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REVIEW

Heterochromatin and Epigenetic Control of Gene Expression

Shiv I. S. Grewal[1]* and Danesh Moazed[2]*

Eukaryotic DNA is organized into structurally distinct domains that regulate gene expression and chromosome behavior. Epigenetically heritable domains of heterochromatin control the structure and expression of large chromosome domains and are required for proper chromosome segregation. Recent studies have identified many of the enzymes and structural proteins that work together to assemble heterochromatin. The assembly process appears to occur in a stepwise manner involving sequential rounds of histone modification by silencing complexes that spread along the chromatin fiber by self-oligomerization, as well as by association with specifically modified histone amino-terminal tails. Finally, an unexpected role for noncoding RNAs and RNA interference in the formation of epigenetic chromatin domains has been uncovered.

Chromatin and higher order chromosome structures play a central role in nearly every aspect of DNA biology in eukaryotes. Processes ranging from gene expression to chromosome dynamics during cell division are regulated by the folding of DNA into chromatin. Chromatin regulation operates at both local and global levels. Local effects often occur at the level of single genes. Global effects, however, can involve changes in the properties of enormous chromosome domains or even entire chromosomes. Both types of regulation act at the level of the nucleosome, which is the fundamental unit of chromosome folding in eukaryotes. Global regulation of chromosome domains appears to involve the assembly of higher order supranucleosomal structures that control DNA accessibility. This review focuses on recent advances in our understanding of how DNA is assembled into specialized chromatin domains, called heterochromatin or silent chromatin.

Chromosomes are composed of two types of domains, euchromatin and heterochromatin. Heterochromatic domains are in general inaccessible to DNA binding factors and are transcriptionally silent. Euchromatic domains, in contrast, define more accessible and transcriptionally active portions of the genome. Large blocks of heterochromatin surround functional chromosome structures such as centromeres and telomeres, whereas smaller heterochromatic domains are interspersed throughout the chromosome (*1*). After several decades of speculation, it has recently become clear that heterochromatin plays a crucial role in centromere function. Heterochromatin proteins are associated with DNA repeats that surround centromeres and are required for proper sister-chromatid cohesion and chromosome segregation (*2*–*5*). Heterochromatin also stabilizes repetitive DNA sequences at centromeres, telomeres, and elsewhere in the genome by inhibiting recombination between homologous repeats (*6*, *7*).

In addition to its role in the maintenance of genome stability, heterochromatin plays a central role in the regulation of gene expression during development and cellular differentiation. Heterochromatin-like structures are involved in the stable inactivation of developmental regulators such as the homeotic gene clusters in *Drosophila* and mammals, and the mating-type genes in fungi (*8*). Moreover, dosage compensation in female mammals involves the heterochromatic inactivation of one of the two X chromosomes in somatic cells (*9*).

Several properties of heterochromatin make it particularly suitable for processes that require the stable maintenance of expression states over long periods. First, the heterochromatic state is epigenetically and stably inherited through many cell divisions, which may take place under different developmental conditions and environmental inputs. Second, the mechanism of assembly of heterochromatin and the spreading of heterochromatin from nucleation sites to surrounding DNA regions allows a transition from sequence-specific genetic control to sequence-independent epigenetic control. Although many studies over the past few decades have established the basic properties of heterochromatin and have identified many of its structural building blocks, we are only now beginning to understand how these DNA domains, which in some eukaryotes account for about half of total genomic DNA, are assembled and epigenetically propagated.

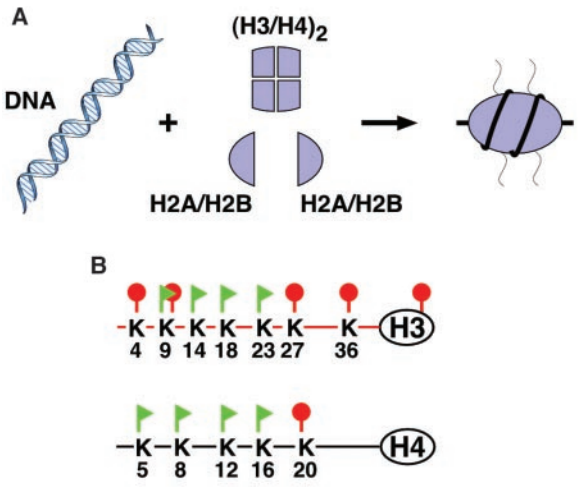

Fig. 1. Diagrammatic representation of the nucleosome and sites of acetylation and methylation on histone tails. (**A**) Nucleosome, the fundamental unit of chromatin, consists of 147 base pairs of genomic DNA wrapped twice around the highly conserved histone octamer complex. Each core histone (H2A, H2B, H3, and H4) is composed of the histone fold domain and the less structured tails that extend outward from the superhelical turns of DNA. (**B**) Green flags and red lollipops indicate the location of acetyl and methyl histone modifications in the amino termini or the globular domains of histones H3 and H4, respectively.

