SUMMARY

The requirement of ARS element-containing cis-acting regions flanking the donor cassette for silencing in *S. pombe* suggests that a generalized mechanism involving DNA replication in establishment of hetrochromatin structure may operate in *S. pombe* and other eukaryotes. So to check the involvement of DNA replication in silencing we tested several temperature sensitive (ts^-) mutant alleles of DNA polymerase α for defect in mating type silencing. *swi7H4* ts^- mutant allele of DNA polymerase α (originally isolated by Okayama) was found to be defective in mating type silencing. We checked the silencing defect by RTPCR analysis and by expression of *ura4*$^+$ marker gene inserted at silent locus under different genetic backgrounds. We found that *swi7H4* mutant exhibits derepression of the silent loci and the *ura4*$^+$ marker placed in their vicinity. In combination with deletion of cis acting silencer/ARS element, it exhibits two stable states of silencing that are inherited as epigenetic chromosomal loci through mitosis and meiosis. Interestingly, we observed that derepression phenotype is dominant and persists as a stably marked chromosomal locus even when the *swi7H4* mutation is segregated away in genetic cross. By using *in vivo* chromatin probe in form of integrated copy of *E.coli* dam methylase gene, we found that derepressed silent loci is more accessible to dam methylase as compared to repressed silent loci suggesting a change in chromatin structure at the silent mating type loci in *swi7H4* mutant strain.

We have also found that efficiency of mating type switching is reduced drastically in double mutants of *swi7H4* with *clr1-4* and *swi6*, having no effect on the level of double- strand break at mat1 locus, suggesting that pol-α may interact with these hetrochromatin proteins to bring about switching. This genetic interaction was

also supported by the finding that there was no cumulative effect of *swi7H4* mutation on silencing with *clr1-4* and *swi6*. So we checked the physical interaction of *swi6* and *clr4* proteins (chromodomain proteins) with DNA polymerase-α protein. We constructed $(His)_6$-swi6, GST-clr4 and MBP-pol-α fusion proteins. Our binding assay shows that polα protein is retained by $(His)_6$-swi6 column demonstrating a direct interaction of swi6 protein with DNA polymerase α protein *in vitro*.

Furthermore, in *swi7H4* mutant the cells have elongated telomeres as compared to wild type suggesting a role of DNA polymerase α in maintenance of telomere length. An effect of swi7H4 mutation on telomere and centromere silencing was also observed. We also found that *swi7H4* strain exhibit shows cold sensitivity as was also observed in *swi6* mutant by Allshire, indicating that DNA polymerase α and swi6 participate in the same pathway. In *swi7H4* mutant the rate of loss of artificial chromosome increases by 70-fold suggesting that DNA polymerase α is also required for chromosome stability. Finally few suppressors of *swi7H4* were obtained by EMS mutagenesis. Cloning by complementation analysis identifies a Tup1 like protein, which suppresses the ts^+ phenotype of *swi7H4* suppressor.

Another mutant allele of DNA polymerase α was found to increase the rate of revertants of ade6-210 mutant in selective medium, indicating its role in adaptive mutation.

Significance:

1. We have demonstrated for the first time, a direct role of DNA polymerase-α in assembly of the hetrochromatin structure at the three main hetrochromatin loci in yeast, namely, mating type loci, telomeres and centromeres. Our results suggest a

novel model for hetrochromatin assembly wherein DNA polymerase-α can recruit one or more components of hetrochromatin proteins during lagging strand synthesis.

2. We have identified a novel mutant of DNA polymerase-α which exhibits elevated rate of adaptive mutations, suggesting that DNA polymerase-α may actively suppress the process of adaptive mutations in yeast and other organisms.