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CarD-Mediated Transcription Iniation in Mycobacterium Tuberculosis

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CarD-Mediated Transcription Initiation in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (*Mtb*), the bacteria that causes tuberculosis (TB), infects about one-third of the world's population. People infected by *Mtb* have a 10% lifetime risk of falling ill with TB, making it one of the top ten causes of death worldwide. CarD, an essential transcription factor known to enhance transcription in *Mtb*, could prove to be an important drug target as mutant CarD protein leads to a decrease in antibiotic resistance. The goal of this study is to elucidate the molecular mechanisms behind CarD-enhanced transcription initiation, including its ability to stabilize the RNA polymerase open promoter complex and affect the rates of promoter escape. Using single-molecule magnetic-tweezers assays, DNA tethered between the surface of a glass cover slip and a paramagnetic bead can be manipulated and tracked at constant force. DNA unwinding can be detected on torsionally constrained DNA templates via changes in the end-to-end distance of the tethered DNA.

Preliminary data suggests that CarD acts by increasing the lifetime of the RNA polymerase (RNAP) open complex. Furthermore, DNA extension changes due to promoter opening on positively supercoiled DNA are greater than those on negatively supercoiled DNA. These changes are indicative of DNA compaction possibly stemming from DNA wrapping around the polymerase, consistent with published measurements on *E. coli* RNAP. Additionally, a single trace taken in the presence of CarD and all four rNTPs shows possible DNA scrunching behavior in the process of promoter escape. This occurs during an intermediate state when RNA polymerase remains stationary, while unwinding and pulling downstream DNA into itself before escaping the promoter and beginning processive transcription. Further experiments will be performed as a function of CarD concentration to fully determine the mechanism of CarD in the context of an important human pathogen.