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Inheritance of epigenetic transcriptional memory through read-write replication of a histone modification

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Abstract

Epigenetic transcriptional regulation frequently requires histone modifications. Some, but not all, of these modifications are able to template their own inheritance. Here I discuss the molecular mechanisms by which histone modifications can be inherited and relate these ideas to new results about epigenetic transcriptional memory, a phenomenon that poises recently-repressed genes for faster re-activation and has been observed in diverse organisms. Recently, we found that the histone H3 lysine 4 dimethylation that is associated with this phenomenon plays a critical role in sustaining memory and, when factors critical for the establishment of memory are inactivated, can be stably maintained through multiple mitoses. This chromatin-mediated inheritance mechanism may involve a physical interaction between an H3K4me2 reader, SET3C, and an H3K4me2 writer, Spp1⁻ COMPASS. This is the first example of a chromatin-mediated inheritance of a mark that promotes transcription.

Keywords

Epigenetics; chromatin; transcription; memory

A genome encodes multitudes. The numerous phenotypes, morphologies and functions of cells in the tissues of a multicellular organism represent distinct expression states of the same genome¹. Likewise, unicellular organisms respond to changes in their environment by altering their gene expression. Thus, while the DNA sequence of the genome defines the phenotypic potential of an organism, that potential is realized through the regulation of gene expression, often through selective transcription. Changes in transcription can be rapid and transient but, under certain circumstances, cells undergo long-term changes in gene expression that persists in the absence of the initiating stimulus. For example, in multicellular organisms, transient developmental cues lead to the progressive establishment of stable and (generally) irreversible transcriptional programs that commit cells to differentiation and restrict their developmental potential^{2,3}. In single cell organisms, transcriptional responses can also result in stable, long-term changes in phenotype⁴. For example, wild isolates of *Saccharomyces cerevisiae*^{5,6} as well as pathogenic yeasts such as *Candida albicans*^{7,8} and *Cryptococcus neoformans*⁹ exhibit switching between several

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Competing interests

The author declares no competing interests.

alternative colony morphologies due to changes in transcription^{10,11}. These meta-stable colony morphologies are stably inherited but their relative frequency can be modulated by environmental stimuli^{12,13}. Thus, the transcriptional responses to environmental stimuli can be either transient or longer-term; in some cases, they are inherited through mitosis and meiosis¹⁴. Changes in transcription that are inherited through mitosis or meiosis are termed epigenetic transcriptional regulation.

Heritable transcriptional regulation can be mediated by transcription factor activity, DNA methylation, non-coding RNAs and histone modification. Such regulatory factors function either in *trans* or in *cis*; *trans*-acting factors such as transcription factors bind to genetically encoded DNA elements to promote or repress transcription, while *cis*-acting DNA methylation or histone modifications “mark” regulatory regions to impact transcription of nearby genes¹⁵.

Chromatin as a regulator of transcription

How do *cis*-acting marks impact transcription? Transcription is a complex process involving the coordinated recruitment of numerous factors and complexes¹⁶. Eukaryotic genomes are packaged into nucleosomes¹⁷, which limit access to the DNA, inhibiting transcription^{18,19} and many regulators of transcription impact DNA accessibility by regulating nucleosome occupancy²⁰. The first indication that this was the case was the discovery that enzymes that control acetylation or deacetylation of histones either promote or repress transcription^{21,22} and that large ATP-dependent complexes that could slide or remove nucleosomes regulate transcription^{23,24}. Acetylation of lysine residues in histones promotes transcription^{25,26}, in part because it neutralizes their charge and reduces their affinity for DNA²⁷ and disrupts interactions between neighboring nucleosomes²⁸, making them easier to displace by transcription factors or RNA polymerase^{29–32}. Also, acetylated histones serve as binding sites to recruit coactivators – including acetyltransferases themselves and nucleosome remodeling complexes³³ – that possess bromodomains³⁴. Thus, histone deacetylation represses transcription both by stabilizing chromatin to limit access to the DNA and by preventing recruitment of bromodomain coactivators^{35–37}. Regulating nucleosome occupancy through nucleosome remodeling and histone modification plays a critical role in regulating transcription.

Myriad covalent modifications of histones have been identified³⁸, including methylation, phosphorylation and ubiquitylation on their unstructured terminal tails. Mutations in either the enzymes that catalyze these modifications or the amino acids that are modified perturb transcriptional regulation, highlighting their functional significance³⁸.

