

Chromatin Silencing: RNA in the Driving Seat

Dispatch

David S. Stevenson and Paul Jarvis

Recent studies have provided major new insights into the mechanism by which eukaryotic organisms initiate heterochromatin formation. Surprisingly, RNA appears to be a central component of the chromatin silencing machinery.

Eukaryotic chromatin is characterised by regions containing high densities of reiterated sequences and transposable elements [1]. These are highly condensed regions, referred to as heterochromatin, which stain differentially and show alterations in histone composition and — in vertebrates, fungi and plants — increased levels of DNA methylation. Heterochromatin displays very low levels of transcriptional activity, and can cause the sporadic silencing of flanking genes — a phenomenon termed position effect variegation (PEV). It therefore comes as something of a surprise to learn that this transcriptionally quiet state depends on the production of small RNA molecules that are complementary to the heterochromatic DNA [2–4].

RNA interference (RNAi) is the process by which double-stranded RNA (dsRNA) molecules trigger the sequence-specific degradation of transcribed RNA targets, thereby silencing a target gene or family of genes. RNAi has been documented in plants, filamentous fungi, nematode worms, fruit flies and mammals [5,6], indicating that it is an evolutionarily conserved process. It was first identified as the phenomenon termed ‘co-suppression’ in plants — the attenuation of expression of an endogenous gene following the introduction of additional transgenic copies of the gene [7] — and has since been shown to be an important antiviral defence mechanism and a regulator of normal developmental processes [8].

RNAi is dependent upon several cellular enzymes for activity. Organisms that exhibit RNAi all seem to have similar requirements for the process to operate: all those tested have been found to require a gene of unknown function called *Argonaute*, a ribonuclease III enzyme called Dicer, and a multi-protein RNase complex called ‘RNA induced silencing complex’ (RISC). Dicer cleaves dsRNAs corresponding to the target RNA transcript into 21–26 base-pair chunks. These small dsRNAs then target the homologous RNA target for endonucleolytic attack by the RISC complex. In addition, nematode worms, filamentous fungi and plants all require an RNA-dependent RNA polymerase (RdRp), perhaps to amplify the silencing signal [9].

As RNAi operates at the post-transcriptional level, entirely separate mechanisms were thought to control the transcriptional silencing of heterochromatic DNA.

However, an association between RNAi processes and DNA methylation was observed in several studies, hinting at a possible link between these different levels of regulation [9,10]. Recently, Reinhart and Bartel [2] identified twelve small (~20 base pair) dsRNAs with homology to the centromeric repeats in the fission yeast, *Schizosaccharomyces pombe*. In parallel, Volpe *et al.* [3] demonstrated that transcriptionally silent transgenes within the centromeric heterochromatin of *S. pombe* were activated in mutants lacking *Argonaute*, Dicer or RdRp. These fission yeast mutants were found to contain overlapping ‘forward’ and ‘reverse’ centromeric transcripts — homologous to the small dsRNAs identified by Reinhart and Bartel [2] —

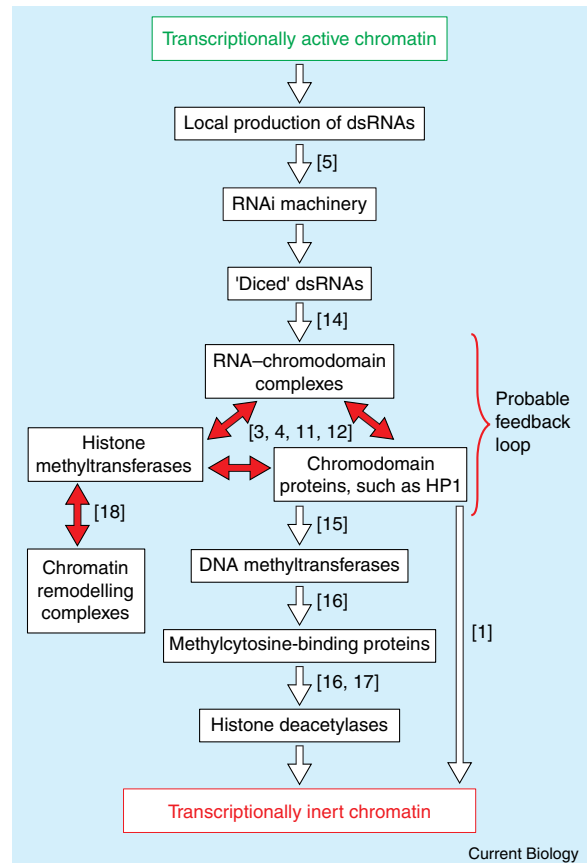


Figure 1. The route to silencing.

The steps leading to the establishment of the heterochromatic state. The link between dsRNA production and histone methylation is currently poorly understood. Strahl and Allis [12] suggest that many of the components may be physically associated in a single proteinaceous complex. Such integration would ensure efficient operation of the silencing process, and facilitate regulation. Heterochromatic silence is maintained by a combination of histone modifications — the ‘histone code’ — and non-histone proteins such as the chromodomain protein, HP1. References for each step are indicated in parentheses.

