the cytoplasmic membrane. Since all Pmt proteins from streptomycetes have an N-terminal extension not present in the mycobacterial homologs we constructed two chimaeric proteins of *M. tuberculosis* Pmt that include the N-terminal region of *S. coelicolor* Pmt. These chimaeric proteins also failed to complement the *S. coelicolor* Pmt mutant. An *in vitro* assay for mannosylation of a synthetic peptide with radioactive mannose revealed that *M. tuberculosis* Pmt is not functional when expressed in *S. coelicolor* membranes even in the absence of protein secretion, meaning that its lack of activity is not due to inability to interact with the secretory apparatus of *S. coelicolor*.

Conclusions. Glycosylation of Apa by *S. coelicolor* requires the same enzyme activities as in *M. tuberculosis* (Ppm and Pmt) but not Lnt, which is dispensable. Specific interactions limit the functionality of *M. tuberculosis* Pmt when expressed in *S. coelicolor*, but not of Ppm, which is fully functional and able to complement a *S. coelicolor* Ppm mutant.

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