Mechanisms of Bacterial Polyprenyl Transferases and Multi-Target Drug Discovery for Tuberculosis

Xinxin Feng Final Seminar April 9, 2014

Bacterial polyprenyl transferases transfer polyprenyl groups onto molecular acceptors, such as proteins and small molecules, and they are very important functional entities that are involved in bacterial cell wall biosynthesis, biofilm formation, virulence formation and natural product formation.[1] Structurally, bacterial polyprenyl transferases can be categorized into eight groups: $\alpha_{cyclase}$, α, αβ, αβγ, βγ, ε, ζ and TIM barrel fold proteins. [1] Six bacterial polyprenyl transferases were investigated in the current research (Figure 1), including *Streptomyces ghanaensis* MoeO5[2] and *Bacillus subtilis*/*Staphylococcus aureus* PcrB[3] (TIM barrel fold), *Bacillus subtilis* YisP[4] (ε fold), *Bradyrhizobium japonicum* Kaurene synthase[5] ($\alpha_{cyclase}$ fold), *Mycobacterium tuberculosis* Rv3378c and *cis*-decaprenyl diphosphate synthase (DPPS)[6] (ζ fold) (Figure 2). The function, mechanism of action, structure, inhibition and functional engineering of these six polyprenyl transferases were studied by X-ray crystallography, mutagenesis, activity assays, thermal dynamics measurements *etc*. Besides that, a multi-target drug discovery approach was also proposed as an attempt to combat drug resistance in anti-infective drug discovery campaigns, both based on structural homologies of *Mycobacterium tuberculosis* Rv3378c and DPPS[6] (ζ fold), and the multi-target effect of SQ-109 and its analogs[7] (Figure 3).

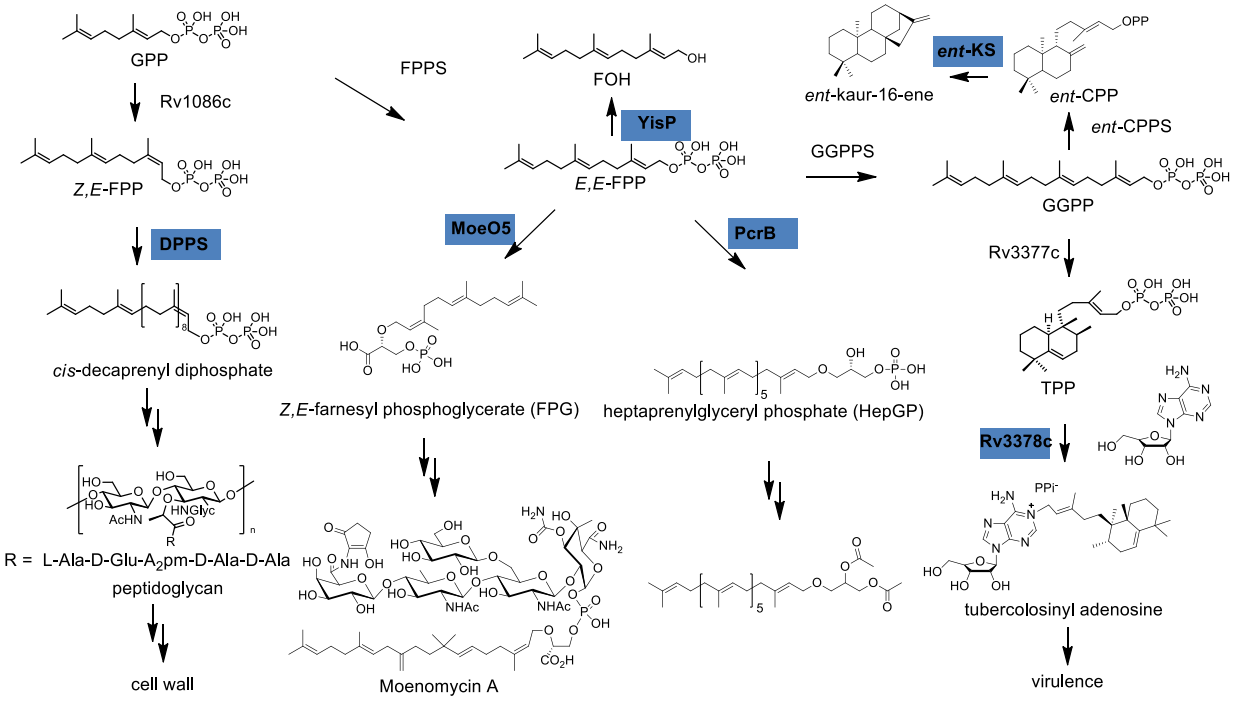

Figure 1. Reactions catalyzed by the six bacterial polyprenyl transferases investigated.

