Synopsis

Leukemia inhibitory factor (LIF) is the most pleiotropic member of the interleukin-6 family of cytokines and can have paradoxically opposite effects in different cell types; stimulating or inhibiting each of cell proliferation, differentiation and survival. Many of the the actions of LIF are not apparent during ordinary development but are induced during tissue damage or injury thereby necessitating a very precise control of its spatial and temporal expression patterns. Regulation of gene expression occurs at different levels (from epigenetic to post-translational). Though regulation of LIF gene transcription has been studied in some detail and its promoter has been characterized, its regulation at post-transcriptional and translational levels remains largely obscure.

The 3′untranslated region (3′UTR) of *lif* mRNA is unusually long and fairly conserved with several conventional *cis*-acting elements and miRNA binding sites. PMA (phorbol-12-myristate-13-acetate) stabilizes *lif* mRNA in human histyocytic lymphoma cells, U937. To reveal the mechanisms of post-transcriptional regulation of *lif* expression, *lif* 3′UTR was characterized *in-silico* to reveal five conserved regions. Functional characterization of these conserved regions of *lif*-3′UTR by GFP-reporter analysis for mRNA decay demonstrated significant mRNA destabilizing potential of the proximal AU rich element containing region, which on treatment with PMA showed stabilizing effects. Increased binding of specific cytoplasmic proteins to ^{32}P-labeled-proximal-ARE transcripts by PMA treatment was observed and those were identified as Nucleolin and PCBP1. Nucleolin over-expression *in-vivo* stabilized GFP-reporter and intrinsic *lif*-mRNA while knockdown of Nucleolin and PCBP1 showed significant decrease in *lif*-mRNA and protein levels in U937 cells. The RNA-protein interactions were found to be direct and both proteins demonstrated RNA dependent *in-vivo* co-association with *lif*-mRNA, which increased on PMA treatment. PMA induced time dependent nucleo-cytoplasmic translocation of Nucleolin and PCB1 in U937 cells was not only a determinant of *lif*-mRNA stabilization, but also resulted in increased association of *lif*-mRNA, Nucleolin and PCBP1 with polysomes indicating their role in enhancement of translation of the message.

In-silico characterization of the *lif* 3′UTR for putative miRNA and RNA binding proteins binding sites revealed that the distal 204 bases at the 3′end of *lif* 3′UTR held significant promise containing the seed sequence (90% context score) for hsa-mir-181 family of micro-RNAs and highly AU rich sequences that could bind several RBPs in a context dependent

manner. At least four putative HuR binding sites could also be mapped on *lif*-E sequence. Using approaches similar to that for lif-B, it was found that HuR (a well known mRNA stabilizing factor) is the major *trans*-acting protein which not only binds *lif* mRNA directly and specifically but also stabilizes it significantly. Hur is also translocated to the cytoplasm on PMA treatment and binds *lif* mRNA, thereby stabilizing it. The potential of miRNA target of *lif* mRNA, hsa-mir-181 family was validated by over-expression of mir-181a in U937, which decreased the intrinsic levels of *lif* mRNA and GFP reporter *in-vivo,* indicating that lif-E was indeed a mir-181a responsive element and that the miRNA was capable of repressing *lif*-mRNA in U937 cells. PMA induced de-repression of mir-181-a mediated *lif* mRNA repression was observed on treating mir-181a overexpressing cells with PMA. The fate of intrinsic mir-181a levels in the context of PMA treatment in U937 cells was determined by comparing the mature mir-181a levels in untreated *vs*. PMA treated cells. Decrease in its levels indicated PMA mediated down-regulation. Another proposed mechanism of *lif* mRNA stability in U937 cells in response to PMA treatment therefore, is through the simultaneous occurrence of two events affecting the 3′end of *lif* 3′UTR namely, decrease in cellular levels mature mir-181a levels and the cytoplasmic translocation of HuR resulting in its elevated levels in the cytosol. Decreased target miRNA levels with increased concentration of an interacting mRNA stabilizing protein culminate in stabilization of *lif* mRNA.

The present study therefore functionally characterizes the *lif* 3′ UTR and identifies two regions in it that are significantly involved in *lif* mRNA stabilization, further delinating the mechanisms by which these regions mediate PMA induced stabilization of *lif* mRNA.