CONCLUSION
VIII. CONCLUSION

Interferons (IFNs) were discovered in 1957. They are a group of unique cytokines that induce a large number of Interferon-Inducible Genes (ISGs) through their cell surface receptors and JAK-STAT signal transduction pathways to stimulate antiviral, antiproliferative and immunomodulatory responses in mammalian cells. IFNs can also either upregulate or downregulate many other biochemical machinery in cells. Since 1979, when recombinant IFN was made available its importance in many cellular, biochemical and clinical research is being increasingly realised. In recent past IFN-α has been used for treatment of leukaemia and IFN-β for multiple sclerosis in human patients.

The 2',5'-oligoadenylate-dependent ribonuclease L (RNase L) is an important IFN-inducible unique RNase in mammalian cells. It is involved in antiviral, antiproliferative and proapoptotic functions through degradation of RNA when induced at transcriptional level by IFN or virus and activated at protein level by the 2-5A cofactor. IFN-inducible RNA degradation by RNase L is considered to be a unique mechanism for preventing proliferation of certain viruses. RNase L has been implicated in chronic fatigue syndrome (CFS) and is absent from certain human cancer cell lines. RNase L has a unique structure of a hybrid ribonuclease and kinase, which includes ankyrin repeats, phosphate-loop motifs, protein kinase domains, cystein-rich domains and RNA binding-ribonuclease domain.

In the present study, structure of the mouse RNase L gene has been investigated by Southern blot analysis. To understand RNase L function in prokaryotic environment, recombinant murine and human RNase L proteins have been expressed in the E. coli, its effect(s) on the cell growth and RNA degradation have been studied. Two types of human cancer cell lines: the Burkitt lymphoma (Daudi) cells and the cervical adenocarcinoma (HeLa S3) cells have been used to find out three aspects of RNase L function in human cells - (a) induction of RNase L gene expression by agents which include protein synthesis inhibitor (cycloheximide), double stranded RNA (poly I:C), cancer-chemotherapeutics (cisplatin, doxorubicin, vinblastine, vincristine), oxidative stress (H2O2), osmotic/membrane stress (calcium chloride) as well as the inflammatory cytokine (TNF); (b) correlation between RNase L expression and activation of the transcription factor Nuclear Factor kappa B (NF-κB); (c) correlation of RNase L expression and IFN-α-inducible antiproliferative
response and chemotherapeutic drug-induced apoptosis. Major findings of the present study can be concluded as follows:

1. Studies on RNase L gene structure in the mouse revealed that it is a relatively large gene extending over at least 16 kb. In addition to the earlier reports, it has 5' upstream proximal sites for Nco I, Hinc II, Bgl II and Eco RI, the 5' upstream distal sites include Bam HI and Xba I. A tentative 6.8 kb 5' upstream DNA is mapped.

2. Expression of mouse and human RNase L cDNA in E. coli revealed that although the gene is eukaryotic in origin, the human RNase L recombinant protein is toxic to the bacterial cells and it caused remarkable growth-suppression in E. coli. Comparison of RNA profiles from the E. coli cells expressing the recombinant mouse RNase L and human RNase L proteins revealed that the toxicity was due to degradation of the host cell RNAs in the absence of any exogenous 2-5A cofactor. Thus, human IFN-inducible RNase L is functional in prokaryotic environment. This raises exciting possibilities about evolution of the RNase L gene.

3. IFN treatment of the Daudi cells exhibited a dose-dependent and time-dependent antiproliferative response. Western blot analysis revealed that RNase L is constitutively expressed in human Burkitt lymphoma Daudi cells. IFN + cycloheximide treatment further induced this RNase L expression. Studies on the DNA binding activity of NF-κB in IFN-treated cells revealed that there is constitutive expression of NF-κB in these cells which may be accounted for its neoplastic nature. Thus, there is a correlation between the constitutive expressions of RNase L and NF-κB activity in the lymphoma cells.

4. The human cervical adenocarcinoma (HeLa S3) cells exhibited inducible RNase L expression and inducible NF-κB DNA binding activity. Expression of RNase L was induced by various stress-inducing agents e.g. dsRNA, cancer-chemotherapeutic drugs, oxidative stress (H2O2), osmotic/membrane stress (CaCl2) and the inflammatory cytokine, TNF. It strongly suggests that RNase L is not only an effector molecule in the IFN-inducible 2-5A antiviral pathway, but also is a stress-responsive molecule linked to apoptosis. This RNase L response to stress was shown to result in RNA degradation ultimately leading to cell death. All cells with inducible RNase L expression also exhibited features common to cells undergoing apoptosis e.g. cell shrinkage, nuclear condensation and DNA fragmentation.
The IFN-inducible 2-5A-dependent RNase L gene is thus responsive to oxidative stress and osmotic stress, inflammatory cytokine (TNF) and cancer chemotherapeutic drugs in human cells. This opens a broad range of functional significance for RNase L and RNA degradation under stress and apoptotic conditions. Further investigation into these aspects should provide novel insights into the pathophysiological role(s) of this IFN-pathway antiviral ribonuclease gene.