Whereas histone acetylation generally promotes transcription²⁶, histone methylation promotes both transcription and silencing, depending on which amino acid is methylated. The histone H3 lysine 9 methyltransferase from mammals, flies and yeast promotes transcriptional silencing^{39,40}. The histone H3 lysine 4 methyltransferase Trithorax and the histone H3 lysine 27 methyltransferase Polycomb play antagonistic roles during development⁴¹; loss of Trithorax leads to decreased expression of critical transcription factors within the homeotic gene cluster, while loss of Polycomb has the opposite effect

⁴¹. Thus, these two marks are associated with alternative, stable transcriptional states that regulate differentiation; H3K4 methylation is associated with actively transcribed loci, while H3K27 methylation is associated with silent loci. Genomic analysis reveals that, in differentiated cells, H3K4 methylation (H3K4me) and H3K27 methylation (H3K27me) are generally mutually exclusive and that the boundary between these marks in the homeotic gene cluster in flies and mammals defines which genes are expressed and which are silenced ⁴². This is due to recruitment of co-activators by H3K4me or co-repressors by H3K27me, regulation of histone H3K27 acetylation by H3K27me and through changes in chromatin folding and compaction ⁴³. In pluripotent stem cells, certain loci possess “bivalent” loci in with both H3K4me and H3K27me, reflecting their potential for transcription or silencing ^{42,44}.

Regions near centromeres and telomeres are constitutively silent ⁴⁵. This stable silencing requires histone deacetylation and, in most species, histone H3 lysine 9 methylation (H3K9me) ^{46,47}. Reporter genes adjacent to telomeres and centromeres have been a critical tool for screens to identify molecular players essential for this type of stable silencing. For example, a chromosomal inversion in *Drosophila* that places the *white* gene beside the centromere of the X chromosome results in variegated silencing of *white* in a subset of cells in the eye ^{48,49}. Likewise, reporter genes inserted near a centromere in *S. pombe* or near telomeres in *S. cerevisiae* show variegated silencing ^{50,51}. ChIP sequencing reveals that H3K9me localizes at many constitutively repressed loci in the genomes of yeast, flies and mammals ⁵².

Genomes are therefore divided into regions having distinct histone marks that correlate with different transcriptional states. Histone modifications are generally directed by *cis*-acting genetic information. For example, histone acetyltransferases are recruited to enhancers or promoters by sequence-specific transcription factors ⁵³. Likewise, at the silent mating type locus in budding yeast, which is silenced by histone deacetylation, the histone deacetylase Sir2/Sir4 is recruited through binding of the Origin Replication Complex and Sir1 to elements that flank the locus ^{54–56}. In the case of histone methylation, the mechanism is related but less simple ⁴⁶. While the enzymes responsible for H3K27 and H3K9 methylation can be recruited to chromosomal loci by sequence-specific transcription factors ^{57,58}, other recruitment mechanisms also occur. H3K9 methylation near centromeres in *S. pombe* depends on low level transcription of pericentromeric repeats that results in double stranded RNAs that are processed by Dicer ⁵⁹. These small RNAs are incorporated into an RNAi transcriptional silencing complex that recruits the H3K9 methyltransferase Clr4 to the transcribed locus ^{60,61}. Likewise, H3K27me is stimulated by recruitment of PcG complexes by both sequence-specific DNA binding proteins and mechanisms such as RNA and other histone modifications ^{62,63}.

H3K4 methylation has been proposed to *reflect* transcription; in yeast, the sole H3K4 methyltransferase Set1/COMPASS is recruited through interaction with the Paf1 complex (Paf1C) ⁶⁴, which binds to elongating RNA polymerase II (RNAPII). Loss of Paf1C leads to global loss of H3K4 methylation ⁶⁴. Furthermore, Paf1C physically interacts with the E3 ubiquitin-conjugation/ligase Rad6/Bre1, which mediates ubiquitination of H2B lysine 123

⁶⁵. This mark is also required for all H3K4 methylation in budding yeast ^{66,67}. These results suggest that active RNAPII recruits Set1/COMPASS, leading to H3K4 methylation.

Heritable chromatin states

The heritability of DNA methylation and its requirement for the maintenance of certain epigenetic states suggests a general conceptual model for the inheritance of *cis*-acting information: following DNA replication, hemi-methylated CpG sites are recognized and re-methylated on the complementary cytosine (Figure 1A). Therefore, a mechanism that both recognizes a *cis*-acting mark and stimulates its re-establishment might allow that mark to be faithfully inherited at that location. Is there evidence that histone post-translational modifications are heritable? In other words, can *cis*-acting histone marks be perpetuated in the absence of *trans*-acting factors? Studies of silencing of telomeres, centromeres and special loci like the hidden mating type loci (*HM*) in flies and yeast suggested that histone modification-dependent transcriptional states are heritable in the absence of some of the *trans*-acting factors necessary for their establishment. For example, although Sir1 facilitates recruitment of the Sir2/Sir4 HDAC to the silent *HM* loci through interaction with sequence-specific DNA binding proteins ⁶⁸, loss of Sir1 does not lead to immediate loss of silencing. Rather, in *sir1* mutant strains, there are two stable populations, one that continues to silence the *HM* loci and the other that expresses the *HM* loci ⁶⁹. This suggests that chromatin domains defined by deacetylated histones are epigenetically inherited.

