**The role of ATF1 in the regulation of early events of gene transcription**

The regulation of metazoan gene transcription by RNA Polymerase II (Pol II) is a multi-step process that is carefully orchestrated by numerous transcription factors. Despite significant progress in research, there remain unresolved gaps in our understanding of the complex molecular processes that govern gene expression in human cells. As the human immunodeficiency virus (HIV) is tightly regulated at early steps of the transcription cycle, its gene expression program has emerged as a valuable model for comprehending the mechanisms that direct cellular transcription. This knowledge also holds profound clinical implications, as transcriptionally repressed yet replication-competent HIV persists within infected cell reservoirs that are resistant to anti-retroviral therapy, therefore a main obstacle for a complete cure of viral infection.

In a search for new regulators of HIV gene expression, we monitored changes in the transcriptome of primary CD4+ T cells infected with HIV in response to T cell stimulation. Along with broad changes in cellular gene expression, our preliminary analysis identified Activating Factor 1 (ATF1) as a significant transcription factor that undergoes a substantial increase in its RNA levels upon T-cell stimulation. Additional findings suggest that ATF1 binds to the HIV promoter and activates viral gene expression. At the molecular level, ATF1 is associated with the cellular TFIIH transcription initiation factor, and its depletion reduces TFIIH levels at the HIV promoter and alters the phosphorylation state of the C-terminal domain of Pol II.

In light of these observations, **we hypothesize that ATF1 activates early events of gene expression by directly binding to gene promoters and recruiting TFIIH.** In **Aim 1,** we will decipher the molecular mechanisms underlying ATF1-mediated gene activation. Cellular and biochemical approaches will confirm the association of ATF1 with TFIIH through direct binding with its cyclin H subunit. A possible role for the cdk7 kinase subunit of TFIIH in phosphorylating ATF1 and regulating its transcription activity will be investigated. In **Aim 2,** we will shift our efforts to unravel the global role of ATF1 in controlling cellular gene transcription. Preliminary RNA-Seq analysis revealed that depletion of ATF1 expression correlates with marked changes in the host transcriptome, with enrichment in transcription regulation, T cell activation, and chromatin activation processes. We will employ genome-wide approaches to map the binding loci of ATF1 within the genome and identify its downstream gene targets. The role of these genes in controlling HIV-specific and global gene expression will be investigated by employing gain-and-loss-of-function experiments. Additional high-resolution ChIP-Seq analysis will determine the global occupancy of TFIIH and ATF1, and nascent RNA sequencing will define the role of ATF1 in the early steps of transcription. Finally, in **Aim 3,** we will rely on preliminary data showing that upon T cell stimulation, depletion of ATF1 in primary CD4+ T cells inhibits HIV gene expression and latency reversal. We will thus place our work in a clinically relevant context, assessing the functional relevance of ATF1 to HIV infection and latency in resting CD4+ T cells, which are the genuine target cells that comprise the HIV reservoirs.

This current proposal covers basic and translational questions in gene transcription regulation and the mechanisms that lead to HIV persistence in infected cells. In particular, it will define ATF1 as an essential transcription activator of HIV-specific and cellular gene expression. New target genes that are regulated by ATF1 will be identified, potentially opening ways for developing novel therapeutic interventions against HIV infection and other human diseases.