Title of thesis: Functional characterization of a novel and essential DNA Topoisomerase IA from *Leishmania donovani*
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Abstract

Evolutionarily kinetoplastids are a living bridge between the prokaryotes and higher eukaryotes. It has adapted to the compartmentalized structure of eukaryotes yet harbors several prokaryote homologous enzymes and processes. One such enzyme we find to be retained in *Leishmania* is DNA Topoisomerase IA. Prokaryotic Topoisomerase IA is known to prevent R-loops by relaxing negatively supercoiled DNA formed behind the transcription bubble. Owing to lack of introns, polycistronic transcription, prokaryotes tend to form large R-loops compared to eukaryotes where monocistronic transcription and co-transcriptional splicing prevents large DNA-RNA hybrid formation. Additionally, co-transcriptional splicing shortens the transcribing pre-mRNA which may prevent it to interact with its complimentary DNA and thus reduces the extent of R-loop formation for which RNaseH II is sufficient. This explains why higher eukaryotes have lost TOPIA in evolution. R-loops in context of *Leishmania* are unknown. In this study functional characterization of LdTOPIA, exhibits its Mg^{2+} dependent DNA relaxation of negatively supercoiled DNA and preference for single stranded DNA. *E. coli* RFM475 strain (TOPIA null, GyrB ts) incapable of growing at 30°C is complemented by LdTOPIA as it helps prevent R-loop formation that in RFM475 at 30°C can genome instability and death. Unlike Trypanosoma Topoisomerase IA which is solely kinetoplast specific LdTOPIA was found to be solely nuclear by virtue of having functional bipartite NLS at its C-terminus. Tetracycline induced conditional anti-sense of *Leishmania* TOPIA causes gradual nuclear R-loop accumulation inside parasite nucleus which leads to genome instability that leads to G2/M cell cycle arrest and parasite death. Such R-loop accumulation and parasite death was also observed by inhibiting the function of LdTOPIA by a FDA approved tricyclic anti-depressant, norclomipramine. Our in- silico data shows that norclomipramine can bind to the active site pocket of homology modelled LdTOPIA structure. Hence, we unraveled the role of novel and essential LdTOPIA in preventing nuclear R-loops during polycistronic transcription of *Leishmania*. Thus, LdTOPIA surfaces as a specific target against leishmaniasis as it is absent in the human host. This comprehensive characterization of LdTOPIA and its inhibition by norclomipramine paves an avenue for specific therapeutic targeting of *Leishmania* through drug repurposing.