PERSPECTIVE

Histone acetylation in heterochromatin assembly

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Histone acetylation is generally considered a mark involved in activating gene expression by making chromatin structures less compact. In the April 1, 2010, issue of *Genes & Development*, Xhemalce and Kouzarides (pp. 647–652) demonstrate that the acetylation of histone H3 at Lys 4 (H3K4) plays a role in the formation of repressive heterochromatin in *Schizosaccharomyces pombe*. H3K4 acetylation mediates a switch of chromodomain proteins associated with methylated H3K9 during heterochromatin assembly.

Heterochromatin assembly in *Schizosaccharomyces pombe*

Heterochromatin is characterized as being transcriptionally inactive and a highly condensed chromatin structure (Grewal and Moazed 2003; Grewal and Elgin 2007; Grewal and Jia 2007; Cam et al. 2009). Histone modifications generally associated with heterochromatin include hypoacetylation of lysines and di- or trimethylation of histone H3 at Lys 9 (H3K9me2 and H3K9me3) (James et al. 1989; Jenuwein. 2001; Peters et al. 2003; Rice et al. 2003). Heterochromatin protein 1 (HP1) specifically recognizes methylated H3K9 via its chromodomain, and is enriched in heterochromatic regions (Bannister et al. 2001; Lachner et al. 2001; Nakayama et al. 2001). This interaction represents an absolute requirement for heterochromatin formation in many organisms.

The fission yeast *S. pombe* has been used widely as a model organism for the study of heterochromatin assembly, as many factors involved in the formation of heterochromatin are conserved with the metazoa. In *S. pombe*, the centromere consists of a central region (*cnt1*) that functions as the site of kinetochore formation, and the pericentromeric outer region (*otr*) containing *dg* and *dh* repeats that are packaged into heterochromatin (Clarke and Baum 1990). Heterochromatin formation requires the coordinated actions of histone-modifying enzymes Clr4 and Clr3 and HP1 proteins (Chp1, Chp2, and Swi6), as well as the RNAi machinery (Ekwall et al. 1996; Ivanova et al. 1998; Volpe et al. 2002; Motamedi et al. 2004; Verdel et al. 2004; Sugiyama et al. 2005). During S phase of the cell cycle, limited exposure of sequences in heterochromatin allows for the transcription of the centromeric repeats by RNA polymerase II (RNA pol II). This in turn facilitates the recruitment of RNA-induced transcriptional gene silencing complex (RITS), which contains the Chp1 and Clr4 proteins (Verdel et al. 2004; Grewal and Elgin 2007; Chen et al. 2008; Cam et al. 2009). The transcribed centromeric repeat RNA is processed into siRNA by RITS and the RNA-dependent RNA polymerase complex (RDRC), which contains RNA-dependent RNA polymerase (Rdp1) and dicer (Dcr1) (Sugiyama et al. 2005). Methylation of Lys 9 on histone H3 by Clr4 recruits Swi6 and Chp2, and establishes a positive feedback loop by stabilizing the chromatin association of Clr4 and RITS through their chromodomains (Grewal and Jia 2007; Cam et al. 2009). During G2 phase, Chp2 recruits the Snf2/Hdac-containing complex (SHREC), which also includes the Clr3 histone deacetylase. Clr3 maintains the hypoacetylated state associated with heterochromatin (Sugiyama et al. 2007; Motamedi et al. 2008; Fischer et al. 2009).

Although several H3K9me-binding proteins required for heterochromatin formation have been identified, the mechanisms for their specific spatial and temporal recruitment have not been determined as yet.

The role of H3K4 acetylation in heterochromatin formation

Xhemalce and Kouzarides (2010) initially found that a histone H3K4R (Lys → Arg) mutation exhibited pericentromeric silencing defects. A *set1Δ* mutant deficient in H3K4 methylation did not, suggesting that modification of Lys 4 other than methylation was involved in pericentromeric silencing. Acetylation of histone H3K4 (H3K4ac) has been described previously in both humans and mice (Garcia et al. 2007). Xhemalce and Kouzarides (2010) found that H3K4ac was indeed enriched at *dg* and *dh* heterochromatic repeats. Chromatin immunoprecipitation (ChIP) assays of pericentromeric heterochromatin revealed that levels of H3K4ac peaked right after H3K9me2 during the cell cycle, suggesting sequential

[*Keywords*: H3K4ac; HP1; RITS complex; chromodomain switch; heterochromatin]
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Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.1922110.