Additional compelling evidence for epigenetically heritable chromatin modifications comes from studies of H3K9 methylation in *S. pombe*. Tethering of a the H3K9 methyltransferase Clr4 to an ectopic locus is sufficient to stimulate nearby H3K9 methylation and transcriptional silencing ^{70,71}. In cells lacking a putative H3K9 demethylase Epe1, this mark can be maintained for many generations upon removing the tethered Clr4 ⁷¹. Inheritance requires the endogenous Clr4 ⁷¹ and is dependent on the density of H3K9me3 ⁷², but is independent of the RNAi machinery ⁷¹. Thus, while wild type cells (having Epe1) require constant re-establishment of H3K9 methylation, this mark can mediate its own inheritance.

For histone modifications to be inherited, two conditions must be met. First, nucleosomes must be reincorporated into chromatin near their original location following DNA replication. Classic pulse-chase experiments ⁷³ as well as more recent global experiments ⁷⁴ reveal that nucleosomes are partitioned equally between the two daughter chromosomes after DNA replication. Furthermore, the location of nucleosomes is quite stable over many cell divisions, particularly in silent regions ⁷⁵. This suggests that nucleosomes are preferentially reincorporated near their original location. Indeed, several histone chaperones associate with PCNA and the DNA replication machinery and have been proposed to facilitate efficient reincorporation ^{76,77}. These results suggest that, following DNA replication, approximately half of nucleosomes will be marked as they were before S-phase and the other half will be unmarked.

Second, to facilitate re-establishment of histone marks after DNA replication, the histone modifications on parental nucleosomes must be recognized (by a “reader”) and that must be coupled to recruitment of the enzyme that modifies new nucleosomes (“writer”). This

is referred to as a read-write mechanism (Figure 1B). Read-write mechanisms have been defined for heritable histone deacetylation, H3K9 methylation and H3K27 methylation. Deacetylated nucleosomes of the *HM* loci or telomeres are recognized by the Sir3 protein, which both binds to unacetylated histone H4 lysine 16⁷⁸ and recruits the Sir2 histone deacetylase. Likewise, H3K9 methylation is recognized by both the HP1 protein and a chromodomain within the Clr4 H3K9 methyltransferase. Because HP1 interacts with Clr4, this protein functions as a reader, while Clr4 is both a reader and writer. The recognition of H3K9 methylation stimulates the methyltransferase activity^{79,80}, promotes spreading of the mark⁸¹ and is essential for inheritance of H3K9 methylation⁷¹ (Figure 1C). Finally, H3K27 methylation can also stimulate its own inheritance through a read-write mechanism involving two subunits of the PRC2 complex. The EED subunit of PRC2 binds to H3K27me3. This leads to both recruitment of PRC2 to nucleosomes that possess H3K27me3 as well as stimulation of the catalytic activity of the methyltransferase EZH2^{82,83} (Figure 1D). Thus, read-write mechanisms are a common strategy to faithful replication of histone modifications.

Epigenetic transcriptional memory

To-date, the best examples of heritable chromatin states are those associated with stable silencing. Histone modifications associated with active transcription are, for the most part, not heritable; histone acetylation is unstable and nucleosomes in actively transcribed regions are not reincorporated faithfully at the same location through multiple cell divisions⁷⁵. However, modifications that reflect previous transcription can be inherited. Here, I highlight a phenomenon whereby a heritable histone modification over an inactive gene both reflects previous expression and promotes future transcription. This mark (H3K4me2) is associated with both active and poised loci, although the molecular requirements and heritability in these two circumstances are different.

Some inducible genes are more rapidly or strongly induced in cells that have previously expressed them¹⁴. This phenomenon, called epigenetic transcriptional memory, was discovered in budding yeast⁸⁴, but has since been observed in flies⁸⁵ and human cells⁸⁶ and related phenomena occur in worms⁸⁷ and plants^{88,89}. The general features of this type of memory are the requirement for interaction with nuclear pore proteins, H3K4me2 and recruitment of poised RNAPII. This type of memory persists through mitosis for between 4 and 15 cell divisions, depending on the system. One of the best-understood models for memory is the yeast *INO1* gene^{90,91}, encoding the inositol 3-phosphate synthase enzyme, which is only expressed in the absence of exogenous inositol. This gene has also been a model to understand the role of the nuclear pore complex (NPC) in regulating transcription and chromatin structure⁹².

