Chapter 1: Introduction

Malaria remains a public health problem of enormous magnitude, affecting over 500 millions people every year. In the fight against malaria, there is an urgent need to develop new antimalarials and effective malaria vaccine because of wide spread resistance to common antimalarials. The progress in the development of effective antimalarials/ vaccine has been slow due to the lack of understanding of the functions of different parasite proteins. Reverse genetics is one of the ways to assess the function of parasite genes. Gene function elucidation by antisense approach, ribozyme technology and gene disruption technology has met with limited success. Even though transfection technology to knock out different genes has been developed for different malaria parasites, they are not being applied routinely due to extensive selection procedures. With the availability of malaria genome sequence, there is an urgent need to develop an effective molecular tool to carry out functional genomic studies in malaria.

Recently, RNAi has emerged as a powerful and quick tool to study the gene function in a wide variety of organisms. RNA silencing is a sequence specific RNA degradation process mediated by its own double stranded RNA. It differs from other methods of reverse genetics in terms of specificity and simplicity. Extensive genetic and biochemical studies have revealed a three-step mechanism for RNAi induced gene silencing. The first step involves degradation of dsRNA to small interfering RNA (siRNA) of 21-25nt long. An RNAse III like enzyme called DICER has been shown to be involved in this step. In the second step, RISC (**R**NA **i**nduced **s**ilencing **c**omplex) is activated by forming a complex with siRNA. The third step involves recognition of corresponding mRNA by siRNA-RISC complex and degradation of that mRNA by nucleases present in the complex. Currently RNAi is being used extensively in *C.elegans*, *Drosophila*, in cultured insect and mammalian cell lines to elucidate the function of the genes. In *C.elegans* RNAi has been successfully used to carry out large-scale functional genomic studies.

In this study, we developed and applied RNAi to know the function of two cysteines protease genes of *P.falciparum* and *P.berghei.* Since, RNAi was being used for the first time in malaria parasite, we attempted to silence the expression of cysteines protease genes because the effects of inhibitors of cysteines protease on parasite morphology and biochemistry was well documented. Moreover, both these

falcipain1&2 had been produced by recombinant means and their role in hemoglobin degradation was well established. However, direct evidence using gene disruptions as well as other gene silencing techniques were lacking to show the involvement of this protein in other parasite functions. In spite of extensive research on cysteine proteases, their precise role in parasite development and maturation has remained largely speculative.

Keeping this in mind, we made an attempt to understand the exact role of these proteases in *Plasmodium* using RNA interference. The experimental approaches applied were as follows:

- To develop methodology to carryout RNAi in *Plasmodium*
- DsRNA mediated gene silencing of falcipain 1&2 in *P.falciparum*
- Use siRNA of Falcipain1&2 to carryout RNAi in *P.falciparum*
- *In vivo gene silencing in P.berghei*- a mouse malaria model.