I. Summary

Complex cellular responses are often coordinated by a genetic regulatory network in which a given transcription factor controls expression of a diverse set of genes. Interferon Regulatory Factor-1 (IRF-1) is a positive transcription factor for many mammalian genes. It was identified as a transcription factor that specifically bound the virus response element (VRE) of IFN-β gene promoter and repeated functional hexamer motifs, (AAGTGA)$_4$ or (GAAAGT)$_4$ derived from it following virus induction of mammalian cells. DNA sequence elements recognized by IRF-1 are inducible by virus, double stranded RNA or poly (rI.rC.), IFN-γ, IFN-α, IFN-β and other cytokines like TNF-α, IL-1, hormones like Prolactin, genotoxic stress by UV and other agents. 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. When multimerised, such synthetic hexanucleotides act like virus, IFN, poly (rI.rC.) and IRF-1-inducible enhancers in transcription assays. The tetrameric hexamer, (GAAAGT)$_4$, when used as a promoter element in transfection studies, binds IRF-1 and gets activated by IRF-1 to stimulate a downstream reporter gene following virus and IFN induction.

IRF-1 is an important transcription factor with remarkable functional diversity including host response to pathogen infection, regulation of IFN- and other cytokine-inducible genes, development of lymphocytes, resistance to oncogenic transformation, regulation of cell cycle and programmed cell death following DNA damage. Despite extensive information regarding its involvement in diverse cellular processes, very little is known about the mechanism(s) by which its structural domains function. IRF-1 is a typical modular protein comprising of many individual domains. Distinct domains have been identified for its transcriptional activity i.e. 185-256 a.a. for activation, 1-60 a.a. for repression, 1-124 a.a. for DNA binding, 120-138 a.a. for nuclear localisation, 164-219 a.a. for heterodimerisation and 257-329 a.a. for enhancement of transcription. The diversity in function of IRF-1 is facilitated by its C-terminal transactivation domain spanning 140-329 a.a. Its transactivation potential is modulated by posttranslational modification and interaction with other factors. The specificity of interacting partners vary with the cell type and specific stimuli to evoke an appropriate response. Small regions in the
transactivation domain of IRF-1 are possibly involved in interaction with other factors to bring about activation of transcription. In the present study recombinant IRF-1 has been expressed in *E.coli*, its DNA binding activity has been determined and functional significance of the 198-203 a.a. region in the IRF-1 which is different between mouse IRF-1 and human IRF-1 has been analysed.

The present work originated from a comparison of the amino acid sequences of mouse (329 a.a.) and human (325 a.a.) IRF-1. The N-terminal DNA binding domain of IRF-1 is conserved, but the C-terminal transactivation domain has a stretch of six amino acids \(^{198}MQMDII^{203}\) in the mouse IRF-1 which is different from \(^{198}IPVEVV^{203}\) in the human IRF-1. This indicated a possible functional significance of the six amino acid region in the two IRF-1 molecules. The 329 a.a. mouse IRF-1 was expressed from the pGEX-2TK prokaryotic expression vector as a recombinant GST-IRF-1 soluble fusion protein (66 kd) in *E.coli* XL-1 Blue cells upto 5 % of the total cellular protein. This was referred to as wild type IRF-1. In addition to the 66 kd GST-IRF-1, three more (approximately 62 kd, 42 kd and 32 kd) IPTG inducible protein bands were observed. They may be degradation products. Its DNA binding activity with (GAAAGT)\(_4\) DNA sequence was measured by electrophoretic mobility shift assay (EMSA). Four IRF-1-DNA complexes (Complexes 1-4) were observed. As little as 0.5 Jlg of the GST-IRF-1 *E.coli* clone extract showed IRF-1-DNA complex indicating biologically active IRF-1. The murine IRF-1 sequence (MQMDII) at 198-203 was replaced by IPVEVV, the corresponding region of the human IRF-1 by site directed cassette mutagenesis. Both the wild type and mutant IRF-1s were prepared as GST-fusion proteins which exhibited the similar protein expression patterns. The mutant GST-IRF-1 clone showed four IPTG-inducible protein bands of approximately 66, 62, 42 and 32 kd. They were checked for their DNA binding activity with (GAAAGT)\(_4\) oligonucleotide. The DNA-protein complex formation pattern was similar for wild type and mutant IRF-1. Appearance of four bands with increasing concentration of clone extract in the following order was observed in both cases: Complex 4, Complex 3, Complex 2 and Complex 1. However, Complex 2 followed by Complex 1 were the first to diminish after competition with 25x molar excess of unlabeled (GAAAGT)\(_4\) indicating the higher specificity of the two complexes. A similar but nonspecific oligonucleotide (GAAA)\(_6\) did not show the
complex formation. Rabbit polyclonal antiserum was raised against the wild type GST-IRF-1 to study recombinant IRF-1 expressed in E. coli. By western blot analysis five bands approximately of 42, 48, 62, 66 and 80 kd. IRF-1 specific protein bands were observed. The wild type and mutant IRF-1 were ectopically expressed from a mammalian expression plasmid, pCDNA3.1 in human embryonic kidney (HEK-293) cells. Their DNA binding activity was measured by EMSA. IRF-1 specific complex which corresponded to the Complex 1 of GST-IRF-1 was observed in all sets: vector (pCDNA 3.1), wild type IRF-1 and mutant IRF-1 in EMSA with nuclear extracts from transfected cells. Untransfected HEK-293 cells showed formation of two bands with weaker intensity. The effect of replacement of this region on expression of a few genes that are transcriptionally activated by IRF-1 viz. IFN-β, iNOS, COX-2 was measured by RT-PCR. We found that expression of the wild type mouse IRF-1 activated IFN-β, iNOS and COX-2 genes but the mutant IRF-1 did not show this effect. Therefore, MQMDII (198-203 a.a.) in the mouse IRF-1 is required for the expression of the target genes used in this study and replacement of this sequence by IPVEVV (198-203 a.a.) did not show this function.

This is the first report of comparison of the mouse and human IRF-1 transactivation domain with identification of the 198-203 a.a. stretch to be different in mouse and human IRF-1. Assessment of the relevance of this 198-203 a.a. region by overexpression of wild type and mutant IRF-1 on the downstream target genes is also reported for the first time. Thus although the two murine and human IRF-1s are physiologically similar, replacement of a cluster of six a.a. in the mouse IRF-1 by the corresponding region from the human IRF-1 can abrogate the effect of overexpression of murine IRF-1 in a human cell line (HEK-293) i.e. upregulation of IFN-β, iNOS and COX-2 gene expression. Thus, the region MQMDII203 in mouse IRF-1 is important for expression of IFN-β, iNOS and COX-2 genes in the present experimental model system. This provides new information on the function of transactivation domain of IRF-1.