When cells are starved for inositol, the transcriptional activation of the *INO1* gene is coupled with repositioning to the nuclear periphery and a physical interaction with the NPC^{92,93} (Figure 2, left). The interaction with the NPC requires two transcription factors (Cbf1 and Put3) that bind to *cis*-acting *DNA zip codes* upstream of the promoter, as well as several nuclear pore proteins⁹³⁻⁹⁵ (Figure 2, left). Disrupting the interaction with the NPC leads to a defect in *INO1* transcription^{93,94}.

If inositol is added back, *INO1* is immediately repressed^{84,96}. However, the gene remains associated with the NPC for approximately four generations (8h), both in the cells that had been expressing *INO1* and in their daughters, granddaughters, and great granddaughters⁸⁴ (Figure 2, right). Ultimately, this interaction is lost and *INO1* repositions to the nucleoplasm. Thus, the interaction of recently-repressed *INO1* with the NPC is an epigenetically heritable state. Importantly, this interaction is mediated by a different transcription factor called Sfl1⁹⁷. This TF binds upon repression and is necessary and sufficient to mediate interaction with the NPC⁹⁷. Also, the nuclear pore protein Nup100 is required for localization to the periphery only during memory and has no role in targeting active *INO1* to the periphery⁹⁶. Finally, the systems controlling positioning of active and recently repressed *INO1* are independent of each other. Thus, genes can be targeted to the NPC by at least two mechanisms and the *INO1* gene utilizes both under different circumstances.

Transcriptional memory maintains *INO1* in a poised state (Figure 2). The RNAPII preinitiation complex associates with the *INO1* promoter during memory, leading to faster induction and faster adaptation if the cells are challenged with inositol starvation⁹⁶⁻⁹⁸. Also, memory is associated with distinct histone modifications over the promoter compared with those associated with either the long-term repressed or active promoter. Whereas the long-term repressed *INO1* promoter shows low histone acetylation and H3K4 methylation and the active *INO1* promoter shows high acetylation and H3K4me3, during memory, the promoter and 5' end of the *INO1* gene show low levels of acetylation and high levels of H3K4me2, but not H3K4me3^{86,97,98}. During memory, an alternative form of the Set1/COMPASS histone methyltransferase lacking the Spp1 subunit, is recruited⁹⁷. Spp1⁻ COMPASS catalyzes dimethylation, but not trimethylation, of H3K4⁹⁹. Furthermore, the histone variant H2A.Z is incorporated upstream of the promoter during memory^{84,96}. Poised RNAPII, H3K4me2 and H2A.Z are also associated with the promoters of other yeast genes that exhibit memory as well as genes in human cells that exhibit memory^{86,97,98,100}.

The interaction with the NPC is required for these chromatin changes. Loss of Nup100 or Sfl1 leads to loss of H3K4me2 and H2A.Z incorporation during memory^{86,96,97}. In contrast, loss of these factors has no effect on the H2A.Z at the +1 nucleosome of the repressed *INO1* promoter or H3K4 methylation over the active *INO1* promoter^{96,97}.

Chromatin-dependent transcriptional memory

These chromatin changes play an essential role in promoting *INO1* memory. Mutations that inactivate the Set1/COMPASS histone methyltransferase or the SWR1 complex - which exchanges H2A.Z for H2A - lead to loss of memory^{86,96,97}. Likewise, deletion of the *HTZ1* gene or mutation of the lysine 4 in histone H3 also disrupt memory^{84,97}. Finally, while H2A.Z incorporation requires H3K4me2, H3K4 dimethylation is independent of H2A.Z, suggesting that H3K4me2 is a critical upstream step⁹⁸. In fact, the SET3C complex, which binds preferentially to H3K4me2¹⁰¹, is essential for both maintaining H3K4me2 and for binding of RNAPII^{86,97}. Importantly, this role is specific to memory; loss of Set3 has no effect on H3K4me2/3 or RNAPII binding over the active *INO1* promoter^{86,97}. Finally, conditional inactivation of Set1/COMPASS during memory leads to loss of

H3K4 methylation and RNAPII association⁹⁷. Thus, H3K4me2 is continuously required for RNAPII poising.

