Recent studies have suggested a mechanistic link between silencing and DNA replication, which occurs possibly through modulation of chromatin assembly. Our laboratory has recently shown that a mutation in the DNA repair gene *rhp6*, which is required for post-replication DNA repair and believed to conjugate ubiquitin to histones and other unknown targets, also causes derepression of the silent loci. Unlike global regulators of silencing in *S. pombe* namely *swi6, clr1- clr4* and *rik1*, *rhp6* plays a unique role in mating type silencing that is dependent on the switching competence of mating type loci. It was suggested that *rhp6* acts globally either directly or indirectly in re-establishment of chromatin structure at the three mating type loci after DNA replication and switching. It was to address the mechanism of *rhp6* in silencing that this study was undertaken. The following conclusions can be drawn from this study.

- Several extragenic suppressors of the *sng1-1/rhp6*$^-$ mutation were isolated. Complementation studies revealed that they belong to four complementation groups and accordingly denoted as suppressors *rhp6*$^-$, *sur1-sur4*. Surprisingly, the suppressors suppressed the *rhp6*$^-$ mutation by restoring the splicing defect *rhp6* pre-mRNA to varying levels.

- One of the genes *sur2*, was found to belong to the AAA (ATPase associated with different cellular activities) motif-containing proteins. It is for the first time AAA protein is shown to be involved in pre-mRNA splicing. *sur2* also shows considerably homology to the human spastin gene which is associated with spastic paraplegia.

- A 22 kDa protein was identified as an *in vivo* target and mediator of *rhp6* in mating type silencing. Both the overexpression and deletion of the gene encoding

the p22 kDa protein elicit switching dependent loss of silencing. The protein undergoes ubiquitination in a cell cycle-dependent manner and is nuclear localized during late S phase in wild type cells, while in the *sng1-1/rhp6*$^{-}$ mutant it is present in both cytosol and nucleus throughout cell cycle. Interestingly, its sequence indicated presence of histone-fold motif similar to that of histone H2A. Just like H2A, p22 interacts strongly with histone H2B *in vitro*. This protein, renamed as ubiquitinated histone-like protein, uhp1, is thus an *in vivo* mediator of rhp6 in silencing. The spatiotemporal control of nuclear entry of uhp1, its association with chromatin and ubiquitination, followed by degradation, is important for reestablishment of the inactive parental chromatin structure at the silent mating type loci after DNA replication.

- Our studies have identified another important mediator of *rhp6* required for its silencing function as rum1. A reciprocal connection between uhp1 and rum1 levels was observed in our studies suggesting rum1 may regulate the level of *uhp1* in a cell cycle-dependent manner. rum1 is an important cell cycle regulator. Further *rum1* mutation was found to derepress silent mating type loci but not other genes, suggesting that uhp1 and rum1 may be a part of complex that regulates silencing by bringing about chromatin remodeling in a cell cycle dependent manner. Thus, our studies for the first time suggest coupling of chromatin remodeling with cell cycle.

- Further, *uhp1* was found to genetically interact with *clr4* but not with other genes like *clr1-clr3* or *swi6*. Overexpression of *uhp1* in *clr4*$^{-}$ mutant and h^{90} strain caused a stable change in staining, *i.e.*, from dark to light, suggesting a role of

uhp1 in establishing an epigenetic chromosomal state. However, further molecular and genetic studies need to be carried out to confirm this.

- uhp1 may also be involved in directionality as indicated by increased level of sporulation in h^{09} strain in which *uhp1* is overexpressed. This effect is *swi6-mod*$^{+}$ dependent, since it is not observed in h^{09} *swi6-mod*$^{-}$ strain. Since *swi6* has also been shown to be involved in directionality, uhp1 probably acts in the same pathways as swi6, in not only affecting directionality but also in silencing.