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Regulation of Heterochromatin Assembly in Fission Yeast

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Chromatin is a highly organized architecture that is composed of DNA and histone proteins, and acts as the primary physiological platform of genetic information among all eukaryotes. A diverse array of posttranslational modifications on histone amino-termini influences chromatin structure, thereby regulating access to the underlying DNA. Distinct histone amino-terminal modifications can generate synergistic or antagonistic interaction affinities for chromatin-associated proteins, which in turn control dynamic transitions between transcriptionally active or transcriptionally silent chromatin states (Jenuwein & Allis, 2001). For example, histone acetylation and phosphorylation correlate with transcriptional activation whereas deacetylation and methylation of histones are required for transcriptional repression (called gene silencing) and heterochromatin silencing. Thus the combinatorial nature of various histone amino-terminal modifications revealed a "histone code" that extends the information potential of the genetic code contained in DNA molecules (Jenuwein & Allis, 2001). Moreover, it is proposed that histone code is the black box of gene or chromatin regulation that can determine proper use of genetic information within DNA. Thus, the histone code can act as a key controller, which is required for regulation of gene expression, and assembly and maintenance of heterochromatin. On the basis of recent studies, it is proposed that the epigenetic marking system via the histone code represents a fundamental regulatory mechanism that has an impact on most of cellular processes based on chromatin as a template. Furthermore, the histone code system has a broad implications for cell fate decisions and both normal and pathological development (Jenuwein & Allis, 2001).

Methylation of histone H3 lysine 9 (H3 Lys9) by the conserved H3 Lys9-specific methyltransferase, Su(var)3-9 in flies, SUV39H1 in human, and Clr4 in the fission yeast *Schizosaccharomyces pombe* correlates with heterochromatin assembly. The methylated Lys9 residue recruits another conserved heterochromatin protein, which is called Swi6 in *S. pombe* and HP1 (heterochromatin protein 1) in higher eukaryotes, leading to regional silencing of chromatin (Grewal & Moazed, 2003). In the fission yeast, recent studies addressing the silencing of the mating-type and centromere regions provide insights for understanding the regulation of heterochromatin assembly in eukaryotes (Grewal &

Moazed, 2003). At present, our lab study aims to understand the regulatory mechanisms of heterochromatin assembly and disassembly using fission yeast as a model system. **In this abstract, I would like to summarize our two recent projects: (1) Identification of two ubiquitin-conjugating enzymes, Rhp6 and UbcX as destabilizing factors of heterochromatin; and (2) Discovery of stress-activated transcriptional factors, Atf1 and Pcr1, as key regulators of Swi6/HP1 dependent heterochromatin assembly independent of RNA interference machinery.**

As mentioned above, methylation of histone H3 has been linked to the assembly of higher-order chromatin structures: H3-Lys9-methyl (Me) is associated with silent chromatin while H3-Lys4-Me is prominent in active chromatin. Although the mechanism for heterochromatin assembly is being intensively studied at this time, the destabilization process of heterochromatin has little been understood yet. Here, to identify putative enzymes responsible for destabilization of heterochromatin, we screened genes whose overexpressions disrupt silencing at the silent *mat3* locus in fission yeast. Interestingly, we identified two genes, *rhp6*$^+$ and *ubcX*$^+$, both of which encode ubiquitin-conjugating enzymes (**Fig. 1**). Their overexpression disrupted silencing at centromeres and telomeres as well as at *mat3*. Additionally, the overexpression interfered with centromeric function, as confirmed by elevated minichromosome loss and antimicrotubule drug sensitivity. On the contrary, deletion of *rhp6*$^+$ and *ubcX*$^+$ enhanced silencing at all heterochromatic regions tested, indicating that they are negative regulators of silencing. More importantly, chromatin immunoprecipitation showed that their overexpression alleviated the level of H3-Lys9-Me while enhancing the level of H3-Lys4-Me at the silent regions. On the contrary, their deletions enhanced the level of H3-Lys9-Me while alleviating that of H3-Lys4-Me. Taken together, the data suggest that two ubiquitin-conjugating enzymes, Rhp6 and UbcX, affect methylation of histone H3 at silent chromatin, which then reconfigures silencing (**Choi et al., 2002**).