To understand the molecular function of H3K4me2, we employed conditional genetics. The recruitment of preinitiation RNAPII to the *INO1* promoter during memory requires the Mediator kinase Cdk8^{97,98}. Either conditional depletion of Cdk8⁹⁷ or inhibiting an analog sensitive mutant Cdk8⁹⁸ leads to rapid loss of RNAPII from the poised *INO1* promoter. Inhibition by the analog is rapidly reversible, allowing us to test if continuous RNAPII binding is critical for maintaining the *INO1* promoter in a poised state. If so, then RNAPII should fail to be recruited upon removing the inhibitor. However, RNAPII was recruited back to the recently repressed *INO1* promoter following removal of the Cdk8 inhibitor, suggesting that the conditions necessary for RNAPII poising are maintained in the absence of RNAPII⁹⁸.

Given that RNAPII binding is not required to maintain the *INO1* promoter in a poised state, we next tested if H3K4me2 is required. To do this, we combined temporary inhibition of Cdk8, which leads to loss of RNAPII binding, with inactivation of SET3C, which leads to rapid loss of H3K4me2⁹⁷. Thus, we assessed the ability of RNAPII to be recruited back to the *INO1* promoter after removal of the Cdk8 inhibitor in the presence and absence of H3K4me2. In the absence of H3K4me2, RNAPII failed to associate with the *INO1* promoter⁹⁸, suggesting that recruitment of poised RNAPII during memory requires H3K4me2.

How does H3K4me2 promote RNAPII recruitment? Our data suggest that H3K4me2 functions in a positive feedback loop during memory. While loss of Sfl1 or Nup100 prevents H3K4me2 from being deposited during memory, conditional removal of H3K4me2 disrupts Sfl1 binding⁹⁸. Therefore, Sfl1-mediated interaction with the NPC is required for H3K4 dimethylation and H3K4me2 is required for continued Sfl1 binding. This suggests that H3K4me2 permits Sfl1 binding and Sfl1 interacts with Cdk8⁺ Mediator¹⁰² to promote RNAPII recruitment.

Chromatin as a source of heritable transcriptional memory

Could H3K4me2 be the source of epigenetically heritable information? In other words, is H3K4me2 inherently stable and capable of being re-established after DNA replication? We first assessed the stability of H3K4me2 associated with active transcription by examining this mark over the *INO1* promoter in a mutant that lacks memory. In such a mutant, H3K4me2 is rapidly lost upon repressing transcription⁹⁸. This suggests that H3K4me2 *per se*, is neither stable nor heritable.

If H3K4me2 associated with memory is epigenetically heritable, it should persist in the absence of *trans*-acting initiating factors¹⁰³. To assess the heritability of H3K4me2 during memory, Sfl1 was depleted by auxin-induced degradation¹⁰⁴ after establishing *INO1* memory. Under these conditions, interaction with the NPC, RNAPII binding and H2A.Z were lost, confirming that Sfl1 function was disrupted⁹⁸. However, H3K4me2 was maintained without dilution through four cell divisions⁹⁸. Thus, while interaction with the NPC is essential for *establishment* of H3K4me2 during memory, this mark can be

maintained and re-established through cell divisions in the absence of this interaction. This is reminiscent of the perpetuation of silent chromatin upon loss of Sir1 on *HM* silencing or removal of tethered Ctr4 and suggests that H3K4me2 may stimulate its own inheritance.

How might H3K4me2 be inherited during memory? Could this involve a read-write mechanism? During memory, the writer of H3K4me2 is Spp1⁻ COMPASS and the putative reader is SET3C^{86,97}, a histone deacetylase complex with a PHD finger that recognizes H3K4me2¹⁰¹. Simultaneous inactivation of Sfl1 and SET3C led to loss of H3K4me2 during memory⁹⁸, consistent with this complex facilitating inheritance. Furthermore, co-immunoprecipitation revealed that SET3C physically interacts with Spp1⁻ COMPASS⁹⁸. This interaction suggests a read-write model in which SET3C recognizes H3K4me2-marked nucleosomes after DNA replication and recruits Spp1⁻ COMPASS to re-establish this mark, facilitating both its spreading and inheritance (Figure 3).

These observations suggest that the same mark (H3K4me2) at the same location in the genome can be either unstable or heritable, depending on the pathway by which it is deposited. While all H3K4 methylation in budding yeast requires COMPASS, at least two distinct mechanisms can lead to COMPASS-mediated H3K4me2; H3K4me2 during active transcription and H3K4me2 during memory. H3K4 dimethylation during active transcription requires RNAPII, but H3K4 dimethylation during memory does not; inactivation of Cdk8 or Sfl1 led to loss of RNAPII but did not affect H3K4me2 during memory⁹⁸. H3K4me2 during memory requires Nup100 and Set3C, but these factors are not required for H3K4me2 over transcribed genes^{86,97}. Likewise, a subunit of the Paf1C, Leo1, is specifically required for H3K4 methylation during memory⁹⁸. Therefore, the heritability and functional impact of H3K4 methylation depends on additional, context-specific factors.