Factors in Heterochromatin Assembly

Studies in organisms ranging from yeast to mammals suggest strongly that histones and their posttranslational modifications play a pivotal role in the assembly of heterochromatin. In eukaryotes, DNA is assembled with histones to form the nucleosome, in which DNA is wrapped approximately two turns around an octameric complex composed of two molecules of each of the four histones H2A, H2B, H3, and H4 (Fig. 1). The amino termini of histones contain a diversity of posttranslational modifications (*10*). The most prominent among these are acetylation and methylation of lysine residues in the highly conserved amino termini of histones H3 and H4 (Fig. 1). Increased acetylation almost invariably correlates with transcriptional activity, whereas decreased acetylation correlates with a tran-

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scriptionally repressed state. Thus, in nearly all organisms, the heterochromatic state is associated with hypoacetylation of histones (*11*).

Many of the trans-acting factors required for heterochromatin assembly are either enzymes that directly modify histones or factors that bind to histones. In budding yeast, the products of the silent information regulator (SIR) genes Sir2, Sir3, and Sir4 are required for assembly of heterochromatin at the silent mating-type loci and telomeric DNA regions. The Sir proteins form a complex, and the Sir3 and Sir4 subunits of this complex can bind to deacetylated histone tails in vitro (*12*–*14*). The Sir2 subunit is a nicotinamide adenine dinucleotide (NAD)–dependent histone deacetylase, and its deacetylase activity is required for heterochromatin assembly (*7*, *11*, *14*). In fission yeast and metazoans, several histone deactylases, including both Sir2-like NAD-dependent and Hda1/Rpd3-like NAD-independent deacetylases are required for silencing (*15*).

In addition to histone hypoacetylation, in fission yeast, *Drosophila*, and mammals methylation of histone H3 lysine 9 (H3 Lys9) correlates with heterochromatin assembly. This residue is methylated by the conserved methyltransferase Su(var)3-9 in *Drosophila*, SUV39H1 in human, and Clr4 in fission yeast (*16*, *17*). These H3 Lys9 methyltransferases are associated with another conserved protein, called Swi6 in fission yeast and HP1 in *Drosophila* and human, respectively (*18*, *19*). The Swi6 and HP1 proteins bind specifically to histone H3 tails that are methylated at lysine 9 by Clr4/Suv39h enzymes in fission yeast and metazoans, respectively (*20*, *21*).

DNA methylation is also believed to contribute to the stability of silenced chromatin states in higher eukaryotes with complex genomes. Evidence from plants and fungi suggests the existence of feedback mechanisms between DNA and histone methylation, such that one promotes maintenance of the other (*22*–*24*). The interdependence of these epigenetic marks suggests that DNA methylation and chromatin-mediated epigenetic mechanisms act in concert to maintain a silenced chromatin state.

Role of Silencers, Repeats, and RNAs in Nucleation of Heterochromatin

How are heterochromatin complexes targeted to a specific chromosomal domain? Although the role of specific regulatory sites such as silencers and sequence-specific DNA binding proteins in the nucleation of heterochromatin is well documented (*11*, *14*) (Fig. 2), evidence suggests a role for repetitive DNA elements and noncoding RNAs in regional targeting of heterochromatin complexes (*25*, *26*). Transposons and satellite repeats that comprise a major fraction of heterochromatic sequences are believed to preferentially recruit the heterochromatin machinery, leading to silencing of nearby genes. How repeats and transposons attract heterochromatin is not known, but it is believed that the repetitive nature of these elements is important (*26*, *27*). In *Schizosaccharomyces pombe*, the centromere homologous repeat element is a heterochromatin nucleation center involved in regional silencing throughout a 20-kb domain (*6*, *28*).

Noncoding RNA molecules of various sizes appear to play a broad role in the regulation of chromosome behavior. For example, RNAs play an important role in chromosome-specific localization of chromatin-modifying activities required for dosage compensation in *Drosophila* and mammals (*29*) and in some cases of genomic imprinting in mammals (*30*). In mammals, *Xist* RNA originating from the X-inactivation center is required for initiation but not for the subsequent inheritance of X-inactivation (*9*), and silencing is also regulated by *Tsix*, an *Xist* antisense transcript (*31*). The assembly of *rox* RNAs with the MSL (male-specific lethal) complex that associates with the male X chromosome along its length is an early step in dosage compensation in *Drosophila* (*29*), and in plants the production of double-stranded RNA (dsRNA) can cause posttranscriptional and transcriptional silencing (*32*).

