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Mechanisms for the Inheritance of Chromatin States

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Studies in eukaryotes ranging from yeast to mammals indicate that specific chromatin structures can be inherited following DNA replication via mechanisms acting in *cis*. Both the initial establishment of such chromatin structures and their inheritance require sequence-dependent specificity factors and changes in histone posttranslational modifications. Here I propose models for the maintenance of epigenetic information in which DNA silencers or nascent RNA scaffolds act as sensors that work cooperatively with parentally inherited histones to re-establish chromatin states following DNA replication.

Cells with identical genomes can display distinct gene expression patterns and phenotypes that persist during numerous cell divisions. This capacity is critical for cellular differentiation and for development of multicellular organisms with stable tissues, organs, and morphologies, all of which arise from a single founder cell, the fertilized egg. The distinct gene expression and phenotypic states of genetically identical cells, which develop without change in DNA sequence and persist in the absence of initial inducing signals, are referred to as epigenetic states (Gottschling, 2004; Ringrose and Paro, 2004).

Following establishment during embryogenesis, a variety of mechanisms mediate the epigenetic inheritance of gene expression states. These mechanisms can be broadly divided into *trans*-acting and *cis*-acting. The first class relies on positive feedback mechanisms involving diffusible regulatory factors and includes transcription factors, such as the phage lambda repressor (cI) and Cro proteins, and eukaryotic cell type-specific master regulators, such as the fungal Wor1 and mammalian myoD proteins (Lassar et al., 1989; Ptashne, 2007; Ptashne and Gann, 2001; Zordan et al., 2006). The second class of mechanisms involves the *cis*-maintenance of chromatin modifications or DNA methylation (Beisel and Paro, 2011; Margueron and Reinberg, 2010; Schaefer et al., 2007). Both types of mechanisms are important for maintenance of gene expression patterns, but genetic studies of heterochromatin in fungi and *Drosophila,* and embryonic development in *Drosophila* and mammals, suggest that heritable changes in chromatin structure play profound roles in maintenance of the expression states of master regulators such as the homeobox *HOX* genes (Beisel and Paro, 2011; Grewal and Moazed, 2003). These studies further indicate that changes in chromatin states are inherited in *cis* through mitotic and even some meiotic cell divisions (Cavalli and Paro, 1998; Grewal and Klar, 1996; Xu et al., 2006; Klar, 1998), thus representing systems for inheritance of information that may be as powerful as replication of DNA. Whereas *cis*-replication of DNA methylation patterns is well understood, models for *cis*-inheritance of histone modifications that are consistent with the available evidence are lacking. Here I propose models for *cis*-inheritance of chromatin states that provide an explanation for the observation that in addition to histone modifications, sequence-specific elements such as DNA silencers and noncoding RNA, which mediate the establishment of silent chromatin domains, are also required for the maintenance of such chromatin structures.

Histone Modification-Based Chromatin Inheritance

Current models of chromatin inheritance are based on experimental evidence on the fate of nucleosomal histones following DNA replication. Studies using pulse-chase experiments followed by fractionation to measure chromatin-bound histones strongly suggest that at the bulk level parental histones H3 and H4 do not exchange with newly synthesized H3 and H4 but remain bound to the newly replicated daughter DNA strands (Jackson and Chalkley, 1974) (Figure 1). These studies and electron microscope images of replicating chromatin further suggest that during DNA replication parental histones are distributed randomly between the two daughter DNA strands (Jackson and Chalkley, 1985; Sogo et al., 1986). More recently, genome-wide studies in budding yeast using an epitope tag exchange strategy that allows parental histones to be distinguished from newly synthesized ones have defined the patterns of parental histone inheritance, demonstrating histone retention at a gene-specific level (Radman-Livaja et al., 2011). Together with extensive evidence on the role of histone posttranslational modifications in the regulation of transcription, these studies have given rise to the proposal that histone modifications can be re-established by complexes that recognize a specific modification on an inherited parental histone and catalyze the same type of modification on adjacent newly deposited nucleosomes (Dodd et al., 2007; Grewal and Moazed, 2003; Kaufman and Rando, 2010; Kouzarides, 2007; Rusche et al., 2003; Strahl and Allis, 2000; Suganuma and Workman, 2008) (Figure 1). With some important differences (discussed later), this model is similar to how the maintenance DNA methyltransferase, Dnmt1, is thought

