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Transcription regulation of cytokines and their associated proteins

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Comparison of closely related genomes reveals that speciation has largely resulted from evolution of gene regulatory regions, whereas the exons have been more conserved. Indeed, regulation of gene expression is a complex field, whose study is crucial in every area of biological research.

In view of our long-term interest in cytokines and their associated proteins, we recently focused on studying the regulation of their gene expression. In the past, we have identified various components of the cytokine network, including the interferon alpha/beta receptor and more recently, an IL-18 binding protein (IL-18BP), which regulates pro-inflammatory Th1 responses. IL-18BP is specifically induced by IFN-gamma. We found that the IL-18BP promoter includes within its proximal region (bases -1 to -122) a gamma-activated sequence (GAS) followed by an IRF-1 response element (IRF-E) that are essential for basal and IFN-gamma-induced promoter activity. Our studies further revealed that IFN-gamma induces the association of IRF-1 and C/EBP beta, and the resulting heterodimer translocated from the cytoplasm to the nucleus. Electrophoretic mobility shift assays (EMSA) revealed that this heterodimer binds to the proximal GAS-IRF-E pair.

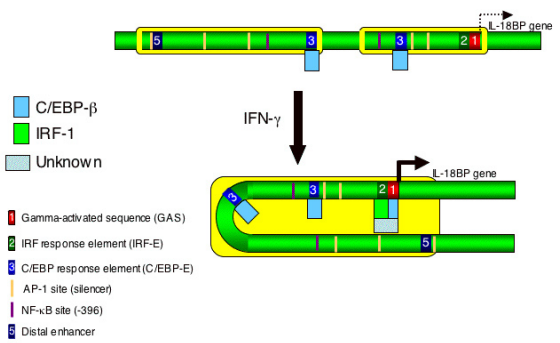

Fig. 1 *Schematic representation of the IFN-gamma-induced activation of the IL-18BP promoter*

Further characterization of the promoter revealed a clockwork of silencers and enhancers that mediated responses to various cytokines (Hurgin, V. et al, 2002).

In addition to the GAS-IRF-E pair, the proximal promoter contained two AP-1 sites, acting as silencers, two additional C/EBP-Es and two NF-κB-Es, all acting as enhancers. Mutation of any one of these elements affected both basal and IFN-gamma-induced transcription of the IL-18BP gene by orders of magnitude. The NF-κB-E probably mediated responses to IL-1 beta and TNF alpha, as these cytokines synergized with IFN-gamma in inducing IL-18BP, but were unable to induce the gene in the absence of IFN-gamma. Indeed, SN50, a specific NF-κB inhibitor, significantly reduced IL 18BP mRNA induction. Furthermore, we found that IFN-gamma induced a complex between NF-κB and the proximal AP-1-E, suggesting a possible mechanism by which IFN-gamma may overcome the AP-1-mediated repression of IL-18BP gene expression. Further analysis has identified a distal silencer/enhancer pair within bases -1081 to -1272, that was physically associated with the proximal IRF-1. Chromatin immunoprecipitation confirmed the presence of these separate proximal and distal promoter regions, which merged into a single transcription-activation complex following induction by IFN-gamma.

Selected Publications

Novick, D. Kim, S-H., Fantuzzi, G., Reznikov, L.L., Dinarello, C.A. and Rubinstein, M. (1999) Interleukin-18 binding protein: a novel modulator of the th1 cytokine response. Immunity, 10, 127-136.

Novick, D., Schwartsburd, B., Pinkus, R., Suissa, D., Belzer, I., Sthoeger, Z., Keane, W.F., Chvatchko, Y., Kim, S-H., Fantuzzi, G., Dinarello, C.A. and Rubinstein, M. (2001) A novel IL-18BP ELISA shows IL-18BP in sepsis and extensive decrease of free IL-18. Cytokine, 14, 334-342,.

Hurgin, V., Novick, D. and Rubinstein, M. (2002) The promoter of IL-18 binding protein: activation by an IFN-gamma-induced complex of IFN regulatory factor 1 and C/EBP beta. Proc. Natl. Acad. Sci. USA., 99, 16957- 16962.

Novick, D. and Rubinstein, M. (2004) Receptor isolation and characterization: from protein to gene. Methods Mol. Biol. 249, 65-80.

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