The RNA interference (RNAi) pathway, in addition to its well-known role in post-transcriptional gene silencing (*33*), is also involved in initiating heterochromatin assembly at repetitive DNA (*28*, *34*) (Fig. 2). Components of the RNAi machinery, including a member of the PAZ/Piwi family Argonaute (*ago1*), an RNaseIII-like enzyme Dicer (*dcr1*), and an RNA-dependent RNA polymerase (*rdp1*), are required for heterochromatin formation and for the targeting of H3 Lys9 methylation in *S. pombe* (*28*, *34*). Centromeric repeat sequences that are transcribed at low levels and produce dsRNA are sufficient to recruit heterochromatin at an ectopic site in *S. pombe*, and this recruitment of repressive chromatin is strictly dependent on the RNAi machinery (*28*). Interestingly, "small heterochromatic" RNAs (shRNAs) resembling *Dicer* cleavage products that correspond to centromeric repeats have been isolated (*35*). The role of RNAi in epigenetic gene silencing appears to be conserved among diverse species. Genes encoding members of the PAZ/Piwi family are also required for cosuppression in *Drosophila* (*36*), for silencing and chromatin modification in *Arabidopsis* (*37*), and for programmed DNA elimination in *Tetrahymena* (*38*).

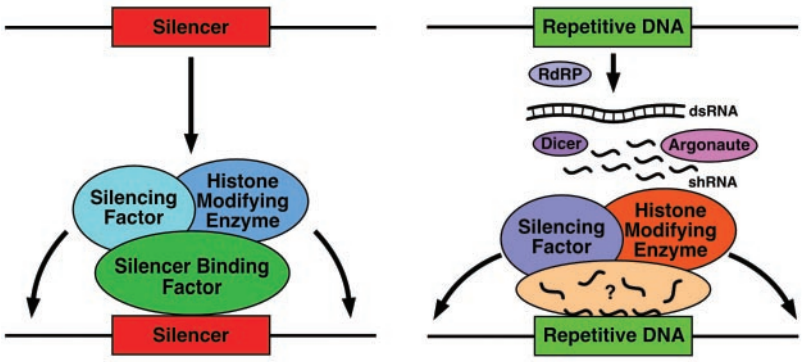

Fig. 2. Mechanisms for the initiation of heterochromatin. Heterochromatic structures can be nucleated by specific cis-acting sequences, called silencers, which are recognized by DNA binding proteins (left). Alternatively, repetitive DNA elements such as transposons in the genome are believed to serve as signals for heterochromatin formation (right). Transcripts generated by repetitive DNA are processed into shRNAs by a mechanism requiring components of the RNAi machinery such as RNA-dependent RNA polymerase (RdRP), Dicer, and Argonaute proteins.

The possible mechanisms by which repeated sequences and RNA trigger silent chromatin assembly include physical pairing of homologous DNA sequences and/or DNA-RNA or RNA-RNA interactions (*27*, *32*). The connection between RNAi and heterochromatin assembly has suggested a model for the RNA-mediated epigenetic structuring of the eukaryotic genomes. Double-stranded RNA is believed to be processed into small RNAs, which in turn provide specificity for targeting histone-modifying activities and epigenetic modification of the genome through homology recognition (Fig. 2). Although it is unclear how RNAs target chromatin modifications, among the possible candidates for linking shRNAs to chromatin are chromodomain proteins. The chromodomain motif has been shown in some cases to interact with RNA (*39*), and chromodomains of Clr4/Suv39h1 are required for methylation of H3 Lys9 at heterochromatic loci (*17*). In this model, shRNA targeting of silencing complexes is analogous to the targeting of silencing complexes to DNA by silencer-binding proteins in *Saccharomyces cerevisiae*, an organism that appears to lack the RNAi pathway (Fig. 2). Two classes of hypothetical models can be proposed to account for the specificity of shRNAs in initiating heterochromatin. By analogy to the targeting of mRNA by the RNA-induced silencing complex (RISC) (*33*), these models would involve pairing between shRNAs with either DNA or nascent RNA transcripts at the target locus (Fig. 3,

top and middle). Regardless of the mechanism, it should be noted that repetitive DNA sequences capable of producing double-stranded RNA transcripts are widespread throughout complex genomes, and RNAi-mediated chromatin remodeling may influence a variety of chromosomal functions, including gene expression patterns during development.

RNA has also been implicated in subnuclear localization of heterochromatic proteins such as HP1 (*40*, *41*). The identity of the RNA component is not clear, but it appears to operate in the formation of a higher order structure. Supporting a role for RNAs in the higher order association of heterochromatic sequences, the RNAi machinery is required for the clustering of telomeres in *S. pombe* (*5*). Therefore, in addition to their role in the initiation of heterochromatin formation, RNAs may act in conjunction with silencing factors as a "glue" to promote the clustering of heterochromatic regions into higher order structures that may serve to promote long-range regulatory interactions (Fig. 3, bottom).