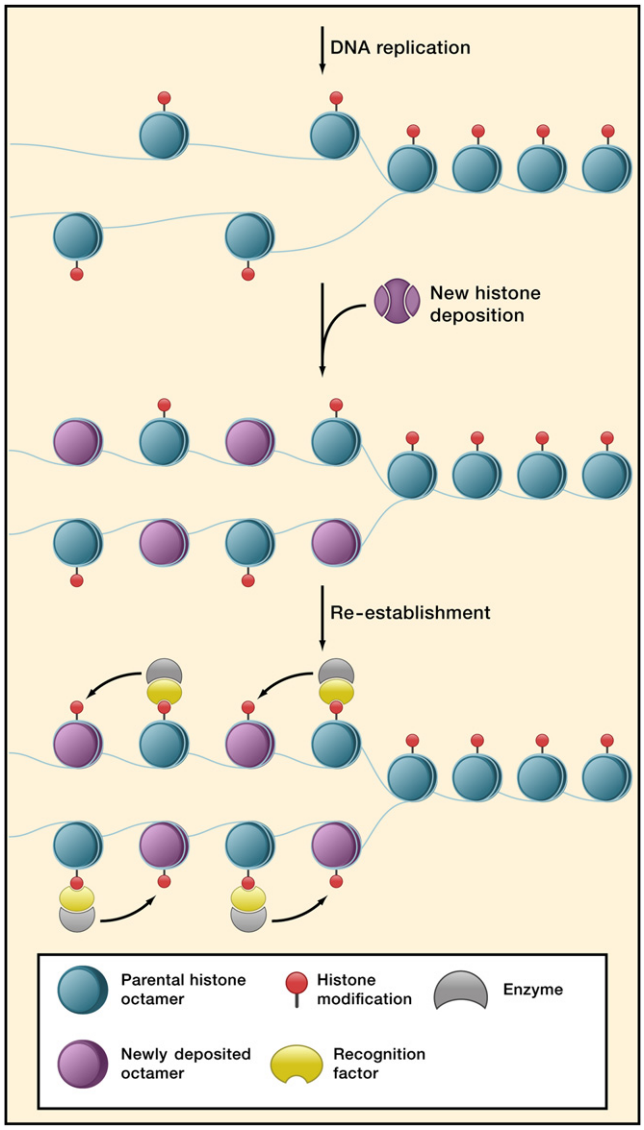

Figure 1. Re-establishment of Epigenetic States from Parental Histone Modifications

During chromatin replication, parental histones and their posttranslational modifications are retained and randomly associate with the newly synthesized daughter DNA strands. The modifications of parental histones are proposed to be copied onto newly deposited histones by chromatin modification complexes that contain a subunit that recognizes the modification on the parental histone and another subunit that is an enzyme that catalyzes the same modification on an adjacent nucleosome. Note that distribution of histones to daughter DNA strands is random. For simplicity, equally spaced nucleosomes are depicted.

to re-establish DNA methylation patterns by preferentially associating with and methylating hemimethylated DNA (Holliday, 1987; Schaefer et al., 2007). The model requires that histone modifications provide sufficient specificity to directly or indirectly recruit cognate-modifying enzymes and that the kinetics of their erasure is slower than the kinetics of postreplication re-establishment. Although in principle this mechanism based entirely on histones could account for the epigenetic inheritance of chromatin states, experiments in yeast and flies, discussed below, suggest that histone modifications alone are not sufficient for epigenetic inheritance.

Establishment and Maintenance of Silent Chromatin Domains

Silent or heterochromatic DNA domains in eukaryotic organisms ranging from yeast to human share a number of central properties, including their mode of epigenetic inheritance (Beisel and Paro, 2011; Grewal and Moazed, 2003). Here I briefly review our current knowledge of how yeast silent chromatin domains are established and maintained and what these studies tell us about epigenetic inheritance. In the budding yeast *Saccharomyces cerevisiae*, heterochromatin-like silent chromatin domains occur at the silent mating-type loci (called *HM* loci) and telomeres (Moazed, 2001; Rusche et al., 2003). The formation of silent chromatin requires input from three different classes of molecular players (Figure 2A). The first class is specificity elements. DNA regions, called silencers, direct the assembly of silent chromatin at the *HM* loci. Silencers are composed of binding sites for two general transcription factors, Rap1 and Abf1, and the origin recognition complex (ORC) (Bell et al., 1993; Brand et al., 1985; Foss et al., 1993; McNally and Rine, 1991; Shore and Nasmyth, 1987). At telomeres, silencing is initiated by tracks of Rap1-binding sites and the chromosome end, which is bound by the Ku70 and Ku80 proteins (Gasser and Cockell, 2001). Silencer- or telomere-binding proteins act combinatorially to recruit a second class of regulators, the Sir1, Sir2, Sir3, and Sir4 proteins, which spread along the chromatin fiber away from the nucleation site and create modified chromatin domains that are refractory to productive transcription (Moazed, 2001; Rusche et al., 2003). The Sir2 and Sir4 proteins assemble together into a heterodimer that associates with Sir3 to form the SIR complex (Hoppe et al., 2002; Moazed et al., 1997; Moretti et al., 1994; Rudner et al., 2005; Strahl-Bolsinger et al., 1997). The Sir1 protein forms a bridge between silencer-bound ORC and the Sir3 and Sir4 subunits of the SIR complex, which is important for efficient recruitment (Gardner et al., 1999; Triolo and Sternglanz, 1996) (Figure 2A). Histones are the third class of regulators. In particular, the conserved N terminus of histone H4 and lysine 16 within this region are critical for silencing (Johnson et al., 1992; Kayne et al., 1988). Any model for the mechanism of inheritance must take into account the fact that all three classes of regulators are required for establishment as well as inheritance of the silent state.

