Functional Characterization of Leukemia Inhibitory Factor (LIF) 3'UTR: Elucidation of its Role in the Regulation of LIF Gene Expression

Abstract

LIF is a potent pleiotropic cytokine involved in diverse biological activities thereby requiring precise spatial and temporal control of its expression. The present study reveals that enhanced expression of LIF in response to PMA (phorbol-12-myristate-13-acetate) in human histyocytic lymphoma cell-line U937 is largely through *lif*-mRNA stabilization. The 3'-untranslated region (3'-UTR) of human *lif*-mRNA previously uncharacterized, is long and fairly conserved with several conventional *cis*-acting elements. Functional characterization of these conserved regions by GFP-reporter analysis for mRNA decay demonstrated significant mRNA destabilizing potential of the proximal AU rich element (ARE) containing region (lif-B) and distal ARE containing lif-E, which on treatment with PMA showed stabilizing effects.

Increased binding of specific cytoplasmic proteins to ^{32}P-*lif*-B with PMA treatment was observed and the proteins 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. 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 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.

lif-E is also a potent *cis*-element containing seed sequence for hsa-mir-181 family and at least four putative HuR binding sites. HuR was found to be the major *trans*-acting proteins that not only binds *lif* mRNA directly but also stabilizes it significantly. On PMA treatment, Hur is also translocated to the cytoplasm promoting mRNA stabilization. Over-expression of mir-181a in U937 on the other hand, decreased intrinsic *lif* mRNA and GFP reporter levels indicating that lif-E is a mir-181a responsive element. PMA induced the de-repression of mir-181-a mediated *lif* mRNA repression since intrinsic mir-181a levels decreased on PMA treatment. Therefore, decreased cellular levels of mature mir-181a and cytoplasmic translocation of HuR simultaneously culminate in stabilization of *lif* mRNA. Taken together, the study conveys a deep insight into the molecular mechanisms of post-transcriptional regulation of *lif* gene expression.

Alina Chakraborty, Department of Biophysics, Molecular Biology and Bioinformatics, CU.