Assembly of Heterochromatic Chromosome Domains

Genetic and biochemical studies in *S. cerevisiae* and *S. pombe* have provided important insights into the stepwise process of heterochromatin assembly. A common theme that has emerged is that heterochromatin assembly is nucleated at specific regulatory sites and then spreads to nearby sequences in a manner that requires the physical coupling of histone-modifying activities and structural proteins such as Sir3, Sir4, and Swi6/HP1 (*1*, *11*). In *S. cerevisiae*, site-specific DNA binding proteins bind to nucleation sites (silencers) and then recruit to DNA the Sir2/Sir4 complex. The Sir2 protein is believed to deacetylate histones to create a binding site for the Sir3 and Sir4 proteins. The Sir3 and Sir4 proteins can oligomerize and, once bound, will recruit additional Sir2/Sir4 complexes (Figs. 2 and 4). Sequential cycles of binding and deacetylation then result in the spreading of these silencing proteins along the chromatin fiber beyond the original nucleation site (*12*, *42*, *43*).

In contrast to *S. cerevisiae*, in *S. pombe*, specialized repetitive sequences and RNAi cooperate to initiate heterochromatin formation. It has been hypothesized that shRNA generated by RNAi-mediated processing of double-stranded transcripts provides the specificity for targeting histone-modifying activities to the corresponding genomic locations. This initial recruitment has been proposed to nucleate heterochromatin assembly by creating a histone code for binding of silencing factors (*28*). Specifically, deacetylation and methylation of H3 Lys9 is believed to create a binding site for Swi6/HP1 (*15*, *17*). Moreover, deacetylation of H3 Lys14 seems to be important for the silencing and localization of Swi6 at heterochromatic loci in fission yeast. However, methylation of H3 Lys9 and acetylation of H3 Lys14 can occur concomitantly at some genomic locations and might provide a mechanism for transcriptional activation of loci carrying methylated H3 Lys9 (*19*). Once bound to chromatin, Swi6/HP1 recruits histone-modifying activities that create additional Swi6 binding sites on adjacent nucleosomes (*28*). This allows histone modifications and Swi6 to spread in cis in a stepwise manner beyond the original nucleation site(s) and explains the dosage-critical role of Swi6/HP1 in epigenetic silencing (*1*). A general model that emphasizes the common features of the stepwise assembly mechanism in both of the above systems is presented in Fig. 4.

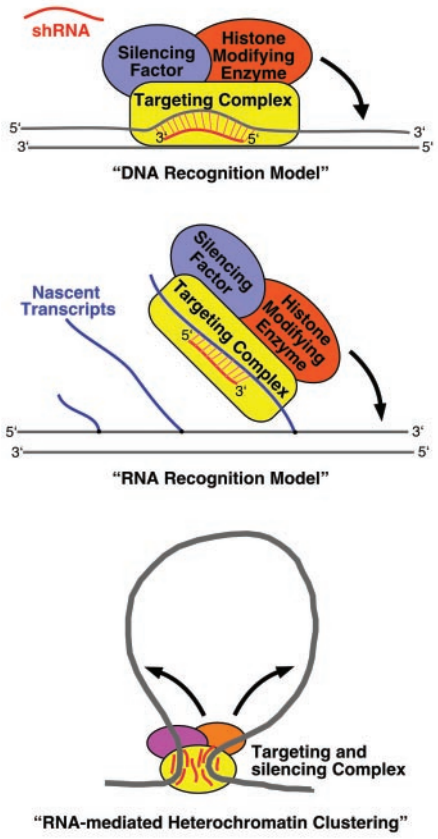

Fig. 3. Hypothetical models for shRNA-mediated sequence-specific initiation of heterochromatin involving recognition of DNA (top) or nascent RNA transcripts by a RISC-like targeting complex (middle). A model for RNA-mediated clustering of heterochromatic domains is presented at bottom.

Propagation of Heterochromatin

Heterochromatic structures such as the inactive X chromosome in female mammals are inherited in cis (*9*). In fission yeast, Swi6/HP1 has been identified as an important component of cellular memory responsible for the maintenance of the heterochromatic state (*28*, *44*). During DNA replication, histones H3 and H4 (H3/H4 tetramers) are randomly distributed to sister chromatids. Therefore, modified parental histones, and possibly assembled heterochromatin proteins such as Swi6/HP1 or Sir3, can serve as "molecular bookmarks" to imprint the parental histone-modification pattern onto newly assembled nucleosomes. Because Swi6/HP1 is required for the maintenance of H3 Lys9 methylation (*28*, *45*), we suggest that this process is accomplished through the same mechanism described above for spreading in cis. Therefore, an "epigenetic loop" between histone modifications and structural proteins that are associated with histone-modifying enzymes may underlie the propagation of heterochromatic domains (Fig. 4).