The SIR complex has three different activities, histone deacetylation, histone binding, and self-association, which play critical roles in establishment and maintenance of silent chromatin. The Sir2 protein is an NAD-dependent deacetylase with preference for histone H4 lysine 16 (H4K16), the H4 residue that is required for silencing (Imai et al., 2000; Johnson et al., 1992; Landry et al., 2000; Tanny and Moazed, 2001). The Sir3 protein binds preferentially to histone peptides (Hecht et al., 1995) and nucleosomes that contain deacetylated H4K16 (Liou et al., 2005; Onishi et al., 2007). In addition to interactions with H4, Sir3 binds to the globular domain of histone H3, around lysine 79, on the surface of the nucleosome, and methylation of histone H3 lysine 79

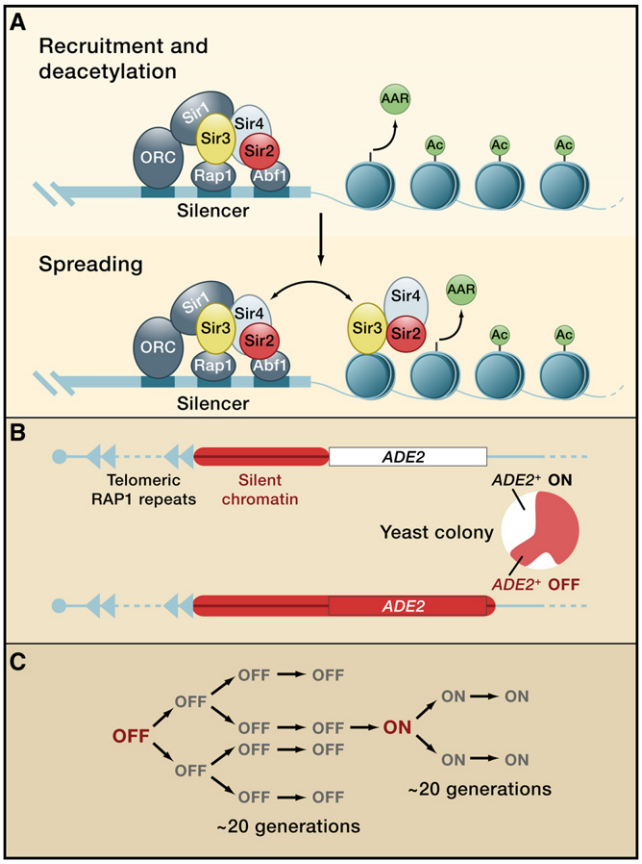

Figure 2. Assembly of Silent Chromatin in Budding Yeast

(A, Top) At the silent mating loci in *Saccharomyces cerevisiae*, silencers (DNA regions composed of binding sites) for the origin recognition complex (ORC), Rap1, and Abf1 recruit the Sir1, Sir2, Sir3, and Sir4 proteins through multiple weak interactions. Sir2 uses NAD to deacetylate histone H4 lysine 16 (H4K16), releasing *O*-acetyl-ADP-ribose (AAR), which binds to one of the Sir proteins and induces a conformational change in the SIR complex that may result in a tighter interaction between Sir3 and Sir4, and Sir3 and the nucleosome. (A, Bottom) H4K16 deacetylation promotes binding of Sir3, and sequential cycles of deacetylation and Sir3 binding to deacetylated nucleosomes are proposed to mediate the spreading of the SIR complex away from the silencer. The interaction of Sir3 with Sir4 is also required for spreading.

(B) Insertion of the *ADE2* gene near a yeast telomere results in stochastic spreading of telomeric heterochromatin into the *ADE2* gene. The resulting ON and OFF states appear as white and red sectors, respectively, in the yeast colony on the right and indicate mitotically stable epigenetic states.

(C) Switches in expression state, ON or OFF, are stable for more than 20 generations, indicating an epigenetic memory during cell divisions after the switch.

(H3K79) antagonizes Sir3 binding (Altaf et al., 2007; Ng et al., 2002; Onishi et al., 2007; van Leeuwen et al., 2002). The Sir4 protein forms a bridge that links Sir3 to Sir2 (Moazed et al., 1997). The interaction of Sir3 with Sir4, and possibly its ability to self-associate, are required for spreading of the SIR complex along the chromatin fiber (Rudner et al., 2005). In addition to spreading, all of the above activities are also required for efficient binding of the SIR complex to the silencer (Figure 2A) (Hoppe et al., 2002; Luo et al., 2002; Rudner et al., 2005; Rusché et al., 2002). As discussed later, the requirement for the deacetylase activity of Sir2 in efficient binding of the SIR complex to silencers themselves is a key observation because it suggests that efficient recruitment of the SIR complex to silencers involves cooperative interactions between silencer-binding proteins and deacetylated nucleosomes.

