Acknowledgements

I thank my advisor, Dr. Kasbekar, for his guidance and training throughout my Ph. D. His scientific acumen, keen perception, creativity, and his boundless enthusiasm for science have deeply impressed me. I sincerely thank him for giving me a lot of freedom, his never-ending efforts for my improvement. He had also trained me to utilize time efficiently. He has been an excellent teacher. I am grateful to him for patiently going through the various versions of my thesis.

I thank my former and present Kaslab members for their warmth, suggestions and help. Ashwin helped me find my feet when I had just joined the lab and taught me most of the techniques. I sincerely thank him. Meenal also helped me learn various methods, inspired me to participate in conferences. Prakash was a great source of inspiration for preparing quality seminars; Felicite was the one who identified the Adiopodoumè strain as a dominant RIP-suppressor. Bhavani always reminded me about the time limitation and helped me to be focused. Ravindran was very good company after Ashwin left. Srividhya, Parmit and Mukund has been very friendly, cooperative, helped me various ways and provided me support after the senior members of the lab had left. Ganga was invariably kind to me and assisted me several times in official matters. Padma provided invaluable assistance in maintaining a constant supply of autoclaved media and clean glassware.

I thank all the members of the CCMB Genetics group for their inputs. I am grateful to Dr. Sonti, Dr. Ramanan, Dr. Siddiqi, Dr. Ray, Dr. Reddy, Dr. Shashidhara and Dr. Gowrishankar for their valuable inputs.

I thank the director of CCMB, Prof. Lalji Singh, for providing state-of-the-art facilities and also for maintaining the unique CCMB work culture that allowed me to complete various experiments smoothly.
The excellent support facility of CCMB in the form of the instrumentation section, the digital imaging (Giridharan and Rao), xerox, photography and the fine biochemical section made my work a lot easier. I thank Meher for the oligonucleotide primers, Nagesh, Bhaskar, Pradeep, Srikar, Pardu and other staff members for the sequencing. I also thank the library, hostel and canteen staff.

I thank Sahu and Pavan who have been the sources of my sustenance during my stay in CCMB. I will always treasure the friendship. I thank Prabhu, Amit, Gopal, Vishnupriya, and Rajendra for their help.

I am grateful to the Fungal Genetics Stock Center (FGSC), University of Missouri, for readily providing most of the Neurospora strains free of charges. The Sad-2 mutants were kind gifts of the late Dr. Robert L. Metzenberg. The RIP5 A and RIP 28 a strains were kindly provided by Prof. Hirokazu Inoue, Department of Regulation-Biology, Saitama University, Japan. I also thank my teacher Dr. Suvendra and his brother for inspiration.

I thank Dr. Ravi, Dr. Prabir, Suhasini, Chitnis, Anjana, Ram and Raju and all the people on the South Wing first floor for helping me. I thank all my friends at CCMB who made my stay very pleasant.

I thank Council of Scientific and Industrial Research (CSIR), India, for providing fellowship during my stay.

I am grateful to my Grandfather who played the fundamental role in shaping my educational life. I also thank all my family members for their constant support and inspiration.

Sadly, I had missed my Grandmother and uncle at the time of writing the thesis.

Finally, I express my gratitude to everyone else who helped me for Ph. D.
Synopsis

An unusual strain of *Neurospora crassa* was isolated in the 1950’s from Adiopodoumé, a village and research station in Cote D’Ivoire, West Africa. It was the only *Neurospora* strain found harboring active copies of a LINE-like transposable element named *Tad* (Kinsey and Helber, 1989). All the other wild-isolated *Neurospora* strains examined, including those of species other than *N. crassa*, contained only mutation-inactivated relics of *Tad* (Kinsey, 1989; Kinsey et al., 1994). Genome sequence analysis revealed the complete absence of intact transposable elements from the sequenced strain of *N. crassa* (Galagan et al., 2003; Galagan and Selker, 2004). The Adiopodoumé strain was one of seven wild-isolated strains identified in our lab as dominant suppressors of repeat-induced point mutation (RIP) using the *Dp(erg-3)*-based RIP assay (Noubissi et al., 2000). RIP is a genome defense process of *N. crassa* and other fungi that targets multiple G:C to A:T mutations and cytosine methylation to duplicated DNA sequences during the premeiosis of a sexual cross (Selker, 1990). The RIP suppressor phenotype could have contributed to the survival of *Tad* in the Adiopodoumé strain. Preliminary mapping studies revealed that this strain contains a dominant *Suppressor of RIP* (*Srp*) linked to the *mat* locus on linkage group (LG) IL (Bhat et al., 2003).

