Investigation of *Mycobacterium tuberculosis* RNA Polymerase for the Development of Novel Drug Candidates

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An estimated two billion people are infected with *Mycobacterium tuberculosis* (*Mtb*) in an inactive, latent state. Even though someone may be infected with *Mtb*, there is only a five percent chance of the infection becoming active tuberculosis (TB) within the first two years and only another ten percent of people with latent *Mtb* are reactivated. Three of the four first line TB drugs require actively replicating *Mtb* to work. Rifamycins (Rifs) are the exception but active *Mtb* is killed much faster than in its latent state. Rifs are highly potent antibiotics that treat TB by targeting the *Mtb* RNA polymerase (RNAP). There are a few Rifs currently approved for use in the United States; the most commonly used Rif is rifampin (RMP). RMP is an essential component of TB treatment. Rifampin dramatically reduced the treatment time for tuberculosis but even in combination with other drugs, treatment time is still six-to-nine months. This contributes to poor patient compliance, which accelerates the selection of drug resistant mutants. Rifamycin resistance (Rif^R) is caused by single point mutations in the β subunit of RNAP that drastically decrease Rif binding. Rif^R *Mtb* exhibits greatly decreased treatment success rates and further increases the already long treatment time. The drug resistant mutations also cause fitness defects for the bacterium; however, secondary mutations occur that compensate for the fitness defects. These compensatory mutations primarily occur in the β' subunit of RNAP, adjacent to or in the putative guanosine tetraphosphate (ppGpp) binding site. ppGpp is an alarmone and is produced during the stringent or stress response of many bacteria, including *Mtb*. In *Escherichia coli*, ppGpp has been shown to have many different effects on the cell; one of them is binding to and inhibiting RNAP. *E. coli* and *Mtb* RNAPs have a high sequence similarity allowing *E. coli* RNAP to be used as a model for *Mtb*. Our lab has been working under the hypothesis that ppGpp binds to *Mtb* RNAP in the analogous site to that in the *E. coli* RNAP and that the compensatory mutations were disrupting ppGpp's binding. There has been evidence that ppGpp is necessary for Mtb to become latent. I have performed both cross-linking and kinetics studies that confirm the binding and reduction of activity of the E. coli RNAP by ppGpp; however, the *Mtb* RNAP does not bind or respond to ppGpp. Consistent with my results, the newly published X-ray crystal structures of the *Mtb* RNAP reveal that while the "ppGpp pocket" is present there is a loop of the protein that appears to occlude the site.

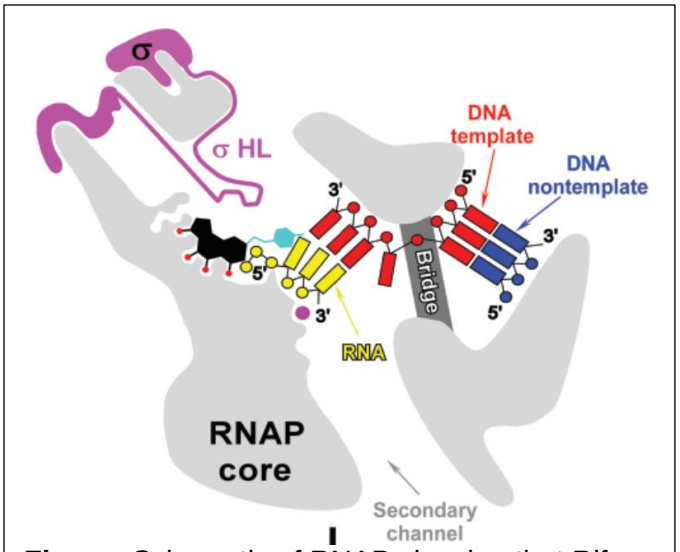
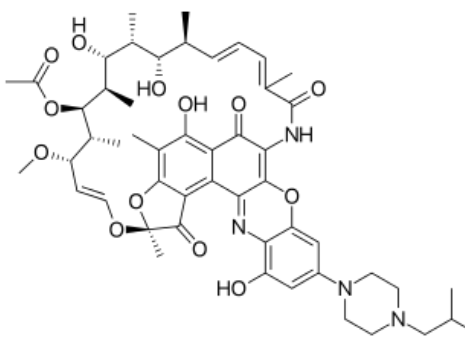


Figure: Schematic of RNAP showing that Rifs (black) block RNA (yellow) development.

Another approach to developing novel *Mtb* RNAP inhibitors that could potentially treat both wild type (WT) and Rif^R strains that our lab is pursuing involves structure based drug design to extend the Rif core. One major drawback of Rifampin is that it activates the human pregnane x receptor (hPXR). This receptor is a ligand-activated transcription factor that regulates the expression of phase I metabolizing cytochrome P450s like Cyp3A4. Cyp3A4 is one of the most common CYPs and it metabolizes almost half of today's prescription drugs. Efforts to reduce this activation by rifamycins have been made but currently there are no such FDA-approved drugs. One example is the investigational benzoxazinorifamycin (bxRif), rifalazil. Our lab has designed, synthesized and characterized many different bxRif analogs that both inhibit RNAP and have almost no hPXR activation. We are continuing to generate analogs that will improve current Rifs by increasing binding affinity for WT and Rif^R RNAP, increase *in vivo* activity, and decrease hPXR activation. Completion of these goals would greatly improve the outcomes for patients being treated for TB by decreasing TB treatment time, decreasing side effects and treating Rif^R strains.



Rifalazil