The histone-based chromatin replication model discussed above provides a possible mechanism for epigenetic inheritance of chromatin states (Figure 1). In the case of budding yeast, silent domains contain several kilobases of DNA-containing nucleosomes with unacetylated H4K16 and unmethylated H3K79. Following DNA replication, recognition of the inherited parental nucleosomes by the SIR complex would mediate deacetylation of the newly deposited histones and leads to the re-establishment of new silent domains (as in Figure 1). This model is, however, not consistent with the continuous requirement for silencers in maintenance of the silent state. Deletion of silencers using inducible site-specific recombination results in rapid loss of the silent state after one cell division, clearly indicating that the modification states of histones cannot be inherited in the absence of input from the silencer (Cheng and Gartenberg, 2000; Holmes and Broach, 1996). Here it may be proposed that the silencer acts on its own to re-establish the silent state after every cell division. However, such an entirely silencer-dependent model is not consistent with the epigenetic properties of silent domains. Once assembled, silent chromatin domains display a variegating effect on gene expression that is similar to position effect variegation, first described for the effect of heterochromatin on gene expression in *Drosophila* (Muller, 1930). Yeast cells carrying an *ADE2* reporter gene near a telomere or a weakened silencer produce sectored colonies in which the *ADE2* gene is either ON (white sectors) or OFF (red sectors) (Figure 2B) (Aparicio et al., 1991). This variegation results from stochastic loss and re-establishment of silent chromatin and indicates that following a switch in gene expression, the daughters of the switching cell have a memory of the expression state of the mother cell (Figure 2C). Similar variegating states have been observed at the silent mating-type loci in cells lacking Sir1, suggesting that epigenetic inheritance is a common property of silent domains in yeast (Pillus and Rine, 1989). There is clearly a memory of the switch between the ON and OFF states that is not stored at the silencer alone, as silencers and silencer-binding proteins do not change in transitions between the ON and OFF lineages. What is the molecular basis of this memory?

Before presenting models that explain the requirement for both specificity elements and histone modifications in epigenetic inheritance (see next section), I will briefly review data that support roles for specificity elements in other systems. In the fission yeast *Schizosaccharomyces pombe*, which is estimated to have diverged from *S. cerevisiae* about 700 million years ago, sequences involved in initiation of heterochromatin are also required for its maintenance. Heterochromatin in *S. pombe* is found at the pericentromeric DNA repeats, telomeres, and the silent mating-type loci. Although site-specific DNA-binding proteins contribute to heterochromatin formation at fission yeast mating-type loci and telomeres, in pericentromeric heterochromatin, small-interfering RNAs (siRNAs) take the place of DNA-binding proteins as specificity factors. siRNAs are produced from noncoding centromeric RNAs (ncRNAs) and load onto the RNA-induced initiator of transcriptional silencing

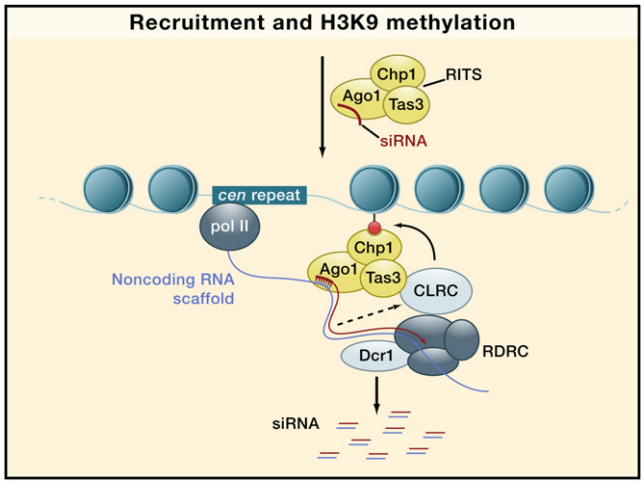

Figure 3. Heterochromatin Assembly at Pericentromeric DNA Repeats in Fission Yeast

In *Schizosaccharomyces pombe,* the small-interfering RNA (siRNA)-programmed RITS (RNA-induced initiator of transcriptional silencing) complex targets a nascent noncoding RNA, transcribed from pericentromeric (cen) DNA repeats by base-pairing interactions. The RITS complex recruits the RNA-dependent RNA polymerase complex (RDRC) and the Dicer (Dcr1) ribonuclease, which generate additional siRNAs. RITS also directly recruits the CLRC complex, containing the Clr4/Suv39h H3 lysine 9 (H3K9) methyltransferase. The methylation of H3K9 (red) allows efficient association of RITS with chromatin.

