CHAPTER 7

FUTURE DIRECTIONS
This above described work has shed some light on the regulation of core clock gene *period* and regulation in circadian oscillation of the pacemaker neurons. This work has provided some answers as also raised some new question regarding circadian clock regulation. It has opened up many possibilities in the molecular clock regulation. Based in these findings we propose some future directions of our findings, which will further explore the circadian rhythm.

First of all the significance of the presence of two antisense transcripts with differential 3' end points and its role in the regulation of feed back loop is to be checked. Though the splicing of antisense transcripts is negated by indications of some experiments (Sq RT-PCR for reverse strand and the northern for mapping the small RNA) no direct experimental evidence has been obtained. So the splicing, if there is any, of the antisense transcripts have to be checked by specific experiments for the purpose. This can be done by doing PCR amplification of the reverse transcript specific first strand. For the amplification PAS2F can be used as the constant primer at the 3' end and at the 5' side variable primers covering the entire *period* gene region can be used and vice versa using PAS2R oligo. If from these direct experiments splicing is observed for the antisense transcripts then the exact sequence of the antisense transcripts have to be obtained in light of the splicing. Further new small RNA forming sequences can be found out by extending the range of the mapping experiment to encompass the whole antisense transcripts. Primer extension assays can also be done to characterize splicing.

The promoter for antisense *per* transcripts and the transcription factor (TF) specific for this antisense transcription has been predicted in this work their *In Vivo* existence can be examined. The molecular & behavioral studies of ADF-1 (predicted TF) and their contribution in the antisense production can be analyzed further.
Because of the finding of a novel sized small RNA hither-to unknown, the probability of the presence of a novel double stranded RNA specific RNase exists. Search has to be carried out to find such novel dsRNase molecule, if any.

It is hypothesized that association of the novel small RNAs with PIWI group of proteins is involved to carry out chromatin modification leading to the regulation of the central clock feedback loop work should be done to show this biochemical association. It is also required to show nuclear localization of the small RNAs and their chromatin modifying property. Rhythmic accumulation of acetyl and methyl histones and their role in chromatin modulation related to biological clock have to be done in flies, to exhibit the probable role of this per small RNA in this processes.

Earlier work has reported that a significant number of genes including that of humans, show overlapping. These have potential of forming sense-antisense overlaps (SA genes), and thus paired transcripts. These are examples of Cis-natural antisense transcripts. An increasing number of experimentally validated and identified antisense transcripts fall into this category. Characterization of such transcripts in various species has indicated the presence of a widespread RNA mediated gene regulatory phenomenon. In case of D. melanogaster there is an annotated gene CG2650 at the 3’ end of period in the reverse orientation showing complementarity. A 50 nt sequence is shared between the sense transcripts of per and CG2650. The 3’ UTR sequence of per sense transcript from 4478-4527 shows 100% complementarity with 1014-965 sequences of CG2650 transcript in a plus/minus pairing. This creates a possibility of formation of a partially overlapped double stranded RNA structure. Further there is evidence of a 0.9 kb rhythmic transcript being formed at this region. This transcript was reported to be strongly implicated in per’s control of biological rhythms. It was also reported that two arrhythmic mutations at the per locus reduces the level of this transcript (Reddy et al. 1984). This transcript is suggested to be of CG2650 origin in the FlyBase. Therefore this can be examined for its role on the feedback loop of the molecular clock.
Any work is meaningless without finding its application. So work has to be done to find how this new light on *per* antisense mediated clock regulation can be harvested to find solutions for various sleep disorders. The work on characterization of neurotransmitters involved in sleep pathway using *D. melanogaster* as an easy model has to be furthered. This will help to understand sleep regulation better, so as to find cures for different sleep disorders. This is more imperative as most of the sleep related medicines target neurotransmitters and not much is known about how they function. This will also help to find new drug targets.