In Chapter 3, I describe the further mapping of *Srp*. I generated *mat A* and *mat a* strains with the suppressor phenotype (*Srp*') and used them to perform *Srp*-homozygous crosses. Surprisingly, a subset of progeny from these crosses lost the *Srp* phenotype and instead showed an intermediate (*Srp*') or non-suppressor (*Srp*) phenotype. This result indicated that *Srp* is subject to variable expressivity and incomplete penetrance. Therefore only the *Srp* segregants were used to genetically localize *Srp*. Four *leu-3 Srp a* strains were crossed with *arg-1 A* or *arg-3 A* strains and four *Srp arg-3 a* strains were crossed with the *leu-3 A* strain. These crosses were designated, respectively, as class 1 and class 2. Most progeny from these crosses are auxotrophic for either leucine or arginine, but prototrophs are produced by crossover between *leu-3* and *arg-1* (or *arg-3*). Since *Srp* and *mat* were closely linked, the auxotrophic markers also bracketed *Srp*. The
Srp phenotype of the prototrophic segregants was determined by performing crosses with Dp(erg-3) strains of the opposite mating type and the crossovers that generated them were mapped with respect to RFLPs between the Adiopodoumé and OR strains. The Srp" prototrophic segregant #117 A was identified among 177 class 1 crossover progeny, and it marked the distal boundary of the Srp region whereas the Srp" prototrophic segregant #51 a, one of 81 class 2 crossovers progeny, marked the proximal boundary. Single nucleotide polymorphisms were used to delimit the Srp region to a ~34 kb segment located ~26 kb proximal to mat.

Chapter 4 describes the sequencing of the Srp region and the testing of a candidate gene for identity with Srp. Sequencing of the Srp region revealed that the Adiopodoumé strain contains a highly variant upr-1 allele (upr-1Ad). Both upr-1Ad and the standard laboratory Oak Ridge (OR) allele (upr-1ORA) share identical introns of 101 bases but differ at 118 positions in their coding sequences, including 66 non-synonymous changes. We disrupted upr-1Ad by homologous replacement with the hph selectable gene. The [(upr-1Ad A + upr-1Ad :: hph A)] heterokaryotic primary disruptants were crossed with OR a and all progeny inheriting the upr-1Ad :: hph A disruption were found to have the Srp" phenotype whereas a significant proportion of the progeny inheriting the intact upr-1Ad allele retained the Srp" phenotype. Since upr-1Ad was essential for the Srp" phenotype, therefore Srp was identical with upr-1Ad. The upr-1 gene encodes the catalytic subunit of the translesion DNA Pol ζ (zeta). Dominant RIP suppression by upr-1Ad was independent of the Pol ζ regulatory subunit encoded by the mus-26 gene.

In addition to Srp, large chromosome segment duplications (Dp’s) also can display the dominant RIP suppressor phenotype, probably because they titrate out the RIP machinery (Bhat and Kasbekar, 2001; Bhat et al., 2003; Vyas et al., 2006; Singh and Kasbekar, submitted). In Chapter 5, I describe studies that showed a difference in dominant RIP suppression by Srp and Dp’s. I constructed the heterokaryotic strain [(leu-3 Srp; Sad-2 a) + (erg-3; his-1 a)] and crossed it with the strain R; Sad-2; Dp(dow) A.
Mating of \( R; Sad-2; Dp(dow) A \) with the \( leu-3 Srp; Sad-2 a \) component is non-productive due to homozygosity for \( Sad-2 \), and crosses of \( R; Sad-2; Dp(dow) A \) with adventitiously formed \( erg-3; his-1 a \) homokaryons also are non-productive because both \( R \) and \( erg-3 \) confer female-sterility. Since the heterokaryon is female-fertile, progeny are obtainable only from the mating between \( R; Sad-2; Dp(dow) A \) and the \( erg-3; his-1 a \) component of the heterokaryon. In this cross, RIP was suppressed in the \( R; Sad-2; Dp(dow) A \) nuclei, thus showing that \( Srp \) can act in \textit{trans}. In contrast, RIP was not suppressed in the \( R; Sad-2; Dp(dow)A \) nuclei when the heterokaryon \([(Dp(IBj5); trp-1; Sad-2 a) + (erg-3; his-1 a)] \) was crossed with the \( R; Sad-2; Dp(dow) A \) strain. The \( \sim 400 \) kb duplication \( Dp(IBj5) \) was shown previously to be an effective dominant suppressor of RIP (Vyas \textit{et al.}, 2006). In other words, RIP suppression by a presumed titration of the RIP machinery was limited to the \( Dp \)-bearing asci whereas RIP suppression by \( Srp \) is able to spread to the \( Srp^+ \times Srp^+ \) asci of the mosaic perithecia that form in \([Srp^+ \times Srp^+] \times Srp^+ \) crosses.

Translesion DNA polymerase are able to replicate through DNA lesions (Goodman, 2002). They are error prone polymerases that lack 3'-exonuclease proofreading activity. They carry out aberrant DNA synthesis with poor replication accuracy and can efficiently extend from mismatches. I generated RIP-induced mutations in the genes for the translesion DNA polymerases Pol \( \eta \) (eta), Pol \( \iota \) (iota) and Pol \( \kappa \) (kappa). Additionally, I also generated mutants in Pol \( \zeta \) like those made previously by Hirokazu Inoue and colleagues (Sakai \textit{et al.}, 2002; 2003). I used these mutants to test the role of their gene products in RIP. None of the translesion DNA polymerase tested was essential for RIP (Tamuli \textit{et al.}, 2006). This work is described in Chapter 6.