(RITS) complex (Verdel et al., 2004; Volpe et al., 2002). RITS contains the fission yeast Argonaute protein Ago1, the chromodomain protein Chp1, and the GW domain protein Tas3 and is directed to specific chromosome regions via base-pairing interactions between Ago1-bound siRNAs and nascent noncoding centromeric RNAs (Motamedi et al., 2004). This siRNA-containing complex promotes H3K9 methylation and heterochromatin formation by directly recruiting the CLRC complex, which contains the Clr4/Suv39h histone H3 lysine 9 (H3K9) methyltransferase (Bayne et al., 2010; Gerace et al., 2010) (Figure 3). Although siRNAs can initiate RITS recruitment and heterochromatin formation, stable binding of the RITS complex to chromatin requires Clr4 (Iida et al., 2008; Noma et al., 2004). These observations suggest that Clr4-mediated methylation of H3K9 stabilizes the association of RITS with its target chromosome regions by permitting the chromodomain of Chp1 to bind to nucleosomes containing methylated H3K9 (Figure 3). The role of siRNAs as specificity elements is supported by experiments involving ectopic production of siRNAs from a long hairpin construct complementary to the *ura4*$^+$ gene (Iida et al., 2008; Simmer et al., 2010). Hairpin-generated *ura4*$^+$ siRNAs can initiate silencing at some *ura4*$^+$ targets, but this silencing is lost several generations after removal of the hairpin. This observation, together with the continuous requirement for the RNAi pathway in maintenance of pericentromeric heterochromatin (Volpe et al., 2002), suggests that domains of H3K9 methylation (and histone hypoacetylation) cannot be maintained in the absence of an initiation signal, arguing against a purely histone-based model for *cis*-inheritance of heterochromatin in fission yeast.

Finally, the failure of histone modifications to direct their own inheritance is not limited to yeast. In *Drosophila*, the Polycomb and Trithorax groups of proteins act through specific regulatory sequences, called Polycomb response elements (PREs), to maintain the gene expression patterns that are established during embryogenesis (Ringrose and Paro, 2004). PRE-mediated silencing of a *white*$^+$ reporter gene is rapidly lost after excision of the PRE using cre/lox-mediated site-specific recombination (Busturia et al., 1997; Sengupta et al., 2004), again suggesting that histone modifications (in this case, hypoacetylation as well as histone H3 lysine 27 methylation) are not maintained in the absence of input from DNA sequence. In mammalian cells, it has been suggested that H3K27 methylated domains, induced by the artificial recruitment of the H3K27 methyltransferase complex (PRC2) via a tetracycline-inducible GAL4-EED fusion protein, are maintained after tetracycline-mediated repression of GAL4-EED (Hansen et al., 2008). However, the possibility that leaky expression of GAL4-EED or coupling to DNA methylation contribute to maintenance has not been ruled out.

Cooperativity between DNA or RNA Sequences and Histone Modifications

A requirement for specific DNA sequences in *cis*-inheritance of chromatin states may seem paradoxical. In contrast to *trans*-epigenetic mechanisms, which require the continuous action of a transcription factor through its DNA-binding site, chromatin inheritance mechanisms are generally thought to operate via nucleosome-based templating mechanisms independent of the underlying DNA sequence (Figure 1). Studies on the mechanism of association of silencing complexes with chromatin in the budding and fission yeasts, described above, suggest that the association of these complexes with chromatin, including their interaction with nucleation sites such as silencers, involves input from both sequence-specific factors and histone modifications. This mode of binding suggests a model for *cis*-inheritance of silent chromatin based on well-established cooperativity and allostery mechanisms that are prevalent in biology (Kuriyan and Eisenberg, 2007; Ptashne and Gann, 1998).

In budding yeast, efficient association of the SIR complex with silencer DNA requires two distinct types of activities. First, although subunits of the SIR complex make multiple contacts with silencer-bound proteins, and the silencer-binding proteins are constitutively expressed and bound to silencers, efficient association of the complex with the silencer requires the enzymatic activity of Sir2 (Hoppe et al., 2002; Luo et al., 2002; Rudner et al., 2005; Rusché et al., 2002). This observation suggests that binding of the SIR complex to the silencer is stabilized by Sir2-mediated deacetylation of silencer-proximal nucleosomes. Second, in addition to Sir2-mediated deacetylation, efficient binding of the Sir3 subunit of the SIR complex to silencer-proximal chromatin requires an interaction between Sir3 and a Sir4 protein bound to the silencer (Rudner et al., 2005). Here the silencer-proximal nucleosome is deacetylated by the bound Sir2/Sir4 complex, but consistent with the evidence that hypoacetylated domains cannot be maintained in the absence of silencers (Cheng and Gartenberg, 2000; Holmes and Broach, 1996), Sir3 binds poorly when it cannot interact with silencer-bound Sir2/Sir4 (Rudner et al., 2005).