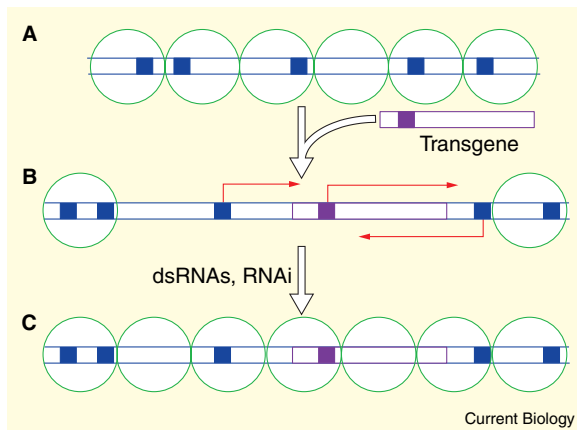


Figure 2. Genome surveillance: silencing mobile DNA.

(A) A repressive chromatin configuration (green spheres) maintains transcriptional silence. (B) Perturbation of the local chromatin structure – in this case by an intruding transgene – exposes ‘cryptic’ promoter elements (filled boxes) leading to the transient activation of transcription (red lines). (C) dsRNA production from the resultant transcripts initiates RNAi, which in turn re-establishes a repressive chromatin configuration through the mechanisms described in Figure 1.

whereas the wild type contained ‘reverse’ transcripts only, and these appeared to be turned over rapidly [3].

Volpe *et al.* [3] also showed that RdRp is physically associated with centromeric heterochromatin. Together, these data indicate that the transcriptional silencing of centromeric heterochromatin is mediated by the RNAi machinery and transcripts encoded by centromeric DNA. Similar results were obtained by Hall *et al.* [4], who demonstrated that silencing of the *S. pombe* mating locus is also directed by RNAi. Once the transcriptionally silent state is established, however, components of the RNAi machinery become dispensable and a heterochromatin protein, Swi6, assumes control [4].

What is Swi6, and what is its role in silencing? Swi6 is the fission yeast homologue of heterochromatin protein 1 (HP1) [3,4], a chromodomain protein, like Polycomb, which was first identified in fruit flies several years ago [1]. The HP1 chromodomain localises the protein to chromatin by interacting specifically with histone H3 methylated at lysine 9 (H3-K9) [11]. In fact, histone H3 may be methylated at a number of different residues: methylation at lysines 9 and 27 is associated with transcriptionally silent chromatin, whereas methylation at position 4 is associated with transcriptionally active chromatin [12,13]. Hall *et al.* [4] found that methylation of H3-K9 is central to chromatin silencing in *S. pombe*, and that silencing is a consequence of Swi6 binding. Interestingly, Cao *et al.* [13] recently demonstrated that methylation of H3-K27 facilitates the binding of Polycomb, another heterochromatin-associated protein.

Quite how RNAi initiates chromatin silencing is open to conjecture. Research in fruit flies has shown that certain chromodomain proteins bind RNA, and that this binding is essential for their activity [14]. One possibility, then, is that the localised production of

small dsRNA molecules enables unspecified chromodomain proteins to recruit histone methyltransferases to the pre-heterochromatic region [4]. This would become a self-sustaining loop in which methylated histones bind chromodomain proteins, which in turn recruit further histone methyltransferases. Such a feedback loop would account for the heterochromatic stability observed by Hall *et al.* [4] in the absence of the RNAi machinery.

Where does DNA methylation fit in? This question was recently addressed by Jackson *et al.* [15], who showed that *Arabidopsis* HP1 binds methylated H3-K9, and that this interaction is required for the binding and activity of the CpNpG-specific DNA methyltransferase, chromomethylase 3. This is the first conclusive evidence of a direct link between histone methylation and DNA methylation. Once methylated, DNA is bound by methylcytosine-binding proteins, such as MeCP1 and MeCP2 which are components of the histone deacetylase complex [16,17]. Deacetylation of histone H4 then enables the chromatin to take on a more compact configuration, and finalises the heterochromatic transition [12] (Figure 1).

The final pieces in the puzzle are the chromatin-remodelling complexes. Best understood are the ATP-dependent SWI/SNF factors first identified in the budding yeast *Saccharomyces cerevisiae* [18]. These factors are thought to disrupt histone–DNA interactions, enabling various other proteins to gain access to the chromatin. The *Arabidopsis* mutant *decreased DNA methylation 1 (ddm1)* – which carries a lesion in a gene for a SWI/SNF protein – displays drastically reduced levels of DNA methylation, transposon reactivation and numerous developmental defects that accumulate over successive generations. Gendrel *et al.* [18] recently showed that histone methylation patterns are altered in *ddm1* heterochromatin: H3-K9 methylation is largely replaced by H3-K4 methylation. Thus, the function of the DDM1 protein may be to enable histone methyltransferases to gain access to the chromatin. Loss of this activity would affect the distribution of methylated histones and, in turn, the binding of DNA methyltransferases [18].

