Abstract
MicroRNAs (miRNAs) are small non-coding RNAs that have emerged as key post-transcriptional regulators of gene expression in eukaryotes. Since the discovery of first miRNA, *lin-4* in *Caenorhabditis elegans* (Lee et al. 1993), miRNA has changed our view on the term, non-coding RNAs. In humans, expression of more than 60% of genes is predicted to be regulated by these non-coding RNAs and their specific function in almost all the cellular pathways have been demonstrated (Friedman et al. 2009).

Biogenesis of miRNA begins with the cropping of primary miRNA(s) containing transcript by Drosha, an RNaseIII class enzyme present in the nucleus, which generates precursor miRNA (pre-miRNA); this 60-90 nucleotides long pre-miRNA gets transported to the cytoplasm by Exportin-5 with the help of Ran (Bartel 2004). In the cytoplasm, pre-miRNA that contains single stem-loop structure becomes the substrate to another RNaseIII class enzyme, Dicer, which cleaves pre-miRNA from both the sides and produces functional mature miRNA (Bartel 2004). Mature miRNA gets incorporated into the RNA-induced silencing complex (RISC), which harbors Argonaute protein. RISC complex determines the fate of target mRNA based on the extent of complimentarity between miRNA and its target (Bartel 2004). In animals, miRNAs generally repress translation via partial complementarity, while in plants, miRNA directs the cleavage of its perfectly complementary mRNA targets with the help of Argonaute proteins (Bartel 2004). Initially miRNAs function was believed to be limited to developmental processes, but after a decade, it was realized that they are macro regulators of almost all biological pathways, and hence have a prominent role in controlling various diseases including cancer (Ambros 2004; Xiao and Rajewsky 2009).

In 2004, virus encoded miRNA was discovered for the first time in Epstein-Barr virus (EBV) (Pfeffer et al. 2004), since then more than 200 miRNAs have been
identified from different groups of viruses (Skalsky and Cullen). Functional analysis of virus-encoded miRNAs has shown that small size and non-antigenic nature of miRNAs make them ideal tool for viruses to counterattack immune arsenal mounted by the host. Due to the small size of their genomes, viruses have very limited number of protein-coding genes, which they express using host machinery. For expressing miRNAs also viruses rely on the host factors, as to date there is no evidence of any virus-encoding miRNA pathway components. Virus-derived miRNAs may be categorized into two groups, first the “analogs” of host miRNAs, and second the virus-specific miRNAs (Kincaid and Sullivan). Mimicking a host miRNA seed region allows a viral miRNA to potentially regulate hundreds of transcripts that have evolved target sites for a particular host miRNA. Virus-specific miRNAs have the capability to modulate host as well as their own genes. Viral miRNA functions are vital for their survival and maintenance of latency (Grundhoff and Sullivan; Skalsky and Cullen). Viral miRNA studies are limited to the mammalian system, while insect viruses have hardly been investigated in this regard. When I started my study on insect viral miRNAs, only one report of insect virus encoded miRNA was published (Hussain et al. 2008).

The domesticated silkworm, Bombyx mori, mainly known for producing silk, is an emerging model to study host-pathogen interaction. B. mori is infected by different pathogens including bacteria, virus and fungi, and among all Bombyx mori nucleopolyhedrosis virus (BmNPV) is the most dreaded, which largely affects silkworm crop every year by infecting its larvae. Genome sequences of both the host, B. mori and the pathogen, BmNPV are available in the public domain (Gomi et al. 1999; Mita et al. 2004; Xia et al. 2004) and genes responsible for diverse functions have been characterized. Recently, miRNAs from B. mori have been identified (He et al. 2008) but BmNPV was not explored for the same.
I started my thesis work with an objective to characterize and carry out functional analysis of BmNPV-encoded miRNAs in *B. mori*. Therefore, in order to examine BmNPV-encoded miRNAs and their putative functions, I started by identifying miRNAs derived from BmNPV. **Chapter 1** describes experimental validation of five BmNPV-encoded miRNAs. These miRNAs were previously identified through computational prediction analysis tools in our lab (Singh et al. 2010), were experimentally validated by Northern blotting using RNA from the BmNPV infected *B. mori* fat body and midgut tissues, and reconfirmation of predicted viral miRNA expression was done using poly(A) tailed RT-PCR. Results showed that four of the five predicted miRNAs were expressed both in fat body and midgut tissues of BmNPV infected *B. mori*. The targets of all the four viral miRNAs in *B. mori* (host targets) as well as in BmNPV (virus targets) were also predicted using the various bioinformatics software. Expression analysis of BmNPV-encoded miRNAs showed that they are expressed in BmNPV infected tissues of *B. mori* and are most likely to play a vital role in virus infection, as they were found to have target sites on various viral and the host genes associated with replication and defense machinery, respectively.