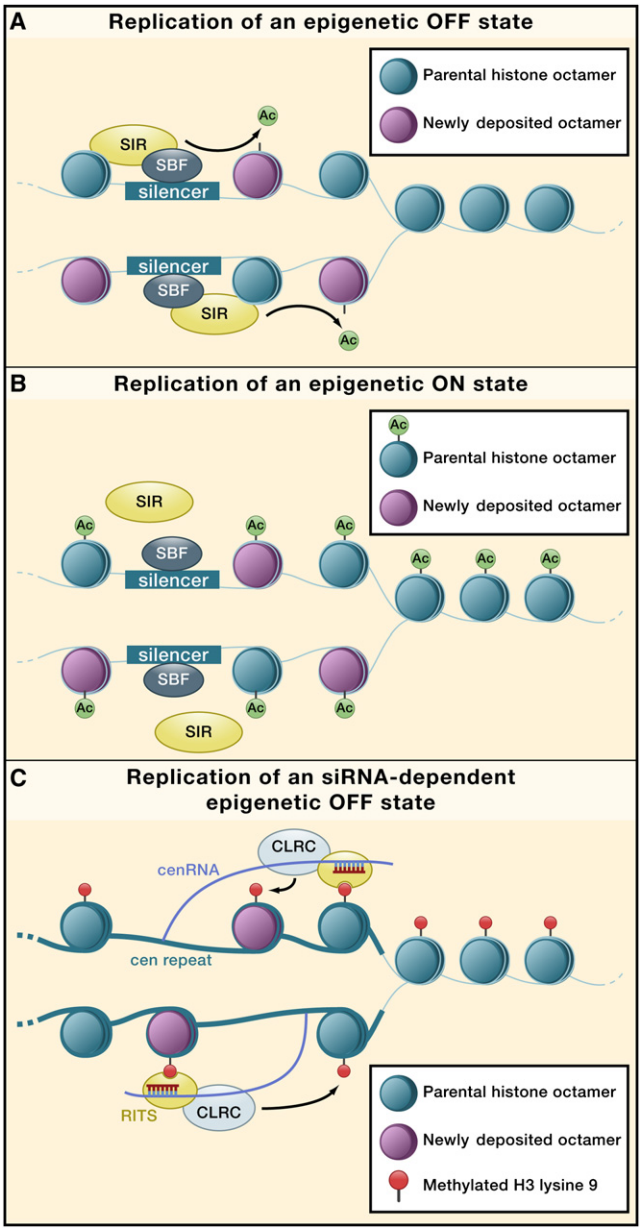

Figure 4. Specificity Factors and Histone Modifications Cooperatively Recruit Silencing Complexes

(A) During replication of *S. cerevisiae* silent chromatin, the silent state is efficiently re-established because the SIR complex is recruited through cooperative interactions with both deacetylated parental histones and silencer-binding factors (SBF).

(B) In contrast, during replication of the epigenetic ON state, even though the silencer is present, the interactions between the SIR complex and silencer-binding proteins are too weak to efficiently re-establish silencing. The ON state is therefore stable for many generations. Note that epigenetic variegation in budding yeast silent mating-type loci is only observed in cells containing weak silencers or lacking Sir1.

(C) In *S. pombe* pericentromeric heterochromatin, small-interfering RNAs (siRNAs) take the place of DNA-binding proteins. During replication of heterochromatin, the silent state is efficiently re-established because the RITS complex can bind cooperatively via siRNA-mediated base pairing and association with H3K9 methylation. RITS-mediated recruitment of CLRC then results in methylation of newly deposited histones and re-establishment of silencing. During replication of active chromatin (not shown), although siRNAs may be present, the RITS complex binds inefficiently and silencing is not re-established. In these models, the silencer and the noncoding RNA scaffold act as sensors for chromatin modification states, while the modifications are carriers of epigenetic information.

These observations suggest that binding of the SIR complex to sequences that initiate silencing occurs cooperatively through interactions with both site-specific DNA-binding proteins and a deacetylated nucleosome. This cooperative mode of binding suggests a model for *cis*-inheritance that takes the requirement for both the silencer and histone deacetylation into account (Figure 4A). Following replication of silent chromatin and distribution of parental histones to newly synthesized daughter DNA strands, the SIR complex binds cooperatively through interactions with silencer-binding proteins and a deacetylated nucleosome. The bound SIR complex then deacetylates newly deposited histones to re-establish silent chromatin (Figure 4A). The association of a new SIR complex with the deacetylated nucleosome also requires its interaction with the bound SIR complex. In genetically identical cells following replication of active chromatin, the silencer cannot efficiently recruit the SIR complex because the silencer-proximal nucleosome is acetylated at H4K16 and methylated at H3K79 (Figure 4B), and thus an ON lineage persists for many generations. In this model, maintenance requires re-establishment after each round of DNA replication using most or the same interactions that mediate establishment. However, re-establishment occurs with much greater efficiency when silencer-proximal unacetylated nucleosomes are inherited.

