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Epigenetic fates of gene silencing established by heterochromatin spreading in cell identity and genome stability

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Abstract

Heterochromatin spreading, the propagation of repressive chromatin along the chromosome, is a reaction critical to genome stability and defense, as well as maintenance of unique cell fates. Here, we discuss the intrinsic properties of the spreading reaction and circumstances under which its products, formed distal to DNA-encoded nucleation sites, can be epigenetically maintained. Finally, we speculate that the epigenetic properties of heterochromatin evolved together with the need to stabilize cellular identity.

Keywords

epigenetic inheritance; Histone 3 Lysine 9 methylation; heterochromatin spreading; cellular identity; chromatin structure; histone turnover; DNA methylation

Introduction

Heterochromatin is a nuclear ultrastructure composed of nucleic acids and effector structural and silencing proteins targeted to chromatin by histone marks. Histone marks signaling heterochromatin assembly, such as Histone 3 (H3) Lysine 9 (K9), Lysine 27 (K27) methylation (me) and Histone 4 (H4) Lysine 16 (K16) deacetylation, have been implicated as among the few documented histone modifications capable of imparting epigenetic behaviors [1]. Heterochromatin occupies central roles in the maintenance of genome stability, such as the repression of recombination at repetitive elements, correct assembly of kinetochore components at centromeres, and genome defense, including silencing of invasive DNA elements, such as ancient retroviruses. Separately and crucially, heterochromatin takes on a key function in the establishment and maintenance of cell identity. It contributes to this process by partitioning the genome such that regions irrelevant to the cell fate remain heritably repressed. This function is particularly central in

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Compliance with ethical standards

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multicellular organisms with lineage specialization such as metazoans. Thus heterochromatin has evolved to regulate the genome in a large variety of ways.

a. Heterochromatin nucleation and spreading, and the question of memory at distal sites

The initial formation of heterochromatin requires at least two steps: 1. A DNA-sequence driven component, called nucleation, where DNA signals encoded at the nucleation site recruit heterochromatin effectors either by direct mechanisms (transcription factor based recruitment) or indirect mechanisms, often involving small RNAs. The available literature favors the notion that heterochromatin at nucleation sites can be faithfully maintained intergenerationally, requiring repressive histone modifications [1–3]. 2. A DNA sequence indifferent process, called spreading, during which heterochromatin propagates along the chromosome. This reaction is essential to establishing large heterochromatic regions, particularly those involved in genome partitioning. It is less clear whether once formed, heterochromatin located distal to nucleation sites is intrinsically capable of epigenetic maintenance and if so, by what mechanisms. If not re-formed with high fidelity each cycle, the distal heterochromatic repression would be variegated and therefore not compatible with maintenance of a genome partition pattern. In turn, epigenetic behaviors such as in the definition of cellular lineages necessitate high fidelity (Figure 1). However, low fidelity of inheritance, or variegation, has been observed in multiple systems. This was first observed in flies at genomically disrupted regions and termed Position Effect Variegation (PEV). Evidence for variegation also exists for intact loci. Observation of the Telomere Position Effect (TPE) in budding yeast, like PEV, also argues in favor of unstable inheritance, although in most studies the separate nucleation process that precedes spreading is not controlled for. In our work, we focused on the intrinsic properties of H3K9 me-marked heterochromatin in fission yeast, which is shared with most metazoans, and find most spreading to be stochastic, multimodal and intergenerationally unstable [4]. However, at a locus essential to cell identity, we find heterochromatin to be remarkably stable. This stability requires the collaboration of a stochastically spreading, but robustly nucleating noncoding RNA (ncRNA) element and a specialized element called *REIII* that nucleates poorly but spreads in a predicable manner.

b. Mechanisms of distal heterochromatin epigenetic inheritance

Our work demonstrates that distal heterochromatin propagated by spreading, at least in fission yeast, is epigenetically unstable. Thus, auxiliary functions have to be built into heterochromatin domains to promote high fidelity at nucleation distal loci. In fission yeast, such auxiliary control is imparted by *REIII* and is absent at ncRNA-only nucleated loci. *REIII,* unlike ncRNA-nucleators, strongly reduces histone turnover [4], which is one pathway that has been implicated in epigenetic inheritance [5, 6] (Figure 2). The suppression of histone turnover in general likely favors retention of H3K9me or H3K27me histones, facilitating re-establishment after S-phase through the read-write capacity of repressive histone modifiers [7, 8]. Another potential mechanism of achieving inheritance is chromatin structure (Figure 2). Chromatin structure formation often depends on the chromosomal context as it may involve interactions with distal elements [9, 10]. Long range looped chromatin structures called “chromatin hubs” have been speculated to reinforce memory of the repressed state in the case of the H3K27me/polycomb pathway [11]. Intriguingly,