Boundaries of Heterochromatin Domains

The highly condensed heterochromatin domains are interspersed along with relatively decondensed euchromatic regions. Given that heterochromatin structures, once nucleated, can spread in cis, resulting in epigenetic silencing of adjacent genes, cells have evolved antagonistic mechanisms that protect active regions from the repressive effects of nearby heterochromatin. Specialized DNA elements known as boundary elements have been shown to mark the borders between adjacent chromatin domains and to serve as barriers against the effects of silencers and enhancers from the neighboring regions (*46*, *47*) (Fig. 4). In budding yeast, silencing proteins Sir2/3/4 are restricted to the silent mating-type loci by boundary sequences (*48*). Mapping of the distinct histone-methylation patterns across the mating-type region of *S. pombe* has revealed that two inverted repeat (IR) elements define the borders between a heterochromatic interval and the surrounding euchromatic regions (*49*). H3 Lys9 methylation and its interacting Swi6 protein are localized strictly to a 20-kb heterochromatic domain surrounded by IR elements, whereas H3 Lys4 methylation is specific to surrounding euchromatic regions. Deletion of either repeat results in the spreading of H3 Lys9 methylation and Swi6 to adjacent euchromatic regions. Therefore, boundary elements might help separate chromatin domains with distinct histone-modification patterns and serve to contain heterochromatin within a particular domain.

How do boundary elements prohibit spreading of heterochromatin? Comprehensive analyses of the histone modifications at the chicken β-globin locus revealed a sharp peak of H3 Lys4 methylation and histone acetylation immediately surrounding the condensed chromatin block that is highly enriched in H3 Lys9 methylation (*50*). Histone acetylation and H3 Lys4 methylation are suggested to act as chain terminators that interrupt the spread of heterochromatin complexes (*46*, *48*). Therefore, boundaries are believed to serve as entry sites for the recruitment of histone acetyltransferase or chro-

matin remodeling activities that disrupt the binding of silencing proteins to histones and thereby terminate the spread of heterochromatin structures. Another idea is that boundaries delimit structural domains by interacting with each other or with some other nuclear structure (*47*) (Fig. 4). In this view, the partitioning into autonomous functional units is a consequence of an underlying structural subdivision of the chromosome into higher order "looped" domains.

Spreading of heterchromatin domains can also be controlled through a balance between the opposing effects of histone-modifying activities. The extent of spreading by the Sir3 protein and silencing near telomeres in budding yeast is regulated by the chromosomal gradient of histone H4 Lys16 acetylation established by the MYST-like acetyltransferase Sas2 and the histone deacetylase Sir2 (*51*, *52*). The global acetylation of H4 Lys16 by Sas2 is believed to serve as a barrier to the spreading of the SIR complex. Similarly, it has been suggested that methylation of H3 Lys79 prevents nonspecific binding of Sir proteins in euchromatic regions (*53*, *54*). In addition to histone modifications, the composition of the nucleosome itself is important for maintaining chromatin states. Histone variant H2A.Z that is enriched in euchromatic regions surrounding silent loci in *S. cerevisiae* is also part of an antisilencing mechanism that prevents the spread of heterochromatin (*55*).

Heritable Gene Silencing and Heterochromatin in Development

The maintenance of heritable transcriptional states is essential for the development of multicellular organisms, and accumulating evidence indicates that misregulation of such processes contributes to cellular transformation and cancer progression. During *Drosophila* development, the Polycomb group (PcG) proteins act in conjunction with DNA sequences termed Polycomb response elements (PREs) to maintain lineage-specific "off" transcriptional states of homeotic genes (*8*). The founding member of the PcG family, Polycomb, contains a chromodomain similar to Swi6/HP1 and is a component of a multiprotein complex called PRC1 (*56*). Another *Drosophila* PcG complex, the ESC/E(Z) complex, and its human counterpart contain methyltransferase activity for histone H3 Lys27 (*57*, *58*) and H3 Lys9 (*59*, *60*). These marks colocalize with Polycomb binding sites (*59*), and methylation of Lys27 facilitates the binding of the Polycomb protein to histone H3 (*57*). The transcriptional repression conferred by Polycomb group complexes shares many characteristics with heterochromatin, including altered chromatin structure and mitotic heritability. In fact, recent studies suggest that PcG complex EED/EZH2 is required for H3 Lys27 methylation and heterochromatin formation during X inactivation (*61*, *62*). Thus, the pathways leading to gene silencing during development appear to follow rules similar to those in the formation of heterochromatin by Swi6/HP1. However, there is currently no direct evidence for the role of RNAs or RNAi in PcG-mediated silencing of developmental regulators in *Drosophila*.

