Role of G-Quadruplexes in Mycobacterial Genome

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Tuberculosis is a highly transmissible disease caused by *Mycobacterium tuberculosis* (Mtb), a member of the mycobacteriaceae family, that can endure in a latent state. Mtb is a slow-growing bacteria with a mycolic acid cell wall contributing to drug resistance, making tuberculosis treatment complex. Intracellular growth within host cells and the ability to persist in the environment further complicate the control of this infectious disease, making it a significant global health concern. G-quadruplexes have emerged as significant players in regulation at all levels of the central dogma. This study compares the distribution of putative quadruplex sequences (PQS) in mycobacteria with that in other bacteria. In contrast to the other bacteria, mycobacteria stand out and contain almost ten times more quadruplex densities in both the genomic and transcript sequences. Interestingly, our data shows that the PQS were enriched in transcript sequences (PQS density in transcripts is more than PQS density in the genome) in the case of slow-growing pathogenic mycobacteria. rG4s enrichment in just the slow-growing pathogenic cannot be a mere coincidence. Notably, the distribution of G-quadruplexes in Mtb is not uniform among the classified gene families and PE/PPE genes contain almost half the PQS found in Mtb. PE/PPE genes are two large families of genes found only in pathogenic mycobacteria and

are associated with pathogenesis. Such RNA G-quadruplexes (rG4s) enrichment in any gene family has not been reported in bacteria. Our CD spectroscopy results confirm that the RNA oligonucleotides of the selected PE/PPE genes form the GQ structures, which are stabilized upon the addition of BRACO19. The results indicate that stabilizing GQs by BRACO19 in PE/PPE genes (*PPE56, PPE67, PPE68, PE_PGRS39,* and *PE_PGRS41*) leads to the downregulation of transcription. BRACO19 also shows selective inhibition of Mtb growth at low micromolar concentrations, while the other bacteria were not affected even at much higher concentrations. Heterologous expression of the selected genes indicates that the GQ-mediated downregulation is not limited to the transcription level. Ectopic expression in both *M. smegmatis* and *E. coli* shows the downregulation of PE/PPE protein levels due to rG4s. In addition, the rG4-mediated reduction in PE/PPE protein levels attenuates proinflammatory response upon infection of THP-1 cells. Thus, our findings on the role of G-quadruplexes in regulating transcription of the PE/PPE genes, the post-transcriptional translation inhibition by rG4s in the transcripts, and its impact on macrophage pro-inflammatory response provide new insights into our current understanding of Mtb adaptation, stress response and survival within the host.