Retrovirology

Poster presentation

Characterization of a Downstream Positive Element Involved in Transcriptional Control of the Human CD3γ Gene Promoter

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Experimental data from our laboratory has shown that TCR/CD3 surface receptors are downmodulated after HIV-1 and HIV-2 infection of CD4+T cells due to a specific defect in CD3γ gene transcripts. In an effort to better understand the mechanism(s) involved, our laboratory has been investigating the critical elements responsible for regulating this gene. We have shown that the CD3 γ gene is transcribed from an independent but weak, lymphoid-specific TATA-less promoter and demonstrated that a cluster of transcription initiation sites is present in the vicinity of the principal core promoter with the major start site situated in a classical initiator sequence. A GT box upstream of the initiator binds Sp family proteins and the general transcription machinery, with the activity of these contiguous elements enhanced by a second Sp binding GC box ten nucleotides further upstream. We found that two previously identified NFAT motifs positively (NFAT γ 1) or negatively (NFAT γ 1 and NFAT γ 2) regulate expression of the CD3 γ gene by their differential binding of NFATc1 plus NF-κB p50 or NFATc2 containing complexes, respectively. Analysis of various mutant and deletion CD3 γ promoter constructs in a transient reporter assay revealed that a 53 bp region downstream from the major transcription start site is critical for positive gene expression. Deletion of ten nucleotides in this region results in a 50% decrease in promoter activity, while deletion of 39 nucleotides completely eliminates promoter activity. EMSA experiments using DNA or RNA probes covering the 53 bp region demonstrate that this element functions through an RNA rather than a DNA intermediate. At least three specific nuclear protein complexes bind to the RNA probe. Deletion of the U at position +9 and the U at +37 completely abrogate binding and promoter activity. Experiments are currently underway to determine whether the composition of the transcription factor(s) complexes bound to the CD3γ element contain components of P-TEFb, which binds to HIV TAR.