Histone deacetylases govern heterochromatin in every phase

Yota Murakami

Faculty of Science, Department of Chemistry, Hokkaido University, Sapporo, Japan. Correspondence to: yota@sci.hokudai.ac.jp

The EMBO Journal (2013) 32, 2301–2303. doi:10.1038/emboj.2013.154; Published online 5 July 2013

Deacetylation of histone tails has been shown to play a role during heterochromatin formation, but the precise mechanism of action has not been understood. Complementary results presented in two recent articles in *The EMBO Journal* (Alper *et al*, 2013; Buscaino *et al*,

2013) together reveal how histone deacetylases (HDACs) affect the various phases of heterochromatin formation: establishment, maintenance and spreading.

Heterochromatin, condensed chromatin that is transcriptionally inactive, plays important roles in epigenetic

Figure 1 Roles of the HDACs Sir2 and Clr3 in pericentromeric heterochromatin formation. (A) Sir2 and Clr3, together with Swi6^{HP1}, contribute to all phases of heterochromatin formation: establishment, spreading and maintenance. They do so by enhancing the action of the histone methyltransferase Clr4, which promotes heterochromatin formation via methylation of Lys9 of histone H3 (H3K9). Clr4 is recruited by RNAi-dependent and RNAi-independent mechanisms during the establishment phase, and Sir2 and/or Clr3 is required for both these mechanisms. During spreading and maintenance, the mechanism for the recruitment of Clr4 is not well understood. (B) Mechanisms of Sir2 to assist Clr4 function. Acetylation of H3K9 as well as H3K14 may specifically inhibit the action of Clr4 to methylate H3K9. In addition, the histone acetylation enhances transcription by RNA polymerase II (RNA pol II), which is associated with an elevated rate of histone turnover that prevents Clr4 from stably methylating H3K9. Sir2 and Clr3 cancel the inhibitory effects of the H3 acetylation by deacetylating H3K9 and 14.

regulation of gene expression and other chromosomal functions, such as chromosome segregation and telomere maintenance. Fission yeast is a good model system to analyse heterochromatin because of the similarity of its heterochromatin structure to other eukaryotes and the ease of genetic analysis. In fact, this organism has contributed greatly to our understanding of the importance of RNA interference (RNAi) during heterochromatin formation. Heterochromatin is defined by methylation of lysine 9 of histone H3 (H3K9me) and hypo-acetylation of histones. H3K9me provides the binding sites for heterochromatin protein 1, HP1 (Swi6 in fission yeast, Nakayama *et al*, 2001). Since deacetylation of histone tails by HDACs generally correlates with the repression of transcription at euchromatic regions, a similar function of HDACs is expected at heterochromatin. Indeed, the HDAC Clr3 is required for efficient repression of transcription at fission yeast heterochromatin (Yamada *et al*, 2005; Sugiyama *et al*, 2007). In addition, HDACs seem to be important for heterochromatin formation because inhibition of their activity using inhibitors causes disruption of heterochromatin (Ekwall *et al*, 1997). However, the precise role of HDACs in heterochromatin formation has not been thoroughly investigated. This is partly due to the redundant function of HDACs. Furthermore, heterochromatin formation consists of three distinct steps: establishment, spreading and maintenance, but it is difficult to study each step separately using native heterochromatin that is already established and stably maintained. Using sophisticated approaches, two recent articles in *The EMBO Journal* (Alper *et al*, 2013; Buscaino *et al*, 2013) revealed the functions of HDACs in heterochromatin formation (Figure 1).

Allshire and colleagues (Buscaino *et al*, 2013) utilized the fact that introduction of pericentromeric repeat sequences into fission yeast with minichromosome-based vectors promotes *de novo* heterochromatin formation on the repeats (Baum *et al*, 1994). Using this system, they identified two independent sequences in the repeats that promoted RNAi-dependent assembly of heterochromatin. They also found that one sequence required the HDAC Sir2 for heterochromatin establishment, while the other required both Sir2 and Clr3. Partridge and colleagues (Alper *et al*, 2013) used a different approach to analyse the requirement of HDACs in heterochromatin establishment. Fission yeast has only one H3K9-specific methyltransferase, Clr4 and deletion of Clr4 causes complete loss of heterochromatin. Re-introduction of Clr4 to Clr4-depleted cells enables monitoring the establishment of heterochromatin (Sadaie *et al*, 2004), and previous analysis using this system demonstrated the existence of a RNAi-independent mechanism at pericentromeric regions (Shanker *et al*, 2010). Partridge's group now showed that Sir2

was essential for both RNAi-dependent and RNAi-independent establishment of pericentromeric heterochromatin (Alper *et al*, 2013).

The minichromosome-based assay makes it possible to analyse spreading of heterochromatin from the nucleation sites. Allshire's group showed that Sir2 and Clr3 were required for spreading of heterochromatin over the repeat sequence (Buscaino *et al*, 2013). In addition, transfer of minichromosomes with established heterochromatin into RNAi mutants, in which establishment of heterochromatin is prevented, showed that Sir2 and Clr3 are required for the maintenance of heterochromatin in the absence of RNAi (Buscaino *et al*, 2013). In all the heterochromatin formation steps, Swi6 is also required, but its precise function is not clear yet (Figure 1A).

How are the HDACs able to function in the multiple phases in heterochromatin formation? It is reasonable to assume that HDACs function in the recruitment of Clr4 to promote H3K9 methylation that is an essential and common step in all three phases. The results that HDACs are not required for siRNA production by RNAi further support this assumption (Alper *et al*, 2013; Buscaino *et al*, 2013).

What is the molecular basis of HDAC function during Clr4 recruitment? Allshire and colleagues suggest that deacetylation of histones by HDACs represses high transcriptional activity and the associated elevated rate of histone turnover, which may prevent Clr4 from stably methylating H3K9 (Figure 1B). In addition, there might be another mechanism. Partridge and colleagues found that Sir2 preferentially deacetylate H3K4, H3K9, H3K14 and H4K16. Among alanine substitution mutants of potential target sites, H3K9A and H3K14A inhibited the establishment of heterochromatin (Alper *et al*, 2013). Since acetylation of H3K9 competes with methylation by Clr4, the importance of deacetylation of K9 for H3K9 methylation is obvious. Interestingly, the H3K14A mutation has previously been shown to disrupt heterochromatin (Mellone *et al*, 2003) and the mutation inhibits recruitment of Clr4 to heterochromatin (Alper *et al*, 2013). Hence, these results suggest that in addition to H3K9 acetylation, deacetylation of H3K14 is essential for Clr4 recruitment (Figure 1B), though further work is necessary to understand the molecular link between H3K14 and Clr4.

These two studies revealed not only the missing link between HDACs and heterochromatin formation, but also the usefulness of fission yeast in investigation of heterochromatin.

Conflict of interest

The author declares that he has no conflict of interest.

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