looping at the very local level has been modeled to promote memory formation in fission yeast [12]. For the case of the fission yeast *REIII* auxiliary element, we favor the notion that its memory characteristics are due in part to a structural constraint. While the common ncRNA-nucleators appear to be independent of location, as they can be inserted at multiple genomic loci and still induce spreading [4], the *REIII* element does not retain its function when transposed to another location [3, 4]. It is possible that this restriction of function to the endogenous site points to *REIII* requiring a specific local chromatin context or structure, such as looping, to operate. Formally, however, it cannot be excluded that additional local elements are required for normal *REIII* function at any given locus. A broad remaining question is how the dynamic rearrangements in architecture observed in the cell cycle [13] affect the ability of chromatin structure to enforce epigenetic memory.

Either by regulating histone turnover or chromatin structure, accessory or specialized elements appear to feature bistability [14, 15], with low transition rates between stable states that are either de-repressed and active (ON) or repressed (OFF). That these elements can exist in a stable ON state is a phenomenon that has been observed in plants and yeast [16, 17]. This inability of a fraction of cells to stably adopt the repressed OFF state counteracts the need for maintaining a population-wide uniform epigenetic state in the development of cellular lineages from less differentiated progenitor pools. Thus, additional features are then required to shift the balance towards uniform maintenance of the OFF state across the population. We believe in the case of fission yeast, this function is fulfilled by nearby placement of one or more ncRNA-nucleator(s). While these elements cannot produce distal heterochromatin structures with strong memory characteristics, they robustly generate local heterochromatin. The coupling of such highly efficient nucleators to a bistable auxiliary element then yields stable epigenetic repression. It still remains unclear why fission yeast ncRNA-nucleators are themselves not capable of producing significant memory, though this type of coupling between two elements or two regions (say nucleation proximal and distal) of heterochromatin for memory formation has been observed in other systems, such as budding yeast and plants [18, 19],

One key mechanism to enhance the robustness of distal heterochromatin inheritance is tying it to DNA replication, a central and high-fidelity cellular process. This can occur via either direct or indirect means, and may be required in complex systems such as mammals and plants that widely use heterochromatin to silence pervasive foreign genetic elements or to partition the genome in differentiation. Examination of post-mitotic replication patterns in mammalian cells reveals generally accurate re-establishment of active and repressive chromatin marks [20, 21]. This may require coordination with histone chaperones [22, 23] and, in the case of heterochromatic marks specifically, proceed via an indirect mechanism, such as DNA methylation (Figure 2). In metazoans, there is a direct connection between the DNA replication process and DNA methylation [24]. Replication-coupled DNA methylation in turn enhances H3K9 methylation [25, 26] and at least in plants, is required for the stability of the H3K9 methylation pattern [27]. However, while DNA methylation can promote the correct “copying” of heterochromatin, its landscape in the genome can be dynamically altered by antagonizing activities and chromatin remodeling among others. Hence, even in systems such as mammals where DNA methylation is thought to support H3K9 methylation, the stability of distal heterochromatin requires the non-enzymatic

subunits of the spreading enzyme complex, including methyl histone reader proteins [28], to be present continuously. Antagonizing activities [29–31] have been shown to be able to encroach on heterochromatin [32], pointing to the involvement of dynamic forces in shaping and mainiting the epigneome beyond replication. Hence, “copying” of heterochromatin is not sufficient to maintain the epigenetic pattern. We posit that the collaboration of elements outlined above may be critically important for safeguarding heterochromatin inheritance both by supporting correct establishment but also by defending the locus in G1 and G2 or in non-replicative settings.

c. Distinct heterochromatin types for different biological needs.