Future work will determine the molecular basis of the switch from RNAPII-dependent, unstable H3K4me2 to RNAPII-independent, heritable H3K4me2 during memory. We hypothesize that this switch is regulated by transcription factors that mediate interaction with nuclear pore proteins. But it is unclear what regulates these transcription factors and how the interaction with nuclear pore proteins impacts histone methylation. Nup98, the Nup100 homolog in flies and mammals, physically interacts with the Trithorax and Set1/COMPASS complexes and impacts H3K4 methylation in those organisms^{105,106}. This suggests that nuclear pore proteins may play critical and conserved roles in regulating epigenetically heritable histone methylation. Also, it will be important to define the molecular mechanism by which SET3C and Spp1⁻ COMPASS physically interact to mediate inheritance of H3K4me2. Integrating how transcription factors, nuclear pore proteins and chromatin modifications stimulate RNAPII poising to lead to changes in future gene expression will have a broad impact on basic cell biology, developmental biology and genetics.

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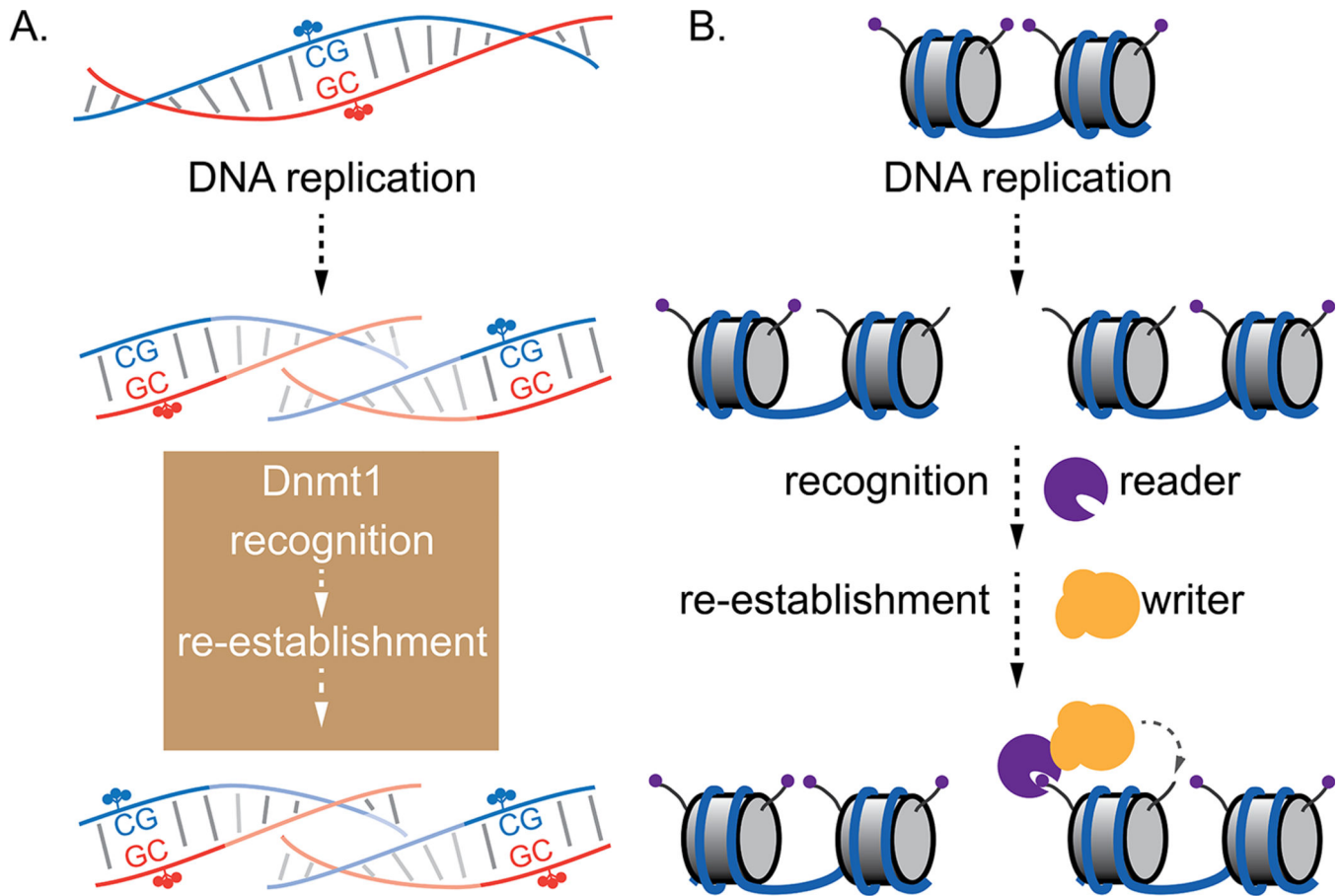
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Brickner, Figure 1

