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 *Sau*3AI sites preferentially in active genes as compared to inactive genes. We found that *Sau*3AI sites located within the *mat2P* and *mat3M* loci are preferentially methylated in h^{90}, *swi*6 as well as the h^{90}, *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 *sng1*⁻ (silencing not governed) and identified as *rhp6* by cloning.
* *sng1*⁻ /*rhp6*⁻ mutation causes the expression of the silent mating type loci in *S. pombe.*
* Silencing defect in *rhp6*⁻/*sng1*⁻ mutant is caused by the alteration in the chromatin structure of the silent mating type loci.
* Silencing defect and chromatin unfolding in the *rhp6*⁻/*sng1*⁻ 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

erturbation of nucleosomes during replication is likely to alter the transcription ctivity of the gene. Thus, it is paramount that the original chromatin structure be re-stablished in the differentiated cell following every replication cycle. The problem of eestablishment of the higher-order structure is particularly challenging in case of nactive genes that have a complex folded structure, which is inaccessible to anscription machinery and probes line DNaseI and *dam* methylase. Therefore, it is kely that eukaryotic cells have a molecular mechanism that couples DNA replication re-establishment of the chromatin structure. Notably, a recent study has shown a oupling of chromatin assembly at the templates undergoing DNA repair by the nromatin assembly factor (CAF-1) which had previously been shown to play a role assembly of nucleosomes on replicating DNA (Smith and Stillman, 1989). More nterestingly, 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 een demonstrated in budding yeast.

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

It is likely that *rhp6* acts globally either directly or indirectly in re-stablishment of chromatin structure of the *mat1, mat2* and *mat3* loci after DNA eplication and switching. The *rhp6* mediated ubiquitination of a key chromatin ssembly protein may be a critical event in this process as proposed in hypothetical nodel (Fig.4.1). Interesting work by Bailly *et al.* (1994) demonstrated that both AD6 (of *S. cerevisiae*) and rhp6 (of *S. pombe*) interact physically with the UBR1 rotein (required for N-end rule proteolysis) and with RAD18 (which binds to single-tranded DNA). Such interactions may target RAD6 to proteolytic substrates to effect rotein degradation during DNA replication, recombination, and repair. which may lso affect chromatin assembly. Additionally, it is possible that other proteins nvolved in DNA repair, such as RAD18, also participate in switching-mediated nromatin alteration.

rhp6/rad6 appears to be involved in several cellular functions: silencing, nromatin compaction, and DNA repairs. This study highlights a specific function:

at of coordination of mating type switching and silencing and suggests underlying nnections between the replication associated with switching, chromatin assembly d silencing and the findings of this study may have relevance for higher eukaryotes.