Examination of the major heterochromatin loci in fission yeasts leads to a model for functional specialization of heterochromatin, with only the minority of heterochromatin capable of epigenetic behavior. Most heterochromatin in fission yeast is ncRNA-nucleated [33] and is involved in the maintenance of chromosome integrity and genome defense. Like in metazoans, pericentromeric heterochromatin is critical for safeguarding proper chromosome segregation and suppressing deleterious repetitive elements [34, 35] (Figure 3). At the pericentromere, multiple ncRNA-nucleators are placed in close proximity. Hence, engineering memory at these loci is likely not necessary, since the distances between nucleators are short. The high efficiency of these ncRNA-nucleators, coupled with their high repeat frequency, likely makes the pericentromere tolerant of the stochastic spreading characteristic of these nucleators [4]. Subtelomeres are also redundantly nucleated [36, 37], but here, spreading appears to propagate towards the centromere for some distance [36] and intrinsically exhibits variegation [38]. Telomeric heterochromatin is generally thought to prevent genomic instability by repressing recombination between the highly homologous subtelomeric sequences [39, 40]. As long as the majority of the time enough spreading occurs to coat repetitive sequences with recombination-protective heterochromatin factors, some manner of stochastic behavior there is likely tolerated.

There is one cell identity locus in fission yeast, which is required for specification of the cell (mating) type. The mating type (MAT) locus, is very tightly repressed through the collaboration of an ncRNA-nucleator and accessory *REIII* element as discussed above. The simultaneous expression of the silent mating type cassettes in the repressed MAT locus can result in haploid meiosis, low spore viability, or death [41]. We hypothesize that this obvious cellular fitness defect that can result from low-fidelity maintenance of the MAT locus, or similar loci in other simple eukaryotes, was one of the pressures that led to the emergence of epigenetic fidelity (Figure 3). We speculate that the mechanisms that enable fidelity later enabled the stabilization of cell types in multicellular organisms through intergenerationally robust patterning of the epigenome. This requirement for stabilizing cell type is probably also connected to the ability to safeguard epigenetic information through environmental changes[42]. We show that *REIII.*, which is found only at the MAT locus, confers strong resistance to environmental perturbations [4]. This is important, because heterochromatin spreading can be highly vulnerable to changes, such as ambient temperature. For example, it was shown 70 years ago that the degree of position-effect variegation, which is an expression of stochastic spreading, is highly impacted by elevated temperature [43]. In fission yeast, both ncRNA nucleation as well as spreading, are also significantly impaired by

elevated temperatures [4, 44]. We propose that accessory elements like *REIII* have evolved to protect against environmentally induced variegation, on top of safeguarding epigenetic inheritance under ideal conditions.

In summary, our results and those from other point to the notion that evolutionarily conserved ncRNA-elements trigger heterochromatin formation that is not intrinsically tethered to the ability to impart epigenetic inheritance. We believe the adverse consequences of transient loss of heterochromatin at cell type specifier regions was one of a number of pressures that pushed the system to evolve memory capacity in heterochromatin. This is executed via the collaboration of efficient nucleators and specialized accessory "memory elements" and may require linkages to the high fidelity cellular process of DNA replication. The specific mechanisms that can confer this memory remain to be investigated.

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References

1. Reinberg D and Vales LD, Chromatin domains rich in inheritance. Science, 2018 361(6397): p. 33–34. [PubMed: 29976815]
2. Coleman RT and Struhl G, Causal role for inheritance of H3K27me3 in maintaining the OFF state of a Drosophila HOXgene. Science, 2017 356(6333).
3. Wang X and Moazed D, DNA sequence-dependent epigenetic inheritance of gene silencing and histone H3K9 methylation. Science, 2017 356(6333): p. 88–91. [PubMed: 28302794]
4. Greenstein RA, et al., Noncoding RNA-nucleated heterochromatin spreading is intrinsically labile and requires accessory elements for epigenetic stability. Elife, 2018 7.
5. Aygun O, Mehta S, and Grewal SI, HDAC-mediated suppression of histone turnover promotes epigenetic stability of heterochromatin. Nat Struct Mol Biol, 2013 20(5): p. 547–54. [PubMed: 23604080]
6. Taneja N, et al., SNF2 Family Protein Fft3 Suppresses Nucleosome Turnover to Promote Epigenetic Inheritance and Proper Replication. Mol Cell, 2017 66(1): p. 50–62.e6. [PubMed: 28318821]
7. Margueron R, et al., Role of the polycomb protein EED in the propagation of repressive histone marks. Nature, 2009 461(7265): p. 762–7. [PubMed: 19767730]
8. Al-Sady B, Madhani HD, and Narlikar GJ, Division of Labor between the Chromodomains of HP1 and Suv39 Methylase Enables Coordination of Heterochromatin Spread. Molecular cell, 2013 51(1): p. 80–91. [PubMed: 23849629]
9. Bonev B and Cavalli G, Organization and function of the 3D genome. Nat Rev Genet, 2016 17(12): p. 772. [PubMed: 28704353]
10. Dekker J and Heard E, Structural and functional diversity of Topologically Associating Domains. FEBS Lett, 2015 589(20 Pt A): p. 2877–84. [PubMed: 26348399]
11. Bantignies F and Cavalli G, Polycomb group proteins: repression in 3D. Trends Genet, 2011 27(11): p. 454–64. [PubMed: 21794944]
12. Erdel F and Greene EC, Generalized nucleation and looping model for epigenetic memory of histone modifications. Proc Natl Acad Sci U S A, 2016 113(29): p. E4180–9. [PubMed: 27382173]
13. Kakui Y and Uhlmann F, SMC complexes orchestrate the mitotic chromatin interaction landscape. Curr Genet, 2018 64(2): p. 335–339. [PubMed: 28936767]
14. Dodd IB, et al., Theoretical analysis of epigenetic cell memory by nucleosome modification. Cell, 2007 129(4): p. 813–22. [PubMed: 17512413]