MoeO5 catalyzes the first step biosynthesis of Moenomycin A, which is a phosphoglycolipid antibiotic. We determined the first crystal structure of MoeO5 and studied the

binding mode of bound substrates. We also assayed its activity with different substrates and compared MoeO5 with its homolog PcrB, which led to a proposal of the MoeO5 reaction mechanism.

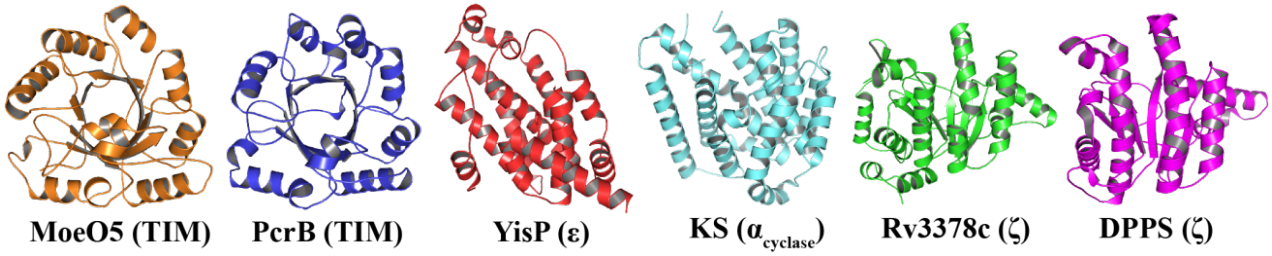

Figure 2. Structures of the six bacterial polyprenyl transferases investigated.

YisP is essential in biofilm formation in *Bacillus subtilis* and is predicted to produce C_{30} isoprenoids. We determined the structure of YisP, and proved that YisP acts as a phosphatase. Using DSC, we confirmed that YisP product farnesol shift and broaden the gel-to-liquid crystal transition of lipids.

The X-ray structure of the bacterial diterpene cyclase *ent*-kaur-16-ene synthase from the soil bacterium *Bradyrhizobium japonicum* was determined in apo-, substrate and inhibitor-bound forms. The catalytic activity of the cyclase was studied by site-directed mutagenesis and inhibition of the enzyme was also investigated.

Structures of tuberculosinol/*iso*-tuberculosinol synthase (Rv3378c) and DPPS from *M. tuberculosis* were determined. They are targets for anti-infective therapies that block virulence factor formation and cell wall biosynthesis, respectively. Given the similarity in local and global structure between these two proteins, the possibility exists that it may be possible to develop inhibitors that target not only virulence, but also cell wall biosynthesis.

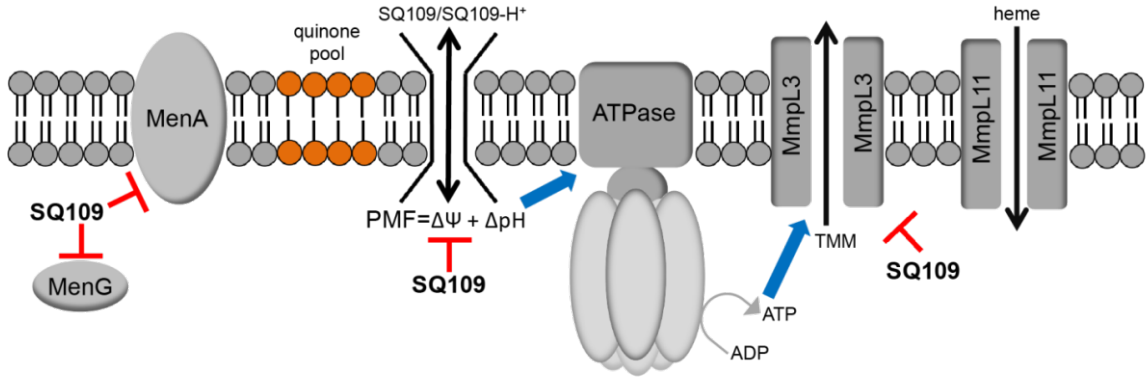

Figure 3. Schematic illustration of proposed sites of action of SQ109 and its analogs.

SQ109, a new anti-tuberculosis ethylenediamine drug, was found to be active against other bacteria as well as yeast. Based on the SAR study and biochemical assays of eleven SQ109 analogs, we found that it might be an example of a multi-target drug that inhibits isoprenoid/quinone biosynthesis and/or respiration (Figure 3).

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