Ashok Kumar (2009). ***Role of chromatin assembly factor-1 and Pap 1 in gene silencing in schizasachharomyces pombe*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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SUMMARY OF THE THESIS

Excellent examples of the role of chromatin in inheritance are provided by the study of yeast functions that depend on heterochromatin regulation. Heterochromatin is a densely packaged form of chromatin, which can restrict the access of DNA to regulatory factors and generally found near telomeres as well as in the centromeric regions of eukaryotic cells. In the fission yeast heterochromatin is assembled at different loci regulating transcriptional silencing, maintaining and preserving genomic integrity. To uncover some of the aspects in fission yeast, we have done this study which cast a light on the role of chromatin assembly factor-1 and Pap1 in the different but essential functions during the life cycle of fission yeast.

The present study was focused on the studying the following aspects of heterochromatin transcriptional silencing in fission yeast:

I. Role of Chromatin assembly factor-1 (CAF-1) in gene silencing in *Schizosaccharomyces pombe*.

II. Role of Pap1 in gene silencing in fission yeast.

I. Role of Chromatin assembly factor-1 (CAF-1) in gene silencing in fission yeast:

Chromatin is a highly dynamic structure that plays an essential role in regulating all nuclear processes such as DNA replication, repair, transcription and recombination. Thus, the mechanisms by which chromatin structures are assembled and modified are questions of broad interest. The fundamental repeating unit of chromatin is the nucleosome, comprising 147 bp of DNA wrapped around an octamer of core histones. The core histone octamer is deposited onto the DNA in a stepwise process. The formation of chromatin is mediated by a class of proteins termed chromatin assembly factors. Chromatin assembly factor-1 (CAF-1) functions as a histone chaperone. CAF-1 is a three-subunit complex whose structure and function have been highly conserved through eukaryotic evolution from yeast to humans. CAF-1 performs the first step by bringing newly synthesized histones H3 and H4 to replicated DNA, followed by H2A and H2B.

In our study we deleted the genes encoding Cac1, Cac2 and Mis16 subunits of CAF-1 in *Schizosaccharomyces pombe* and studied their role in growth, mating-type

switching, chromatin assembly and gene silencing. The major findings of this part of present study are:

- CAF-1 is also evolutionary conserved in fission yeast.
- **CAF-1 disruption affects the transcriptional silencing:** The *cac1* and *cac2* deletion causes derepression of *ade6* at mating-type region. CAF-1 disruption also causes derepression of *ade6* at outer repeats of centromere. Deletion of *cac2* causes modest derepression of *ura4* at inner-most repeats of centromere. Disruption of CAF-1 enhances the silencing of *ura4* at central core repeats of centromere. No effect of CAF-1 disruption on expression of *ura4* and *LEU2* inserted at ribosomal-DNA locus. Mis16 has anti silencing effect on *ura4* at telomere locus.
- CAF-1 disruption is indispensable for the maintenance of heterochromatin at *otr::ade6* of centromere.
- The centromere-phenotype is quite stable.
- Deletion of *cac2* and mutation in *mis16-53* causes enhanced chromosome loss rate.
- CAF-1 is not involved in UV and Gamma ray mediated DNA damage repair.
- Our results show that *mis16-53* mutant is sensitive to Hydroxyurea and our results also indicate that Mis16 is involved in DNA replication fork stability.
- Strains harbouring *cac1*Δ and *cac2*Δ showed sensitivity towards the TSA (Histone deacetylase inhibitor) which suggest that they may affect histone acetylation pattern.
- *cac1* and *cac2* genes do not restore the silencing of Δ*mat2::ura4* and *ura4* at outer repeats of centromere in *swi7-H4* mutant. Thus, Cac1 and Cac2 may not interact with Polα in fission yeast.

From all these results, we could combine the insights provided by fission yeast in order to build our understanding of the DNA-replication machinery and mechanism of chromatin assembly with respect to heterochromatin formation.

II. Role of Pap-1 in gene silencing in fission yeast:

All organisms are constantly exposed to exogenous and endogenous genotoxic assaults that challenge genome integrity. Preservation of genomic integrity involves

multiple biological processes, including DNA-replication, DNA-repair, and signalling pathways that coordinate DNA metabolism with cell cycle progression (Kolodner *et al.*, 2002).