The cooperative association model also provides an explanation for properties of siRNA-mediated heterochromatin assembly at regions of pericentromeric DNA in fission yeast. Similar to the situation in budding yeast where the enzymatic activity of Sir2 is required for efficient binding of the SIR complex to the silencer, efficient association of the RITS complex with chromatin requires the Clr4 histone H3K9 methyltransferase (Motamedi et al., 2004; Noma et al., 2004). In this case, because Clr4 is also required for siRNA generation (Motamedi et al., 2004), the contributions of siRNAs and H3K9 methylation to RITS binding could not be uncoupled. However, a RITS complex loaded with Clr4-independent hairpin siRNAs initiates de novo silencing very inefficiently, but it readily potentiates silencing at a locus that contains pre-existing H3K9 methylation (Iida et al., 2008). Together with the bivalent structure of the RITS complex, this observation supports a cooperative mode of binding for RITS, involving interactions with specificity elements (siRNA-binding sites in nascent RNA scaffolds) and H3K9 methylated nucleosomes (Figure 4C). Thus, similar to DNA silencers, siRNA-binding sites on RNA scaffolds can act as sensors for chromatin modification states after DNA replication and re-establish heterochromatin only in combination with appropriately modified inherited histones (Figure 4C). A cooperative recruitment model may also explain the sensitivity of plant tandem repeat siRNAs to the presence of pre-existing DNA methylation in recruiting further DNA methylation and the observed variability in siRNA-mediated chromatin modifications in mammalian cells (Chan et al., 2006; Moazed, 2009).

Specific DNA and RNA Sequences as Sensors of the Epigenome

Sequence-specific regulatory elements and histone modifications are intimately associated with perhaps all changes in transcriptional regulation (Suganuma and Workman, 2008). The vast majority of these instances lack a heritable epigenetic component, as the associated gene expression states are dynamic and rapidly decay in the absence of the initial inducing signal(s). I propose that three specific properties of *cis*-regulatory sequences allow them to act as epigenetic sensors that mediate *cis*-inheritance. First, epigenetic sequence sensors must interact weakly with chromatin-modifying complexes so that their ability to recruit these complexes relies on additional local interactions such as appropriate histone modifications. Second, the sensors must recruit modifying complexes that make multivalent interactions, that is, they have interaction surfaces for the DNA-binding proteins at the sensor as well as domains that bind to specific histone modifications. Third, the histone-binding modules in the recruited complexes must preferentially associate with histone side chains that are modified by subunits of the same complex. For example, the budding yeast SIR complex weakly associates with silencer-binding proteins and contains an H4K16-specific deacetylase (Sir2) and a subunit (Sir3) that specifically binds to nucleosomes containing deacetylated H4K16. Similarly, the RITS complex contains siRNAs bound to its Ago1 subunit, physically associates with an enzyme (Clr4) that methylates H3K9, and contains a chromodomain subunit (Chp1) that specifically binds to methylated H3K9. Thus the recruitment functions of DNA silencers and RNA scaffolds rely on their chromatin environment. This chromatin environment, which is maintained in a sensor-dependent manner, provides a memory of the transcriptional history of the locus, for example as directed by the activities of patterning gradients during embryogenesis or following a stochastic switch event in yeast.

The cooperative mode of binding described above requires that silencing complexes be able to interact with sensors and nucleosomes over distance. Following DNA replication, each daughter DNA strand receives about half of the parental histones, which randomly associate with the newly synthesized DNA strands over an estimated zone of about 400 base pairs (Radman-Livaja et al., 2011). Furthermore, the randomness of parental histone distribution to the new daughter DNA strands will create situations wherein the two sister chromatids have different densities of inherited histones. Thus, some silencers would be located at a distance from a parentally inherited nucleosome after DNA replication. DNA looping or nucleosome relocalization, requiring the activity of chromatin remodelers, may be required for efficient binding of the modifying complex to both the silencer and parental histones on distally located nucleosomes. This is also likely to place a lower limit on the size of epigenetically heritable chromatin domains, which are indeed usually several kilobases in size.

In summary, the *cis*-inheritance model proposed here (Figure 4) suggests specific roles for sequence-specific elements and histone modifications in re-establishment of chromatin states. The specificity elements, DNA silencers, and RNA scaffolds (containing siRNA-binding sites or binding sites for proteins that recruit chromatin modifiers) act as sensors for the information content of the epigenome and are able to utilize this information to re-establish chromatin states after DNA replication. Nucleosomal histones and their modifications act as the carriers of epigenetic information, which can only be decoded when it is proximal to an appropriate sensor. As is the case for DNA silencers, the importance of histone retention in inheritance of silent chromatin is supported by genetic evidence. Mutations in replication-coupled histone assembly factors in a wide variety of organisms result in defects in maintenance of silent chromatin (Kaufman et al., 1997, 1998; Zhang et al., 2000).