The HP1 protein itself has also been implicated in the regulation of euchromatic genes. Studies on the distribution of HP1 have revealed a banded pattern across a small number of euchromatic sites dispersed throughout the *Drosophila* genome (*63*). This pattern indicates that, although HP1 is primarily concentrated at pericentric heterochromatin, specific locations along the chromosome arms are also under its control. Recent studies have shown that H3 Lys9 methylation and HP1 are recruited to specific promoters for gene silencing, directly implicating integral components of heterochromatin in the regulated silencing of euchromatic genes (*64*, *65*). Despite the involvement of HP1 in euchromatic gene regulation, it is important to recognize that several differences exist in euchromatic gene repression by heterochromatin proteins and the silencing of large chromosomal domains. For example, heterochromatin proteins associated with euchromatic sites do not seem to spread, resulting in localized gene repression.

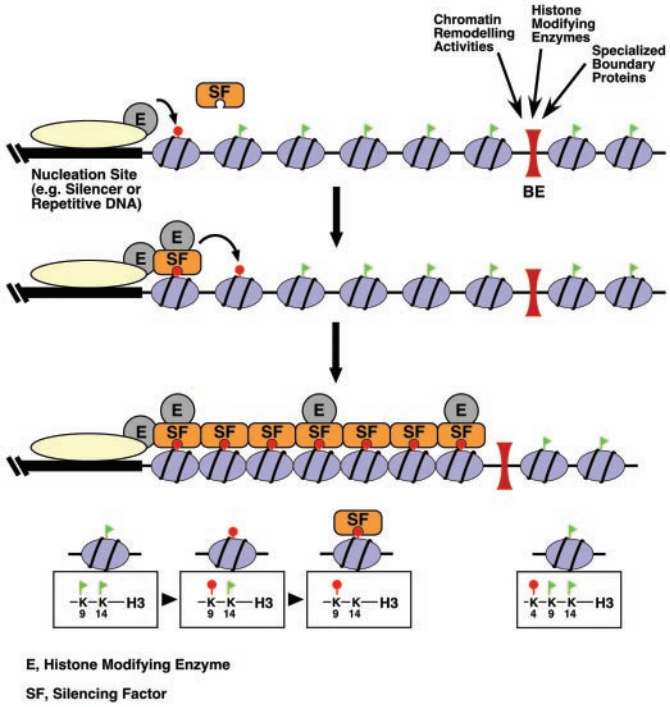

Fig. 4. Model for formation of silenced chromatin domains. After the recruitment to a specific heterochromatin nucleation site by proteins that directly bind DNA or are targeted by way of RNAs, histone-modifying enzymes (E) such as deacetylases and methyltransferases modify histone tails to create a binding site for silencing factors (SF). After this nucleation step, self-association of silencing factors (such as Swi6/HP1 or Sir3) is hypothesized to provide an interface for their interaction with histone-modifying enzymes, which then modify adjacent histones, creating another binding site for silencing factors. Sequential rounds of modification and binding result in the stepwise spreading of silencing complexes along nucleosomal DNA for several kilobases (spreading). Spreading of silencing complexes is blocked by the presence of boundary elements (BE). The modifications associated with the amino terminus of histone H3 in fission yeast heterochromatin (bottom left) and euchromatin (bottom right) are illustrated as an example. Deacetylation and methylation of H3 Lys9 are followed by deacetylation of H3 Lys14 and create a binding site for the Swi6 silencing factor.

Concluding Remarks

Regulation of higher order chromatin structure is directly coupled with regulation of the expression and integrity of the genetic information of eukaryotes and is likely to be a major force in the origin and evolution of genes, chromosomes, genomes, and organisms. In particular, the packaging of DNA into heterochromatin exerts epigenetic control over important biological processes. The past few years have witnessed a revolution in our understanding of how epigenetic chromatin states are assembled and, in particular, how the mechanism acts on histones to generate altered chromosome domains. Furthermore, the discovery of a requirement for the RNAi pathway in heterochromatin assembly has generated new excitement about the role of RNA in the regional control of chromosome structure. Future studies will undoubtedly provide exciting insights about the mechanism of assembly and propagation of epigenetic chromatin domains and the apparently diverse roles of RNA in the assembly of these domains.