Can these findings tell us anything about the mechanisms underlying the sporadic silencing of transgenes and mobile DNA? In 1993, Adrian Bird [19] proposed that DNA methylation functions to silence ‘cryptic’ promoter elements. In any given stretch of DNA, there are multiple DNA sequences that can drive transcription in the absence of epigenetic control. Any event alleviating this control and causing the ‘exposure’ of cryptic promoter elements – such as the introduction of new DNA by transposition or transgenesis – might cause dsRNA production and initiate heterochromatin formation through the newly described RNAi process (Figure 2). After the establishment of a stable heterochromatic state, production of dsRNA would cease and transcriptional silencing would be maintained by the ‘histone code’ [4,12].

Throughout the 1990s, DNA methylation was generally considered within the context of the ‘genome defence’ hypothesis of Krickler *et al.* [20]. This hypothesis states that the principle role of DNA methylation is to

demarcate and ultimately mutagenise mobile DNA (methylcytosine is readily deaminated to thymine). Although this mechanism clearly does degrade the ability of repetitive sequences to mobilise and recombine, it must be subservient to the role of DNA methylation in repressing the transcriptional activity of promoter elements within and flanking mobile sequences. The real surprise, however, lies in the means by which DNA methylation appears to be directed — through the genome's inherent ability to generate dsRNA molecules whenever the status quo is disturbed.

We are at an exciting juncture in the study of gene silencing. From formerly disparate parts, a functionally unified whole is emerging. If RNA interference can be likened to shooting the messenger, such a strategy has never seemed more appealing.

References

1. Henikoff, S. (1990). Position effect variegation after 60 years. *Trends Genet.* **6**, 422–426.
2. Reinhart, B.J. and Bartel, D.P. (2002). Small RNAs correspond to centromere heterochromatic repeats. *Science* **297**, 1831.
3. Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I.S. and Martienssen, R.A. (2002). Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* **297**, 1833–1837.
4. Hall, I.M., Shankaranaryana, G.D., Norma, K.-I., Ayoub, N., Cohen, A. and Grewal, S.I.S. (2002). Establishment and maintenance of a heterochromatin domain. *Science* **297**, 2232–2237.
5. Vance, V. and Vaucheret, H. (2001). RNA silencing in plants — defense and counterdefense. *Science* **292**, 2277–2280.
6. Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**, 494–498.
7. Napoli, C., Lemieux, C. and Jorgensen, R. (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell* **2**, 279–289.
8. Llave, C., Kasschau, K.D., Rector, M.A. and Carrington, J.C. (2002). Endogenous and silencing-associated small RNAs in plants. *Plant Cell* **14**, 1605–1619.
9. Vaistij, F.E., Jones, L. and Baulcombe, D.C. (2002). Spreading of RNA targeting and DNA methylation in RNA silencing requires transcription of the target gene and a putative RNA-dependent RNA polymerase. *Plant Cell* **14**, 857–867.
10. Mette, M.W., Aufsatz, W., van der Winden, J., Matzke, M.A. and Matzke, A.J.M. (2000). Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J.* **19**, 5194–5201.
11. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. and Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120.
12. Strahl, B.D. and Allis, C.D. (2000). The language of covalent histone modifications. *Nature* **403**, 41–45.
13. Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S. and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039–43.
14. Akhtar, A., Zink, D. and Becker, P.B. (2000). Chromodomains are protein-RNA interaction modules. *Nature* **407**, 405–409.
15. Jackson, J.P., Lindroth, A.M., Cao, X. and Jacobsen, S.E. (2002). Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560.
16. Quaderi, N.A., Meehan, R.R., Tate, P.H., Cross, S.H., Bird, A.P., Chatterjee, A., Herman, G.E. and Brown, S.D. (1994). Genetic and physical mapping of a gene encoding a methyl CpG binding protein, *Mecp2*, to the mouse X-chromosome. *Genomics* **22**, 648–651.
17. Shahbazian, M., Young, J., Yuva-Paylor, L., Spencer, C., Antalffy, B., Armstrong, D., Paylor, R. and Zoghbi, H. (2002). Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* **35**, 243–254.
18. Gendrel, A.V., Lippman, Z., Yordan, C., Colot, V. and Martienssen, R.A. (2002). Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene *DDM1*. *Science* **297**, 1871–1873.
19. Bird, A.P. (1993). Functions for DNA methylation in vertebrates. *Cold Spring Harb. Symp. Quant. Biol.* **58**, 281–285.
20. Kricker, M.J., Drake, J.W. and Radman, M. (1992). Duplication-targeted DNA methylation and mutagenesis in the evolution of eukaryote chromosomes. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1075–1079.