miRNAs not only have the capability of targeting mRNAs with high specificity but can also regulate multiple transcripts to varying degree. Taking advantage of a conserved gene regulatory mechanism within the host cell, viral miRNAs can help in establishing a cellular environment conducive to viral replication. Viral miRNAs generally target apoptotic and immunity-related genes, which may otherwise be an obstacle for the virus in proliferating inside the host. **Chapter II** describes the function of a BmNPV-encoded miRNA, *bmnpv-miR-1*, that suppresses the biogenesis of host miRNAs. In this chapter, I have reported a new pathway of host defense modulation by a virus, where virus employs its miRNA to regulate host miRNA biogenesis by
regulating Ran, a co-factor of Exportin-5. The *bmnpv-miR-1* was found to have a target site on 3' UTR of Ran mRNA. I have demonstrated the sequence dependent interaction of *bmnpv-miR-1* with Ran mRNA using cell culture and *in vivo* assays. The results reported here demonstrated that *bmnpv-miR-1* represses Ran leading to a reduction in the host small RNA population and consequent increase of BmNPV load in the infected larvae. To reconfirm the observed effect on Ran and virus proliferation, blocking of *bmnpv-miR-1* was done using Locked Nucleic Acid (LNA) that resulted in higher expression of Ran and decrease in BmNPV proliferation. However, the blocking of host miRNA, *bmo-miR-8*, whose expression was repressed upon *bmnpv-miR-1* and Ran dsRNA administration, resulted in a significant increase in the virus load in infected *B. mori* larvae. Taken together these results suggested that BmNPV makes host environment suitable for its proliferation, by suppressing *B. mori* miRNA biogenesis, which play crucial role in counterattacking BmNPV proliferation.

Viruses are known to manipulate the host environment to persist their infection by employing miRNAs, but recent evidences have shown that viruses also regulate the expression of their own set of genes using miRNAs, to limit their titre initially, thereby avoiding recognition by the host immune system (Grundhoff and Sullivan; Skalsky and Cullen). Identifying the virus genes as the targets of viral miRNAs is easier than searching host targets simply because viral genomes encode fewer candidate mRNAs, and also several viral miRNAs lie antisense to viral transcripts, which are obvious potential targets. **Chapter III** describes regulation of BmNPV late genes by *bmnpv-miR-3*. Here I have reported that BmNPV-encoded miRNA, *bmnpv-miR-3*, that regulates the expression of its own late genes including basic DNA binding protein, P6.9. The P6.9 gene is expressed late in the infection process and speculated to be involved in the packaging of virus nucleocapsid (Maeda et al. 1991). *bmnpv-miR-3*
binds perfectly to 3' UTR of P6.9, because it transcribes from the antisense strand of the P6.9 gene in the viral genome. Further prediction analysis showed that bmnpv-miR-3 also has a binding site on the other BmNPV late genes, which are mostly required for transcription (lef-8), DNA recombination (vlf1), viral nucleocapsid packaging (p40 and p95), and liquefaction of virus-infected insect (fusolin and chitinase k1). The various cell culture and in vivo experiments were performed to analyze the role of bmnpv-miR-3 in BmNPV infection cycle. I have shown that bmnpv-miR-3 expresses early after infection and negatively regulates expression of P6.9 and other viral late genes, leading to controlled multiplication of virus in the early stage of viral infection. A remarkable increase in BmNPV load was noticed when bmnpv-miR-3 was blocked by its antagomir, which imply the involvement of P6.9 and other late genes in BmNPV proliferation.

A search for other agents involved in combating viral infections, revealed the antiviral property of two small accessory silk proteins of B. mori, Seroin1 and Seroin2 (Nirmala et al. 2001). Both seroins were found to be upregulated upon viral infection in B. mori fat body and midgut tissues. Chapter IV describes the characterization of seroins as antiviral agents of B. mori, probably under the regulation of host miRNAs. Seroins were previously discovered in Galleria mellonella as a component of silk producing protein complex and speculated to be involved in defence against microbes based on their induction upon injury to larvae, in fat body tissue (Zurovec et al. 1998). In the present study I have shown that seroin proteins of B. mori, Seroin1 and Seroin2 are inhibitors of BmNPV proliferation. The function of seroin proteins in response to BmNPV infection was first shown by knocking them down using dsRNA and estimating viral titre in BmNPV infected larvae. Antiviral nature of seroins prompted us to investigate the pathway(s) that they may follow. Recently Toll receptors in Drosophila
found to inhibit virus infection (Nakamoto et al.), similarly in my study, I also noticed that knockdown of various Toll receptors facilitate BmNPV infection in *B. mori* larvae, suggesting involvement of Toll pathway in antiviral function, although subsequent expression analysis of seroins in Toll receptor knockdown larvae showed no direct link between seroins and Toll pathway. Besides, I also found several *B. mori* miRNAs, which have potential binding sites on mRNAs of both seroins. *B. mori* miRNAs, bmo-miR-71 and bmo-miR-252 effectively reduced seroin2 transcript levels in *B. mori* cells suggesting that expression of seroins may be regulated by host miRNAs.

Overall, in my thesis work I have shed light on miRNA based host-pathogen interaction using silkworm-baculovirus model. Virus encoded miRNAs were shown to regulate host as well viral gene expression. Discovery of BmNPV-encoded miRNAs will certainly provide new ways to study silkworm-baculovirus interactions at the molecular level. Functional analysis of BmNPV-encoded miRNAs revealed two new mechanisms being operated at the level of host-virus interaction in *B. mori*. Firstly, virus manipulates the host miRNA pathway by employing one of its miRNAs; second, virus modulates its own genes expression in the early stage of infection to escape recognition by host defense machinery. Further, I have also reported the unusual function of small accessory silk proteins, seroins, in host defense against viral infections. Seroins may be used as potential candidates in developing virus resistant silkworm strains. My findings have set a new paradigm in host-pathogen interaction from viral infection perspective and further add knowledge to our understanding of baculovirus biology. Outcomes of this study will help us to design control measures to effectively prevent BmNPV infection in silkworm.