Kinetic Characterization of Rifamycin-Resistant *M. tuberculosis* RNA Polymerases and a Novel Therapeutic Approach for Targeting Transcription

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ABSTRACT

Rifampin (RMP) remains a key component of the front-line treatment regimen for tuberculosis, though resistance has presented challenges for its efficacy. Resistance to RMP (RMP^R) primarily occurs through point mutations within the rifamycin resistance determining region (RRDR) in the β-subunit of its target, RNA polymerase (RNAP). Three mutations constitute the bulk of RMP^R, βD435V, βH445Y, and βS450L, with the latter being most prevalent in RMP^R clinical isolates. The molecular mechanisms that yield the observed distribution of RMP^R mutations in MTB have been speculated upon; however, detailed *in vitro* studies of *Mycobacterium tuberculosis* (MTB) RNAP to elucidate those mechanisms have been lacking.

In vivo fitness defects in RMP^R strains of MTB RNAP have been reported. These defects have also been reported to be ameliorated by the presence of secondary mutations in double-psi β-barrel (DPBB) of the RNAP β'-subunit. Differential fitness defects are likely contributors to the relative prevalence of the RMP^R mutants observed in MTB clinical isolates. To identify factors contributing to these fitness defects, several *in vitro* transcription assays were utilized to probe initiation, elongation, termination and RNA primer hydrolysis with the wild-type and RMP^R RNAPs. Secondary, compensatory mutations are predominantly associated with the βS450L mutant; therefore, this mutant was also studied in the presence of secondary mutations. We found that the RMP^R mutants exhibit significantly poorer termination efficiency relative to wild-type, an important factor for proper gene expression. We also found that several mechanistic aspects of transcription of the Rif^R mutant RNAPs are impacted relative to wild-type, particularly the stability of the open-promoter complex and elongation rate. For the βS450L mutant, these defects are mitigated in the presence of secondary mutations in the DPBB of the β'-subunit, making the intrinsic properties of this mutant similar to those of the wild-type. These data provide insight into the cost of antibiotic resistance to the fitness of the organism, a mechanistic basis for how MTB alleviates fitness defects associated with drug resistance and a rationale for the observed distribution of RMP^R mutations in MTB.

Drug resistant tuberculosis has become pervasive in large part due to a lack of novel therapeutics which act by new mechanisms of action. CarD is a global transcription regulator which acts by stabilizing the open-promoter complex of MTB RNAP and has been shown to be required for MTB viability. This suggests that CarD may be an effective and novel target for therapeutic discovery for the treatment of tuberculosis. A fluorescence polarization assay which monitors the association of MTB RNAP, native rRNA promoter DNA and Bodipy-CarD has been developed, optimized and validated. A high throughput screen has been conducted to identify and characterize small molecule inhibitors which block the CarD•RNAP•DNA interaction. Several preliminary hits have been identified from this screen and initial secondary characterizations have been performed. This project will be the foundation for further investigation of CarD's potential as a therapeutic target.