The silent mating type loci mat2 and mat3 in fission yeast are maintained in repressed state generation after generation, while the same genetic information at the mat1 locus is always expressed. A novel ts' mutant of fission yeast was isolated, in which the silent mating type loci are derepressed. The derepression is dependent on the efficient switching of the silent cassettes. The mutation was identified to lie in the DNA repair gene rhp6. The chromatin structure at the silent mating loci was analyzed by using the E. coli dam methylase probe developed in this study. The E. coli dam methylates Sau3AI sites preferentially in active genes as compared to inactive genes. We found that Sau3AI sites located within the mat2P and mat3M loci are preferentially methylated in h9', swi6 as well as the h9', rhp6' strains. However, in non-switching background the rhp6' mutant exhibits a much lower level of methylation. It is also observed that rhp6 mutation does not alter chromatin structure at the level of nucleosome. FACS analysis and microscopic studies provide evidence that rhp6 gene is involved in chromosome compaction and segregation during mitosis. Main findings of this study in brief are -

* A Novel mutant of S. pombe involved in silencing was isolated.
* Mutant was named sngl' (silencing not governed) and identified as rhp6 by cloning.
* sngl' /rhp6' mutation causes the expression of the silent mating type loci in S. pombe.
* Silencing defect in rhp6'/sngl' mutant is caused by the alteration in the chromatin structure of the silent mating type loci.

* Silencing defect and chromatin unfolding in the rhp6'/sngl' mutant are switching-dependent.
* rhp6 mutation does not alter global nucleosome positioning at mating type loci.
* rhp6 gene is also involved in chromosome segregation/condensation during mitosis.
* rhp6 gene is not involved in replication checkpoint mechanism.

CONCLUDING REMARKS

Maintenance of the differentiated state of a eukaryotic cells requires that the chromatin structure and expression status of cell type-specific genes be re-established after DNA replication, when the nucleosome structure at the replication fork is known to be conceivably perturbed. Simultaneous assembly of nucleosomes at the replication fork must coordinate the duplication of DNA in vivo. Moreover, the transient
perturbation of nucleosomes during replication is likely to alter the transcription activity of the gene. Thus, it is paramount that the original chromatin structure be re-established in the differentiated cell following every replication cycle. The problem of reestablishment of the higher-order structure is particularly challenging in case of inactive genes that have a complex folded structure, which is inaccessible to transcription machinery and probes like DNaseI and *dam* methylase. Therefore, it is likely that eukaryotic cells have a molecular mechanism that couples DNA replication to re-establishment of the chromatin structure. Notably, a recent study has shown a coupling of chromatin assembly at the templates undergoing DNA repair by the chromatin assembly factor (CAF-1) which had previously been shown to play a role in assembly of nucleosomes on replicating DNA (Smith and Stillman, 1989). More interestingly, an intimate role of the chromatin assembly genes *CAC1* and *CAC2* (Kaufman *et al.*, 1997) and *RLF2* (Enomoto *et al.*, 1997) in telomere silencing has also been demonstrated in budding yeast.

Our results provide support for the suggestion that a transient window of opportunity is available during chromosome replication to remodel chromatin structure, a general mechanism postulated for modulating gene expression in development.

It is likely that *rhp6* acts globally either directly or indirectly in re-establishment of chromatin structure of the *mat1, mat2* and *mat3* loci after DNA replication and switching. The *rhp6* mediated ubiquitination of a key chromatin assembly protein may be a critical event in this process as proposed in hypothetical model (Fig. 4.1). Interesting work by Bailly *et al.* (1994) demonstrated that both RAD6 (of *S. cerevisiae*) and *rhp6* (of *S. pombe*) interact physically with the UBR1 protein (required for N-end rule proteolysis) and with RAD18 (which binds to single-stranded DNA). Such interactions may target RAD6 to proteolytic substrates to effect protein degradation during DNA replication, recombination, and repair, which may also affect chromatin assembly. Additionally, it is possible that other proteins involved in DNA repair, such as RAD18, also participate in switching-mediated chromatin alteration.

*rhp6/rad6* appears to be involved in several cellular functions: silencing, chromatin compaction, and DNA repairs. This study highlights a specific function:
that of coordination of mating type switching and silencing and suggests underlying connections between the replication associated with switching, chromatin assembly and silencing and the findings of this study may have relevance for higher eukaryotes.