Figure 1. Read-write mechanisms for replicating chromatin marks.

A. Following DNA replication, hemi-methylated CpG sites are recognized by Dnmt1, which re-establishes symmetric methylation. This protein possesses the ability to both recognize the appropriate substrate and to catalyze the reaction. B. Because histone H3/H4 nucleosome cores are reincorporated nearby but randomly distributed between the daughter products of DNA replication, approximately half of the nucleosomes contain parental, local H3/H4 tetramers and half contain new H3/H4 tetramers. The parental nucleosomes can be recognized by reader proteins that bind to specific histone marks. Recognition by readers leads to recruitment of writers, which re-establish these marks on neighboring nucleosomes after DNA replication. C. Inheritance of H3K9 methylation. Both the writer Clr4/Suv29h and the reader Swi6/HP1 can bind H3K9me3. Because Swi6 physically interacts with Clr4, this provides both a direct and an indirect mechanism for Clr4 recruitment to H3K9me3-marked loci. Binding of H3K9me3 to Clr4 also stimulates catalytic activity of the enzyme. D. Inheritance of H3K27 methylation. The PRC2 Polycomb complex contains both a reader (EED; light green) and a writer (EZH2; dark green). Binding of EED to H3K27me3 both recruits PRC2 to chromosomal sites with this mark and stimulates EZH2 catalytic activity to promote methylation of lysine 27 on neighboring nucleosomes.