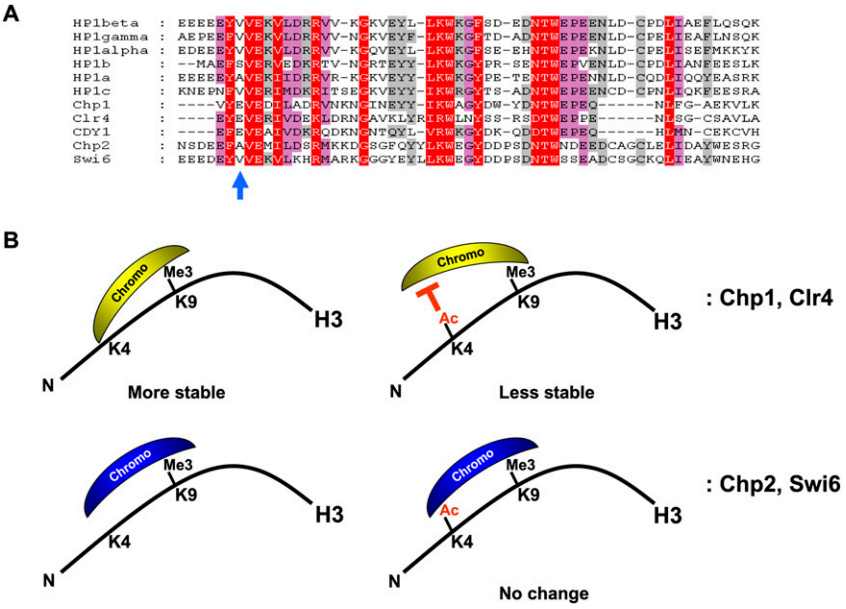

Figure 1. Sequence alignment of the chromodomains and model. (*A*) Amino acid sequences of the chromodomains of H3K9me-binding proteins (human HP1α, HP1β, HP1γ, and CDY1; *Drosophila* HP1a, HP1b, and HP1c; and *S. pombe* Swi6, Chp1, Chp2, and Clr4) were aligned using CLUSTALW and were presented by PSC geneDoc. The blue arrow indicates the location of the Glu residue contacting histone H3 Lys 4. (*B*) A schematic diagram illustrating the model suggested in Xhemalce and Kouzarides (2010). The acetylation of H3 at Lys 4 destabilizes the interaction between H3K9me and the chromodomain of Chp1 or Clr4. However, this model does not apply to the interaction of H3K9me with Chp2 or Swi6.

actions of these two histone modifications. Furthermore, the H3K4R mutation caused increased occupancy of Chp1 and reduced association of Swi6 and Chp2 at both *dg* and *dh* repeats. This indicates that H3K4ac mediates a switch between these chromodomain proteins in the pericentromeric region in vivo. Consistent with this possibility, peptide competition experiments revealed that Chp1 bound less stably to dimethylated H3K9 peptides also acetylated on H3K4 than to peptides only dimethylated on H3K9 (Fig. 1). In contrast, the binding of Swi6 and Chp2 to dimethylated H3K9 peptides was not influenced by H3K4ac. A potential reason for this difference in affinity may be that the Glu23 residue of Chp1 is not conserved in the chromodomains of Chp2 and Swi6 (Fig. 1). In the crystal structure of Chp1 bound to the H3 tail, Glu23 is linked to K4 of histone H3 via van der Waals interactions and a salt bridge (Schalch et al. 2009). Swi6 and Chp2 possess valine and alanine in this location, respectively. Among the chromodomains of proteins from other species also known to interact with H3K9me, only the human CDY1 chromodomain has a glutamate residue at the equivalent sites of *S. pombe*, Clr4 and Chp1, suggesting that a similar chromodomain switch may occur in humans (Fig. 1; Kim et al. 2006). Furthermore, the *Drosophila* HP1b protein contains a serine residue at the same site, which could mimic glutamate when phosphorylated (Fig. 1).

Future perspective

It is no longer surprising that different histone modifications at the same site can play opposing roles. Acetylated H3K9 is enriched at the promoter region of active genes, whereas methylation on the same site is associated with the gene body in inactive genes. Conversely, H3K4me is generated by the passage of RNA pol II and is associated with euchromatin, whereas H3K4ac resides in heterochromatin (Li et al. 2007). This leads to the idea that H3K4me may affect the level of H3K4ac and vice versa. While the *set1Δ* mutant did not show a defect in centromeric heterochromatin formation, the possibility that methylation of K4 affects acetylation in a different region other than the pericentromeric region cannot be excluded. Interestingly, a recent publication showed that Lid2, a homolog of Lsd1 in mammals, demethylates trimethylated H3K4 and regulates heterochromatin formation in *S. pombe*, indicating that the removal of H3K4me is required in heterochromatin assembly (Li et al. 2008).

In addition, Xhemalce and Kouzarides (2010) demonstrated that Mst1, a homolog of the human Tip60 acetyltransferase, and Sir2 deacetylase are responsible for the acetylation and deacetylation of H3K4, respectively. This finding is supported by previous studies, where an *mst1* temperature-sensitive mutant caused a growth defect when combined with *clr3Δ*, *clr4Δ*, or *swi6Δ* (Gómez et al. 2008). Also, it has been shown previously that Sir2 is required for the hypoacetylation and methylation of H3K9 and association of Swi6 with heterochromatic regions (Shankaranarayana et al. 2003). It would be intriguing to explore how Mst1 is targeted to the pericentromeric region during S phase. Interestingly, a direct interaction between trimethylated H3K9 peptide and the chromodomain of human Tip60 has been reported, which raises the possibility that H3K9me may recruit Mst1 (Sun et al. 2009).

Acknowledgments

We thank all members of the Workman laboratory, especially Michaela Smolle for critical reading of the manuscript. This work was supported by NIGMS and the Stowers Institute.

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