References and Notes

1. S. I. S. Grewal, S. C. Elgin, *Curr. Opin. Genet. Dev.* **12**, 178 (2002).
2. P. Bernard *et al.*, *Science* **294**, 2539 (2001).
3. N. Nonaka *et al.*, *Nature Cell Biol.* **4**, 89 (2002).
4. A. Peters *et al.*, *Cell* **107**, 323. (2001).
5. I. Hall, K. Noma, S. I. Grewal, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 193 (2003).
6. S. I. Grewal, A. J. Klar, *Genetics* **146**, 1221 (1997).
7. L. Guarente, *Genes Dev.* **14**, 1021 (2000).
8. G. Cavalli, *Curr. Opin. Cell Biol.* **14**, 269 (2002).
9. P. Avner, E. Heard, *Nature Rev. Genet.* **2**, 59 (2001).
10. T. Jenuwein, C. D. Allis, *Science* **293**, 1074 (2001).
11. D. Moazed, *Mol. Cell* **8**, 489 (2001).
12. G. Hoppe *et al.*, *Mol. Cell. Biol.* **22**, 4167 (2002).
13. A. Carmen, L. Milne, M. Grunstein, *J. Biol. Chem.* **277**, 4778 (2002).

14. S. M. Gasser, M. M. Cockell, *Gene* **279**, 1 (2001).
15. G. Shankaranarayana, M. Motamedi, D. Moazed, S. I. Grewal, *Curr. Biol.* **13**, 1240 (2003).
16. S. Rea *et al.*, *Nature* **406**, 593 (2000).
17. J. Nakayama, J. Rice, B. Strahl, C. D. Allis, S. I. Grewal, *Science* **292**, 110 (2001).
18. L. Aagaard *et al.*, *EMBO J.* **18**, 1923 (1999).
19. S. I. Grewal, unpublished data.
20. A. Bannister *et al.*, *Nature* **410**, 120 (2001).
21. M. Lachner, D. O'Carroll, S. Rea, K. Mechtler, T. Jenuwein, *Nature* **410**, 116 (2001).
22. H. Tamaru, E. U. Selker, *Nature* **414**, 277 (2001).
23. L. Johnson, X. Cao, S. Jacobsen, *Curr. Biol.* **12**, 1360 (2002).
24. W. Soppe *et al.*, *EMBO J.* **21**, 6549 (2002).
25. S. Henikoff, *Biochim. Biophys. Acta* **1470**, 1 (2000).
26. J. Hsieh, A. Fire, *Annu. Rev. Genet.* **34**, 187 (2000).
27. E. U. Selker, *Cell* **97**, 157. (1999).
28. I. Hall *et al.*, *Science* **297**, 2232 (2002).
29. Y. Park, M. Kuroda, *Science* **293**, 1083 (2001).
30. F. Sleutels, R. Zwart, D. Barlow, *Nature* **415**, 810 (2002).
31. D. Cohen, J. Lee, *Curr. Opin. Genet. Dev.* **12**, 219 (2002).
32. M. Matzke, A. Matzke, J. Kooter, *Science* **293**, 1080 (2001).
33. G. Hannon, *Nature* **418**, 244. (2002).
34. T. Volpe *et al.*, *Science* **297**, 1833 (2002).
35. B. Reinhart, D. Bartel, *Science* **297**, 1831 (2002).
36. M. Pal-Bhadra, U. Bhadra, J. Birchler, *Mol. Cell* **9**, 315 (2002).
37. D. Zilberman, X. Cao, S. E. Jacobsen, *Science* **299**, 716 (2003).
38. K. Mochizuki, N. Fine, T. Fujisawa, M. Gorovsky, *Cell* **110**, 689 (2002).
39. A. Akhtar, D. Zink, P. Becker, *Nature* **407**, 405 (2000).
40. C. Maison *et al.*, *Nature Genet.* **30**, 329 (2002).
41. C. Muchardt *et al.*, *EMBO Rep.* **3**, 975 (2002).
42. L. Rusche, A. Kirchmaier, J. Rine, *Mol. Biol. Cell* **13**, 2207 (2002).
43. K. Luo, M. Vega-Palas, M. Grunstein, *Genes Dev.* **16**, 1528 (2002).
44. J. Nakayama, A. J. Klar, S. I. Grewal, *Cell* **101**, 307 (2000).
45. G. Schotta *et al.*, *EMBO J.* **21**, 1121 (2002).
46. A. West, M. Gaszner, G. Felsenfeld, *Genes Dev.* **16**, 271 (2002).
47. M. Labrador, V. Corces, *Cell* **111**, 151 (2002).
48. N. Dhillon, R. Kamakaka, *Curr. Opin. Genet. Dev.* **12**, 188 (2002).
49. K. Noma, C. D. Allis, S. I. Grewal, *Science* **293**, 1150 (2001).
50. M. Litt, M. Simpson, M. Gaszner, C. D. Allis, G. Felsenfeld, *Science* **293**, 2453 (2001).
51. N. Suka, K. Luo, M. Grunstein, *Nature Genet.* **32**, 378 (2002).
52. A. Kimura, T. Umehara, M. Horikoshi, *Nature Genet.* **32**, 370 (2002).
53. F. van Leeuwen, D. Gottschling, *Curr. Opin. Cell Biol.* **14**, 756 (2002).
54. H. Ng, D. Ciccone, K. Morshead, M. Oettinger, K. Struhl, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1820 (2003).
55. M. Meneghini, M. Wu, H. Madhani, *Cell* **112**, 725 (2003).
56. A. Saurin, Z. Shao, H. Erdjument-Bromage, P. Tempst, R. Kingston, *Nature* **412**, 655 (2001).
57. R. Cao *et al.*, *Science* **298**, 1039 (2002).
58. J. Muller *et al.*, *Cell* **111**, 197 (2002).
59. B. Czermin *et al.*, *Cell* **111**, 185 (2002).
60. A. Kuzmichev, K. Nishioka, H. Erdjument-Bromage, P. Tempst, D. Reinberg, *Genes Dev.* **16**, 2893 (2002).
61. J. Silva *et al.*, *Dev. Cell* **4**, 481 (2003).
62. K. Plath *et al.*, *Science* **300**, 131 (2003).
63. F. Sun *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5340 (2000).
64. S. Nielsen *et al.*, *Nature* **412**, 561 (2001).
65. D. Schultz, K. Ayyanathan, D. Negorev, G. Maul, F. Rauscher III, *Genes Dev.* **16**, 919 (2002).
66. We apologize to colleagues whose work could not be cited because of space limitations. We thank members of the Moazed and Grewal laboratories for helpful discussions, and L. Pile, J. Landry, A. Verdel, and members of the Grewal laboratory for their comments on the manuscript. Work in the authors' laboratories is supported by the NCI (S.I.S.G.), the NIH (D.M.), and the Ellison Medical Foundation (S.I.S.G. and D.M.).

