Alternate Transcription and Translation of the LIF Gene Produces a Novel Intracellular Protein

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THESIS SUMMARY

Cytokines are important biological molecules involved in a wide range of cellular processes. The classic mode of cytokine action involves interaction of the secreted molecule with a specific cell surface receptor that transduces a signal into the cell. This signal is transmitted into the nucleus, by a series of signalling pathways, producing an alteration in gene transcription and a cellular response. This mechanism of cytokine action may require modification due to the identification of cytokines that are localised within the cell, with intracellular localisation required for the cytokine response in some cases.

Leukemia inhibitory factor (LIF) is a member of the IL-6 family of cytokines and elicits a wide variety of effects on various cell types, *in vitro* and *in vivo*. The mouse LIF gene has been shown to express two proteins arising from alternate transcripts consisting of novel first exons spliced onto common second and third exons. The first exons of both transcripts contain an in-frame initiation codon that mediates translational initiation in exon 1, yielding secreted proteins of identical sequence that are localised to different cellular locations, one being a diffusible protein (LIF-D) and the other localised to the extracellular matrix (LIF-M). The two transcripts are differentially regulated indicating distinct biological functions for each protein.

In this work a third novel LIF transcript (LIF-T) was identified and cloned from murine, human and porcine sources. This transcript, which also contains an alternate first exon spliced onto common third and second exons, was differentially regulated in cultured cell lines and mouse tissues, indicating an important biological function. Genomic sequence comparison indicated a conserved, complex genomic organisation of the mammalian LIF gene in which three alternate transcripts containing novel first exons are expressed by differential promoter usage. Two classes of transcript are produced from the mammalian LIF gene; transcripts containing an in-frame initiation codon in exon 1, which include LIF-D from all species and murine LIF-M, and those that contain no initiation codon in exon 1, including LIF-T from all species and LIF-M from all species except mouse.

The absence of an in-frame ATG in the first exon of the LIF-T transcript was shown to direct initiation of translation to the first in-frame ATG in exon 2. This produced a truncated,

biologically active 17 kDa LIF protein that does not contain a signal sequence and is retained within the cell. Immunohistochemical detection of overexpressed intracellular LIF protein showed nuclear and cytoplasmic protein localisation.

Transfection studies showed that high and low level overexpression of intracellular LIF caused cells to undergo apoptosis, via a specific cellular pathway that was inhibitable by CrmA but not Bcl-2. Therefore, the cellular effect of the LIF-T protein was different to that of the extracellular cytokine, indicating an alternate mechanism of action and novel biological function for intracellular LIF. LIF-T induced apoptosis was shown to be a function of LIF intracellular localisation, and mutational analysis demonstrated that LIF-T action was independent of LIF receptor interaction and signalling. A novel, conserved structural motif was identified, containing a leucine repeat structure similar to a leucine zipper. Mutation of single leucines in this structure abolished nuclear LIF localisation and intracellular activity, without affecting extracellular LIF activity, demonstrating that extracellular and intracellular activity domains are localised in different regions of the protein. It was postulated that the intracellular LIF protein adopts an alternate structure to the extracellular molecule, involving a leucine repeat protein-protein interaction domain. This structure may act in the nucleus by dimerisation with other leucine repeat containing proteins, affecting gene transcription and causing a cellular response.

This study demonstrates a conserved structural organisation of the mouse LIF gene that produces three differentially localised proteins. This provides a mechanism for specific control of the sites of LIF action and mechanisms for mediating the variety of putative actions for the LIF gene. Intracellular localisation of the LIF protein provides another example of intracellular cytokine action, mediated by a novel mechanism and provides a system for separate analysis of intracellular and extracellular cytokines.