In fission yeast, transcription factor Pap1 has been implicated in the oxidative stress response which also contains bZIP DNA binding domains. In response to low levels of oxidative stress, Pap-1 controls the transcription of many target genes or activates stress-activated signalling cascade MAP (Mitogen Activated Protein) kinase pathway. One of these, a stress-activated protein kinase-Sty1 controls the activity of Pap1 and Atf1. Apart from this it was suggested that these stress sensors act in a parallel mechanism to the RNAi pathway for heterochromatin nucleation and also shown potential roles of ATF/CREB family proteins in chromatin remodelling and gene silencing (Hirota *et al.*, 2004). A recent study has also shown that Atf1 and Pcr1 are indispensable for the initiation of heterochromatin formation via a Swi6-based mechanism in the absence of RNAi-dependent heterochromatin assembly pathway (Kim *et al.*, 2004).

In our study, we observed that the 11 kb long *K*-region contains three Pap1-recognition sequences **(TTAGTCA)** at position 3176, 5370 and 5573, and two of them are present in *cenH* region. Similar sequences are also present at the following regions: 1 in telomere, 7 in *cen-I*, 16 in *cen-II*, and 37 in *cen-III* region. Therefore, we investigated the role of Pap1 in gene silencing at centromere and mating-type region. First, we constructed strains containing deletion of recognition sites in *cen-H* region of *S. pombe* and used these strains to study the role of Pap1 in gene silencing. Our results throw the light on the stress related pathway through which the gene silencing takes place. The major findings of this part of present study are:

- Our results show that *pap1*Δ causes derepression of *ade6, ura4* or *LEU2* reporters located at mating-type, centromere and *r-DNA* loci.
- Deletion of *pap1* affects silencing of marker genes at donor mating-type loci. We found that the derepressed state of *ade6* at *mat-3M* locus, which displays a pink phenotype, is less stable than the white, fully derepressed phenotype.
- Deletion of Pap1 or AP-1 binding sites also affects the silencing of marker genes at outer repeats of centromere-1. We found that there is slight derepression of *ade6* at *otr-1R* of *cen1* in *pap1*Δ and *pbs-otr-1111*Δ mutants. Individual phenotypes are not stable as they tend to inter-convert into

repressed forms of phenotype. Our results also indicate that the sectored phenotype is more stable phenotype as compared to the pink phenotype.

- Deletion of *pap1* exerts an anti-silencing effect on *ura4* marker at inner-most and central core repeats of centromere-1.
- Deletion of *pap1* causes derepression of *LEU2* but does not affect on the expression of *ura4* at *r-DNA* loci.
- A *pap1*Δ mutant does not affect the silencing of *ura4* at telomere locus.
- Pap1 is not involved in chromosome stability.
- *pap1*Δ mutant is super sensitive to histone deacetylase inhibitor (TSA). Therefore, we conclude that Pap1 is one possible target for TSA. One possibility is that Pap1 may interact with and recruit HDAC's like Clr6, and possibly Clr3, Sir2 and Hda1.
- *pap1*Δ mutant is not sensitive to cold-shock and the microtubule destabilizing drug, thiabendazole (TBZ).
- *pap1*Δ and oxidative stress are epistatic with respect to transcriptional silencing at centromere.
- *pap1*Δ and *pbs-otr-1111*Δ affects the localization of Swi6 and H3 Lys9 methylation at centromere.
- Pap1 does not interact with Swi6, Clr4 but shows genetic interaction with Clr3. Our over expression results show that while *swi6* and *clr4* were unable to restore silencing of *ade6* at outer repeats of centromere in *pap1*Δ strain, *clr3* could do so suggesting that Pap1 may help in recruiting Clr3 (and possibly Clr6) to initiate deacetylation of H3-K9 and H3-K14, a step preceding H3-K9 methylation by Clr4 and the subsequent binding by Swi6 to H3-K9me2.

Overall we can conclude that Pap1 and Pap1-recognition sequences are involved in transcriptional silencing at mating-type and outer centromere repeats. Thus, we have made important findings that apart from mating-type, Pap1 is also involved in centromeric silencing.

Based on these results, we purposed a speculative working model for the mode of action of Pap1 in transcriptional silencing as shown in Figure 4.21.