VIEWPOINT

Bacterial Sex: Playing Voyeurs 50 Years Later

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The concept of chromosomes with a ring structure was born during the early studies of bacterial sexuality, and the discovery of fertility factors—episomes or plasmids—provided much later the key tools for gene cloning and biotechnology. But the plasmid-mediated transfer of antibiotic and other resistances, as well as pathogenicity, has served bacteria well in their own adaptive evolution.

Although in the shadow cast by the 50th anniversary of DNA structure, the half-century anniversary of the discovery of the nature of bacterial sexuality must not pass unnoticed. In 1953, L. L. Cavalli-Sforza, J. Lederberg, and E. M. Lederberg (*1*) and W. Hayes (*2*) published in the same issue of the *Journal of General Microbiology* the identification of the F (fertility) factor as a transmissible agent that determines bacterial sexuality. As an *hommage* to these and other pioneers of bacterial genetics, which has made possible a detailed analysis of prokaryotic chromosomes and other genetic elements, as illustrated in the articles by D. J. Sherratt (*3*) and J. Hacker (*4*), we present a short historical account of their discoveries and a visualization of the accomplishment of the *Escherichia coli* sexual act using immunofluorescence microscopy.

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The Amazing Story of the Discovery of Bacterial Sexuality

Progress in classical genetics was largely limited by the generation time of the test organism. For this reason, bacteria became the preferred model system, and consequently, the first proof that DNA is the genetic material was obtained from transformation experiments with *Pneumococcus* in 1944. However, this bacterium was inconvenient to use because it did not grow in synthetic media, which limited the number of useful genetic markers. J. Lederberg and E. Tatum (*5*) chose *E. coli* K-12, which can grow in synthetic medium, and isolated the so-called biochemical (auxotrophic) mutants. Then they mixed cultures of two different auxotrophs and obtained prototrophs (cells growing on unsupplemented medium). Initially, cellular fusion was thought to be responsible for prototrophic growth. Later, the unidirectional transfer of genetic material was demonstrated by mating streptomycin-sensitive and streptomycin-resistant cells (*6*). The existence of the subcellular agent called F, fertility or sex factor, responsible for genetic transfer was demonstrated by the observation that F^+ character can be transmitted from F^+ to F^- cells, without involving the bacterial chromosome (*1, 2*). The choice of *E. coli* K-12 strain by Lederberg and Tatum was critical for this amazing discovery. We now know that, by chance, this strain harbored the conjugative plasmid that, unlike the majority of F-like plasmids, was a naturally occurring mutant transferring at elevated frequency. Furthermore, it contained insertion sequences, which facilitated its integration into the host chromosome.

The nature of chromosome transfer was elucidated by studying conjugational transfer from Hfr (high frequency of recombination) donor strains (*6, 7*). Matings between Hfr and F^- strains with multiple genetic markers showed that there was a hierarchy in transfer efficiencies of different markers. The interpretation was that Hfr injects its chromosome to F^- from a genetically defined chromosomal point; the rationale was that the closer the marker to the injection point, the higher the production of recombinants. This hypothesis was proven by F. Jacob and E. Wollman (*7*) by the famous "blender experiment," i.e., by interrupting mating at different time points. They found that the Hfr character is trans-