

**SIGNALING PATHWAYS REGULATING TRANSCRIPTION, mRNA
STABILISATION AND INTRACELLULAR TRANSPORT OF
CYTOKINES IN ACTIVATED MAST CELLS**

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Mast cells are important elements of innate immunity and key players in allergic reactions. One of the critical features of these cells is their ability to release a large number of active mediators following contact with activators such as antigens or some bacterial wall components. Among the mediators released by activated mast cells are cytokines such as IL-4 and TNF-alpha. The expression of IL-4 is regulated predominantly on the transcriptional level. In resting mast cells, the amount of IL-4 mRNA present is below the detection threshold, but it is generated within minutes of mast cell activation. This up regulation is mediated by the IL-4 promoter, which is activated by specific intracellular signals. Signaling pathways regulating IL-4 promoter activity involve calcineurin and JNK, which activate the nuclear transcription factors NFAT and AP-1. We employed a specific JNK inhibitor and reporter gene assay to study the role of the JNK/c-Jun pathway in the regulation of IL-4 expression in mast cells. Our data support the indispensable role of JNK in antigen-mediated IL-4 induction. The sequences in the IL-4 minimal promoter from -87 to +1 seem to be sufficient to mediate the JNK dependent activation of IL-4 promoter following the addition of antigen. It has been observed that crosslinking of surface IgE with antigen, in addition to regulating promoter activity, also provides signal for stabilization of cytokine mRNA. This stabilization is mediated by specific sequences in the 3'UTR region of IL-4. Another regulatory mechanism, which controls cytokine expression in mast cells, is the intracellular trafficking of cytokine proteins. The regulation of TNF-alpha expression is an interesting example of such post-translational mechanisms. GFP was employed to observe how cytokine protein accumulates in mast cell cytoplasmic granules. Expression of the TNF-alpha-GFP fusion protein but not GFP alone in mast cells resulted in the accumulation of GFP fluorescence in structures resembling cytoplasmic granules. It suggests that the TNF-alpha sequence contains specific signals, which allow for directing protein into cytoplasmic granules.