Positive Charge of Arginine Residues on Histone H4 Tail Is Required for Maintenance of Mating Type in Saccharomyces cerevisiae

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In eukaryotic cells, gene expression is regulated by the structure of chromatin. The chromatin is composed of a nucleosome, which is a histone octamer wrapped by 147 bp of DNA, and its structure is regulated by various factors including dynamic modifications of histones [1, 2]. The histone modifications such as acetylation, methylation, and phosphorylation are implemented by several histone-modifying enzymes, and affect the interaction between histone proteins and DNA molecules, and subsequently change the chromatin structure [3].

The chromatin is generally divided into euchromatin and heterochromatin depending on the DNA packaged level. Unlike euchromatin, in which DNA is packaged at a relatively low level, heterochromatin is packaged at a very high level of DNA, making it difficult to access proteins necessary for the transcription, thereby suppressing gene expression. Regulation of gene expression within heterochromatin and euchromatin is critical to maintaining gene stability [4].

Saccharomyces cerevisiae, budding yeast, is known to have no heterochromatic region. However, the telomeric regions and hidden mating (HM) loci in S. cerevisiae could be considered to be heterochromatic regions in that DNA is packaged at a very high level and the expression of genes within these regions is suppressed. There are two mating types, MATα and MATa, of haploid S. cerevisiae and two different haploid S. cerevisiae can create a diploid by mating together. Both haploid yeast strains contain both genes for
the mating type. However, the mating type is determined according to the gene in the MAT locus regardless of both genes within HM loci, which are always silenced. Therefore, the maintenance of gene silencing within HM loci is critical to determine the yeast mating type. When MATα haploid S. cerevisiae meets a MATα haploid cell, the MATα haploid cell arrests its growth for the preparation of mating. That means that the growth of MATα haploid yeast strains is inhibited by the alpha factor which MATα haploid yeast strains secrete [5, 6].

Unlike eukaryotes including mammals and fission yeast Schizosaccharomyces pombe, S. cerevisiae mediates gene silencing through the Silent Information Regulator (SIR) complex. In the regions of HM loci, telomere, and rDNA repeats, which are known to be the transcriptionally silenced regions in S. cerevisiae, gene expression is suppressed by the SIR complex, although there are some differences in the associated proteins [7]. In addition, H3K9 methylation is prevalent in the heterochromatin region of most eukaryotes including S. pombe, but this common histone modification does not appear in S. cerevisiae [8].

Many studies have been performed to elucidate gene silencing in S. cerevisiae. Previous studies have reported that Dot1, a methyltransferase specific for H3K79, regulates telomeric silencing and HM silencing [9, 10] and another research has shown that Dot1 controls telomeric silencing but not HM silencing [11]. Briggs group found that the basic patch of histone H4 tail regulates telomeric gene silencing through interaction with Dot1 [12]. In this study, to identify whether these factors are also important for the regulation of HM silencing and have the same function as in telomere, we performed some experiments.

In a previous study performing the mating assay with mutant strains deleting some regions of N-terminal tail in histone H4, a significant decrease of mating ability was observed in the strains with removed N-terminal tails on histone H4. These results suggest that the N-terminal tail of H4 is required for suppressing gene expression in HM locus [13]. The 16th lysine residue in histone H4 was considered to be required for maintaining gene silencing in an HM locus because the substitution of the lysine-16 within the N-terminal tail of histone H4 (H4K16) to alanine caused de-repression of silenced genes within HM loci [14]. Based on these studies, we further confirmed the association of the N-terminal tail of histone H4 with maintaining the gene silencing in HM loci using the properties of haploid S. cerevisiae. First, we performed disc assay using two histone H4 mutant strains (H4ΔΔ-23 and H4ΔΔ-23) obtained by removing 1–23 or 4–23 residues from the tail of histone H4 (Fig. 1A). After plating 100 μl of cells diluted into 10^6 cells/ml on the YPD plates, three sterilized paper discs were placed on three points on the plate. 5 μl alpha factor of three different concentrations (1, 0.1, and 0.01 μg/μl, respectively) were spotted onto the paper discs, and the plates were incubated at 30°C for 3 days. H4K16A mutant strain was used as a negative control, and wild type was used as a positive control. The acetylated H4K16 is a substrate of Sir2, a histone deacetylase, and the removal of an acetyl group from acetylated H4K16 by Sir2 is critical in heterochromatin spreading and maintenance of gene silencing [15, 16]. However, the H4K16A mutant strains have lost their acetylated site due to the switch from lysine to alanine. While wild-type yeast strain has clear areas around the discs containing alpha factor, H4ΔΔ-23 and H4ΔΔ-23 do not show clear zones around the discs containing alpha factor like H4K16A mutant strain, which does not maintain HM silencing (Fig. 1A). To identify residues in histone H4 tail affecting HM silencing, we observed loss of HM silencing in the strains containing variously substituted residues of histone H4. We focused on arginine residues including Arg-17, Arg-19, and Arg-23, which may be competent for histone modifications. Arg-17 and Arg-19 of histone H4 tail were reported to influence telomeric silencing as parts of the basic patch on the histone H4 tail [12]. As a result, it was confirmed that HM silencing was not maintained in mutants containing substituted Arg-17, Arg-19, or Arg-23 with alanine of histone H4 (Figs. 1B and 1C). Based on our results, we confirmed that two arginine residues (Arg-17 and Arg-19) located in the histone H4 tail are critical to maintaining HM silencing as well as telomeric silencing. In addition, our data showed that Arg-23 on the histone H4 tail is required for the maintenance of HM silencing (Figs. 1B and 1C).

A previous study verified that the change of positive charge in basic patch residues of histone H4 tail resulted in the loss of telomeric silencing [12]. To determine whether these changes in charge also affect HM silencing, we substituted arginine of H4R17, H4R19, and H4R23 into lysine. As a result, in contrast to H4R17A or H4R19A substitution, substitution of H4R17 or R19 with lysine leads to recovery of HM silencing in both disc assay and serial dilution assay. Surprisingly, H4R23K mutant didn’t recover HM silencing in both disc assay and serial-dilution assay (Figs. 2A and 2B). Also, the substituted mutant of Arg-23 with Alanine (R23A mutant) formed a partly clear zone around the paper disc containing alpha factor compared with R17A or R19A mutants (Fig. 1B). This data suggested that Arg-23 had an important function for the maintenance...