Summary
The work reported in this thesis is an effort to understand two major aspects of SARS nucleocapsid biology. We have identified five probable interactors for SARS N protein using a yeast two-hybrid system based screen. One of the interactors PIAS1 has been studied in detail and we found that in presence of N, the regulation imposed by PIAS1 remains no longer valid. When extrapolated to the real virus scenario, we found that some of the protein products regulated by PIAS1 have been found to be upregulated in SARS patients. A hypothesis in light of the existing literature has been put forward for the same. Further, we have made an effort to study in vitro assembly of N protein which led to formation of spherical particles. The assembly of viral capsids in presence of non specific oligonucleotides is not a novel phenomena but the observation that in vitro assembly of SARS N may require the presence of dATP is an unusual observation. Extrapolation of these results to a natural infection scenario may sound like an overstatement, nevertheless, the phenomenon observed by us can be unquestionably deemed as specific property of N protein.

A brief summary of the results is outlined below:-

**N assembles in vitro to form capsid like structure.**

- Purified recombinant N protein assembles in vitro into distinct spherical entities.
- The assembly of N requires the presence of non specific oligonucleotides and dATP.
- dATP binds to and changes the conformation of N so as to facilitate the process of assembly in vitro. Hence dATP might be acting as a co-factor for the assembly process.
- The assemblies thus formed are stable after multiple freeze thaw cycles and for prolonged periods of time at 4°C.

**N interacts with PIAS1.**

- N interacts with several cellular proteins: PIAS1 or Protein inhibitor of activated STAT1, Protein kinase, interferon-inducible double stranded RNA dependent activator, also known as PRKRA, or PACT, Topoisomerase I binding, arginine/serine-rich, also known as TOPORS, Phosphodiesterase 4D interacting protein or Myomegalin, FLICE-associated huge protein (FLASH).
Interaction of N with PIAS1 is valid *in vitro* and *in vivo* when both are co-transfected in COS-1 cells.

Interaction of N with PIAS1 leads to a change in the sub cellular localization of N within 48 hrs post-transfection from mostly cytoplasmic to mainly nuclear as studied by IFA.

The interaction leads to a release in the block induced by PIAS1 on NFκB DNA binding as seen by EMSA and NFκB mediated gene activation as deciphered by luciferase reporter assay.

Since, PIAS1 regulates a number of pro-inflammatory cytokines some of which have been documented to be upregulated in fatal cases of SARS, we hypothesize that N protein may play an important role in excessive expression/secretion of cytokines and chemokines thereby contributing significantly to the lung injury seen in fatal cases of SARS.