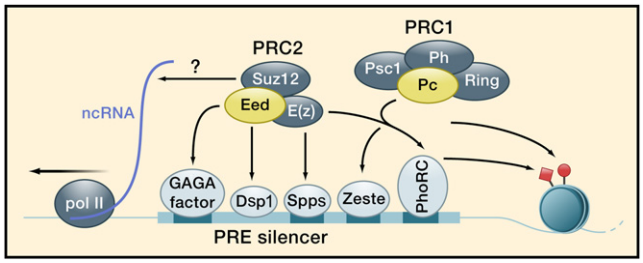

Figure 5. Recruitment of the *Drosophila* PRC1 and PRC2 Complexes
Multiple weak interactions with factors associated with Polycomb response elements (PREs) contribute to the recruitment of the *Drosophila* PRC1 and PRC2 complexes. The PRE contains binding sites for several site-specific DNA-binding proteins and is transcribed by RNA polymerase II (pol II) to give rise to noncoding RNA, which may participate in recruitment. Also depicted are GAGA factor (homolog of mammalian GAGA-related factors), Dsp1 (homolog of mammalian HMGB2), Spps (an Sp1/KLF transcription factor), Zeste (a *Drosophila*-specific transcription factor), and the Pho-repressive complex (PhoRC). H3K27 trimethylation and H3K9/H4K20 monomethylation (red) bind to the Pc and Eed subunits of the PRC1/2 and the dSfmbt subunit of the PhoRC, respectively.

Evolution of Epigenetic Sensors

Silent chromatin domains in fungi are mostly constitutive and do not act as regulated switches that control stable developmental transitions. The epigenetic nature of these domains is revealed only through stochastic switching events that give rise to genetically identical cell populations with different gene expression states (as in Figure 2). On the other hand, epigenetic states such as those associated with PREs are precisely controlled by switch mechanisms that are programmed by transcription during embryonic development. For example, the PRE/Polycomb system is a potent silencer at genes that were not turned on during embryogenesis but has no silencing activity at genes that were activated during embryogenesis (Ringrose and Paro, 2004). Some aspects of the *cis*-inheritance model described for the yeast systems here may also apply to the PRE switch, although additional mechanisms are required to account for precise developmental regulation of this more complex switch. Like yeast silencers, *Drosophila* PREs are composite binding sites for multiple transcription factors that act together to recruit the PRC1 and PRC2 silencing complexes (Müller and Kassis, 2006) (Figure 5). Recent evidence suggests that PREs are conserved in mammals and, like the *Drosophila* PREs, contain binding sites for the YY1 transcription factor (homolog of the fly Pho subunit of the PhoRC complex) and GAGA-related factors (Sing et al., 2009; Woo et al., 2010). In addition, *Drosophila* and mammalian Polycomb complexes associate

with noncoding RNAs, which may play important roles in establishment or maintenance of silencing (Guenther and Young, 2010; Hekimoglu and Ringrose, 2009). Following recruitment of PRC2 and H3K27 methylation, the maintenance of H3K27 methylation patterns may involve interactions between PRC1/2 and methylated H3K27 as well as PRE-binding proteins. Spatially restricted transcription through a PRE region during embryogenesis inactivates the silencing function of the PRE by deposition of transcription-associated histone modifications (Hogga and Karch, 2002; Schmitt et al., 2005). This, together with the absence of H3K27 methylation, then prevents cooperativity between nucleosomal histones and PRE-bound proteins or associated RNAs in recruitment of Polycomb complexes during later chromatin replication cycles.

Additional mechanisms are required to explain an important feature of the PRE switch, which is absent in yeast. If interactions with PRE-binding proteins alone are not sufficient for maintenance of Polycomb silencing, as proposed above, how is the virgin PRE in the early embryo active in Polycomb recruitment? One solution to this problem may be the presence of additional recruitment factors that participate in establishment of Polycomb silencing during embryogenesis but are replaced with H3K27 methylation during the maintenance of Polycomb silencing later in development. Candidates for such postulated factors include an embryo-specific PRE-binding protein or histone modifications (Figure 5).

Finally, silencers use interchangeable recruiting modules and appear to display a high degree of evolutionary plasticity. In budding yeast, telomeres and mating-type silencers share binding sites for only one site-specific DNA-binding protein (Rap1), although both telomeres and silencers recruit the same silencing complex (Moazed, 2001; Rusche et al., 2003). Similarly, *Drosophila* species use a variable arrangement of different binding sites in their PREs (Hauenschild et al., 2008), and mammalian PREs have been difficult to identify based on sequence similarity. This plasticity allows regulatory sites to evolve rapidly to act as either constitutive silencers, not dependent on input from chromatin, or epigenetic sensors, whose activities depend on surrounding chromatin. In budding yeast, silencing at the *HM* loci displays no variegation given that wild-type silencers recruit the SIR complex and re-establish the silent state with very high efficiency. However, variegation is observed in situations wherein silencers are weak, like telomeres or *HM* loci containing mutant silencers, or when the Sir1 adaptor protein (Figure 2A) is lacking. Thus, the evolution of adaptor proteins or gain and loss of interaction surfaces involved in recruitment may readily change a stochastic silencer to a constitutive silencer, and vice versa. In cases where silencers act as epigenetic sensors, their binding affinity for chromatin-modifying enzymes is fine-tuned by evolution so that their recruitment functions rely on the modification states of adjacent nucleosomes.

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