1. SUMMARY

A genetic regulatory network in which a given transcription factor controls expression of a diverse set of genes often coordinates complex cellular responses. Interferon Regulatory Factor-2 (IRF-2) is a negative/positive transcription factor for many mammalian genes. It was identified as a transcription factor, which specifically bound the Virus Response Element (VRE) of IFN-\(\beta\) gene promoter and repeated hexamer motifs, \((\text{AAGTGA})_4\) or \((\text{GAAAGT})_4\) following virus induction of mammalian cells. DNA sequence elements recognized by IRF-2 are inducible by virus, dsRNA and IFNs. Variants of a consensus hexanucleotide of the nature GAAANN (N= any nucleotide) frequently occur in promoter/enhancer(s) of many virus- and IFN-inducible genes in murine and human cells. The tetrameric hexamer, \((\text{GAAAGT})_4\) when used as a promoter element in transfection studies, binds IRF-2 and gets repressed by IRF-2 to stimulate a downstream reporter gene following virus and IFN induction.

Interferon Regulatory Factor-2 (IRF-2) has generally been described as a transcriptional repressor, and is thought to function by competing with the transcriptional activator IRF-1. However, IRF-2 can also act as a positive regulator for interferon stimulated response element (ISRE)-like sequences such as the promoters histone H4, Vascular cell adhesion molecule-1 (VCAM-1), gp91phox, IL-7 and TLR-9. IRF-2 plays important role in cell growth regulation, and has been shown to act as a potential oncogene. Of late, report indicted that IRF-2 drives megakaryocytic differentiation through regulation of the thrombopoietin receptor promoter. Despite extensive information regarding involvement of IRF-2 in diverse cellular process as, very little is known about the mechanism(s) by which its structural domains function. IRF-2 is a typical modular protein comprising of many individual domains. Distinct domains have been identified (1-121 a.a.) DNA binding domain, (182- 218 a.a.) transcriptional activity, (210-265 a.a.) IRF association domain-2 (IAD2) and for transcription repression domain (325-349 a.a.). Its C-terminal domains facilitate the diversity in function of IRF-2. Its repression/activation potential is modulated by posttranslational modification and interaction with other factors and chromatin regulation. The specificity of interacting partners varies with the cell type and specific stimuli to evoke an appropriate response.
In the present study recombinant IRF-2 has been expressed in *E. coli*, its DNA binding activity has been determined and functional significance of the 314-317 a.a. region in IRF-2, which is different between mouse IRF-2 and human IRF-2 has been analyzed.

The present work originated from a comparison of the amino acid sequences of mouse IRF-2 (349 a.a.) and human IRF-2 (349 a.a.). The N-terminal DNA binding domain of IRF-2 is conserved, but the C-terminal domain has a stretch of four amino acids 314PAPV317 in the mouse IRF-2, which is different from 314SSSM317 in the human IRF-2. This indicated a possible functional significance of the four amino acid region in the two IRF-2 molecules. The 349 a.a. mouse IRF-2 was expressed from the pGEX2TK prokaryotic expression vector as a GST-IRF-2 soluble fusion protein (66 kd) in *E. coli* XL-1 blue cells upto 5% of total cellular protein. This was called as wild type IRF-2. In addition to the 66 kd GST-IRF-2, four more (approximately 62, 42, 32 and 30) IPTG inducible protein bands were observed as degradation products. DNA binding activity of IRF-2 with (GAAAGT)4 DNA sequence was measured by Electrophoretic Mobility Shift Assay (EMSA). Five IRF-2 DNA complexes were observed. As little as 0.5 μg of the GST-IRF-2/E. coli clone extract showed IRF-2-DNA complex indicating biologically active IRF-2. The murine IRF-2 sequence (PAPV) at 314-317 was replaced by SSSM, the corresponding region of the human IRF-2, by site-directed cassette mutagenesis. Both the wild type and mutant IRF-2 were prepared as GST-fusion proteins, which exhibited similar expression patterns. The mutant GST-IRF-2 clone showed three IPTG-inducible proteins bands of approximately 62, 32 and 30. They were checked for their DNA binding activity with (GAAAGT)4 oligonucleotide. The DNA-protein complex formation pattern was different for wild type and mutant IRF-2. Appearance of five bands with increasing concentration of the clone extract was observed in the following order: complex 4, complex 5, complex 3, complex 2 and complex 1. However, all complexes diminished with competition with 100 X molar excess of unlabeled (GAAAGT)4 indicating specificity of the complexes. A similar but non specific oligonucleotide (GAAA)6 did not show the complex formation. The mutant IRF-2 showed higher complexes than the wild type IRF-2. Also, the amount of the complexes was higher in the mutants. This reflects both qualitative and quantitative role of the PAPV versus SSSM at c-terminal 314-317 region in the DNA binding function of IRF-2. By western blot
analysis with anti-IRF-2 and anti-GST antibodies four bands were observed. The wild type and mutant IRF-2 were ectopically expressed from a mammalian expression plasmid (pCDNA3.1) in human embryonic kidney (HEK-293) cells, to find out if the c-terminal 314-317 of the IRF-2 has any role on IRF-2 regulated gene expression.

This is the first report of comparison of the mouse and human IRF-2, with respect to the 314-317 a.a. stretch, which is different in mouse and human IRF-2. Although the murine and human IRF-2 are molecularly homologous and physiological similar, replacement of the four a.a. in mouse IRF-2 by the corresponding region from human IRF-2 can influence DNA binding activity of IRF-2. This provides new information on the function of C-terminus of IRF-2. Overexpression studies of the wild type and mutant IRF-2 in HEK-293 cells would find differential effect(s) of the two IRF-2 molecules on the expression of IRF-2 regulated genes.