Elucidation of the Interleukin-13 Receptor α2 Promoter

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Introduction:
Interleukin-13 is a multifunctional cytokine that mediates its immunoregulatory activities via a specific interaction its cognate receptors on macrophages, B-cells, and endothelial cells. Two receptors for IL-13 have been described, Type 1 and Type 2. The type 1 receptor is comprised of the IL-4 receptor alpha chain (IL-4Rα) and the IL-13 receptor alpha 1 chain (IL-13Rα1). This receptor complex binds IL-13 with intermediate affinity and signals. The type 2 receptor is comprised of the IL-13 alpha 2 receptor chain (IL-13Rα2) and binds IL-13 with high affinity but does not confer responsiveness to cells. This receptor is thought to be a decoy receptor since soluble IL-13Rα2 can completely block allergic inflammation in mouse models and membrane bound IL-13Rα2 has no known signaling function. Given the critical importance of IL-13 in allergic inflammation, regulation of IL-13Rα2 expression may be a potent mechanism by which IL-13 is regulated and may have potential therapeutic implications. In order to address how IL-13Rα2 expression is regulated, we wanted to elucidate the elements of the promoter for IL-13Rα2.

Hypothesis:
In the present study, we tested the hypothesis that the DNA elements upstream of the IL-13Rα2 coding sequence contained the active promoter elements necessary for IL-13Rα2 gene expression and we defined the minimal promoter elements needed for gene activation.

Methods:
In order to test our hypothesis we isolated the presumed promoter by PCR utilizing probes designed from sequences obtained from the Celera Database. We subcloned this fragment into a luciferase reporter plasmid and demonstrated that the putative promoter was able to confer strong gene transcription when transfected into U937 human monocytic cells using Effectene (Qiagen). We had previously shown that U937 cells expressed IL-13Rα2 on their surface, thus establishing that these cells contained the necessary transcriptional machinery for for IL-13Rα2 gene expression. In order to define the minimal promoter elements, we created progressive 5' deletions of the 1.6 kb promoter fragment using the Erase-a-Base system (Promega). Each fragment was sequenced and then subcloned into the luciferase reporter plasmid and transfected into a U937 cells. Luciferase activity was then assessed in the transfectants according to standard protocols.

Results:
Our results demonstrated that the minimal promoter fragment necessary for gene expression is approximately 108 base pairs long. The promoter activity conferred by fragments less than 74 bp were not significantly different from the the control vector lacking a promoter.

Conclusions:
Thus, our results confirm that the 5'UTR of the IL-13Rα2 gene contains the active promoter

elements for gene expression. We also conclude that the most important binding sites for transcription factors necessary for gene activity are also located in the region 0 to -108.