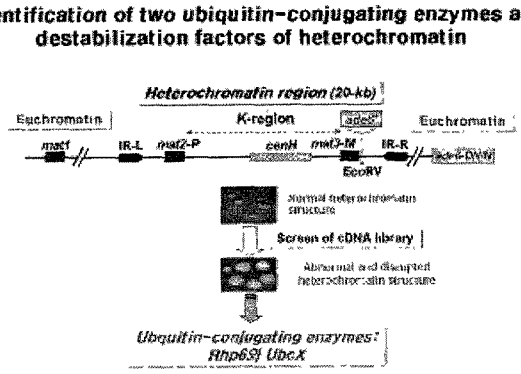

Fig. 1. Identification of two ubiquitin-conjugating enzymes, Rhp6 and UbcX, as destabilization factors of heterochromatin structure.

A study of gene silencing within the mating-type region of fission yeast defines two distinct pathways responsible for the establishment of heterochromatin assembly. One is RNA interference-dependent and acts on centromere-homologous repeats (*cenH*). The other is a stochastic Swi6 (the fission yeast HP1 homolog)-dependent mechanism that is not fully understood. Here we find that activating transcription factor (Atf1) and Pcr1, the fission yeast bZIP transcription factors homologous to human ATF-2, are crucial for proper histone deacetylation of both H3 and H4. This deacetylation is a prerequisite for subsequent H3 lysine 9 methylation and Swi6-dependent heterochromatin assembly across the rest of the silent mating-type (*mat*) region lacking the RNA interference-dependent *cenH* repeat (**Fig. 2**). Moreover, Atf1 and Pcr1 can form complexes with both a histone deacetylase, Clr6, and Swi6, and *clr6* mutations affected the H3/H4 acetylation patterns, similar to the *atf1* and *pcr1* deletion mutant phenotypes at the endogenous *mat* loci and at the $ctt1^+$ promoter region surrounding ATF/CRE-binding site. These data suggest that Atf1 and Pcr1 participate in an early step essential for heterochromatin assembly at the *mat* locus and silencing of transcriptional targets of Atf1. Furthermore, a phosphorylation event catalyzed by the conserved mitogen-activated protein kinase pathway is important for regulation of heterochromatin silencing by Atf1 and Pcr1. These findings suggest a role for the mitogen-activated protein kinase pathway and histone deacetylase in Swi6-based heterochromatin assembly (**Kim et al., 2004**).

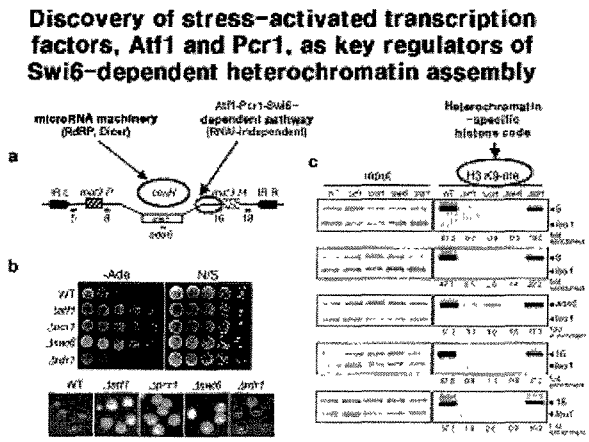

Fig. 2 Discovery of the stress-activated transcription factors, Atf1 and Pcr1, as key regulators of Swi6/HP1-dependent heterochromatin assembly.

Together, we propose that Rhp6 and UbcX are negative regulators of heterochromatin formation via altering the histone methylation patterns at the silent chromatin loci, and the stress-activated transcription factors (Atf1 and Pcr1) play key roles in Swi6-dependent heterochromatin assembly independent of microRNA process (**Fig. 3**).

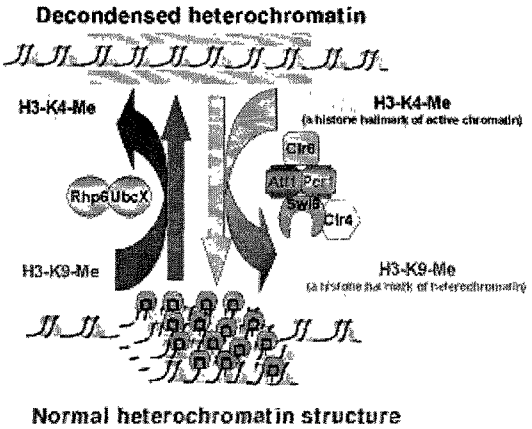

Fig. 3. A proposed model for the roles of Rhp6 and UbcX in decondensation of heterochromatin and the roles of Atf1 and Pcr1 in Swi6/HP1-dependent heterochromatin assembly independent of RNA interference machinery.

References

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