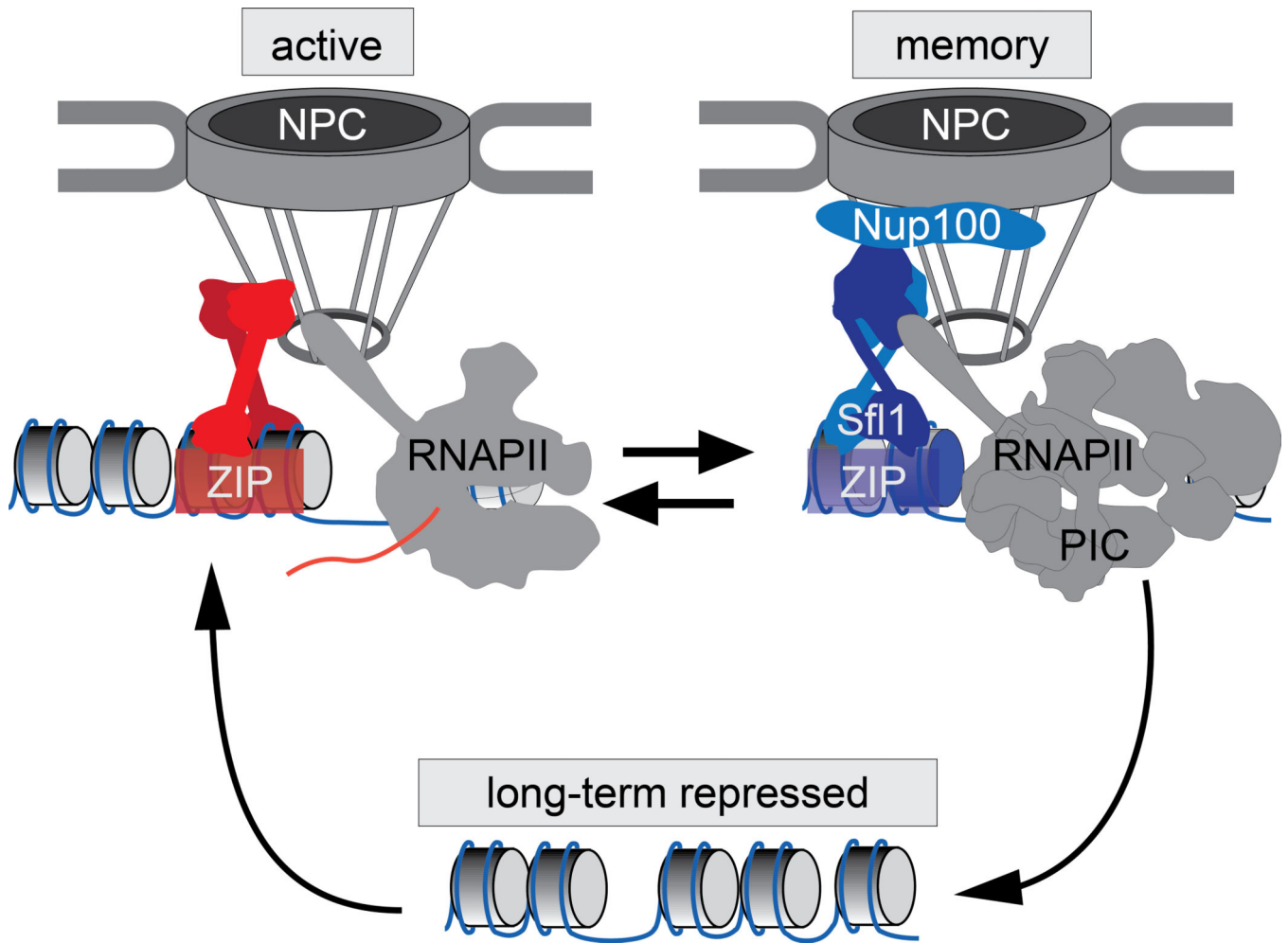


Figure 2. *INO1* transcriptional states.

Long-term repressed *INO1* localizes in the nucleoplasm and is neither associated with RNAPII nor H3K4 methylation. Upon activation, *INO1* is recruited to the nuclear pore complex (NPC) through the action of the Put3 and Cbf1 TFs (red proteins), which bind to *cis*-acting DNA zip codes upstream of *INO1*. Upon transcriptional repression, *INO1* remains associated with the NPC. During memory, interaction with the NPC requires different factors: the Sfl1 TF and the nuclear pore protein Nup100. This interaction leads to changes in chromatin modifications (H3K4me2 and H2A.Z incorporation; indicated in blue) and recruitment of a pre-initiation form of RNAPII.

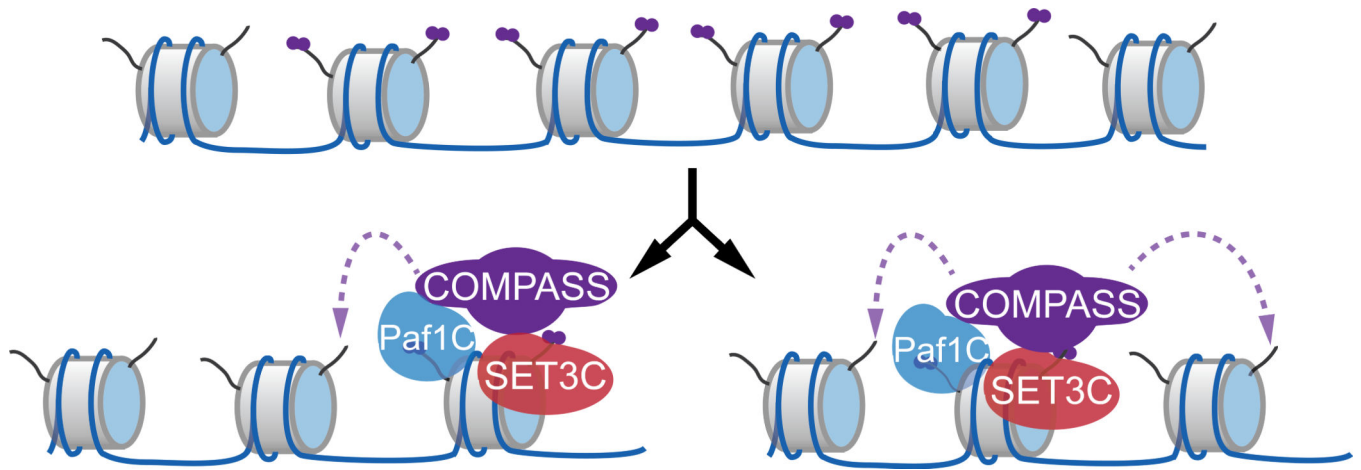


Figure 3. Proposed mechanism of replication of H3K4me2 during transcriptional memory. Following DNA replication, SET3C recognizes the H3K4me2 mark near the promoter of genes that exhibit memory. Through interaction with Spp1⁻ COMPASS, potentially involving the Paf1C complex subunit Leo1, SET3 recruits COMPASS to this site and re-establishes H3K4me2.