15. Angel A, et al., A Polycomb-based switch underlying quantitative epigenetic memory. Nature, 2011 476(7358): p. 105–8. [PubMed: 21785438]
16. Angel A, et al., Vernalizing cold is registered digitally at FLC. Proc Natl Acad Sci U S A, 2015 112(13): p. 4146–51. [PubMed: 25775579]
17. Grewal SIS and Klar AJS, Chromosomal inheritance of epigenetic states in fission yeast during mitosis and meiosis. Cell, 1996 86(1): p. 95–101. [PubMed: 8689692]
18. Boscheron C, et al., Cooperation at a distance between silencers and proto-silencers at the yeast HML locus. EMBO J, 1996 15(9): p. 2184–95. [PubMed: 8641284]
19. Yang H, et al., Distinct phases of Polycomb silencing to hold epigenetic memory of cold in Arabidopsis. Science, 2017.
20. Petryk N, et al., MCM2 promotes symmetric inheritance of modified histones during DNA replication. Science, 2018 361(6409): p. 1389–1392. [PubMed: 30115746]
21. Reveron-Gomez N, et al., Accurate Recycling of Parental Histones Reproduces the Histone Modification Landscape during DNA Replication. Mol Cell, 2018.
22. Serra-Cardona A and Zhang Z, Replication-Coupled Nucleosome Assembly in the Passage of Epigenetic Information and Cell Identity. Trends Biochem Sci, 2018 43(2): p. 136–148. [PubMed: 29292063]
23. Hammond CM, et al., Histone chaperone networks shaping chromatin function. Nat Rev Mol Cell Biol, 2017 18(3): p. 141–158. [PubMed: 28053344]
24. Arita K, et al., Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. Nature, 2008 455(7214): p. 818–21. [PubMed: 18772891]
25. Esteve PO, et al., Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. Genes Dev, 2006 20(22): p. 3089–103. [PubMed: 17085482]
26. Sarraf SA and Stancheva I, Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell, 2004 15(4): p. 595–605. [PubMed: 15327775]
27. Mathieu O, et al., Transgenerational stability of the Arabidopsis epigenome is coordinated by CG methylation. Cell, 2007 130(5): p. 851–62. [PubMed: 17803908]
28. Tchasovnikarova IA, et al., GENE SILENCING. Epigenetic silencing by the HUSH complex mediates position-effect variegation in human cells. Science, 2015 348(6242): p. 1481–1485. [PubMed: 26022416]
29. Zukowski A and Johnson AM, The interplay of histone H2B ubiquitination with budding and fission yeast heterochromatin. Curr Genet, 2018 64(4): p. 799–806. [PubMed: 29464330]
30. Li F, et al., Lid2 is required for coordinating H3K4 and H3K9 methylation of heterochromatin and euchromatin. Cell, 2008 135(2): p. 272–83. [PubMed: 18957202]
31. Geisler SJ and Paro R, Trithorax and Polycomb group-dependent regulation: a tale of opposing activities. Development, 2015 142(17): p. 2876–87. [PubMed: 26329598]
32. Narendra V, et al., Transcription. CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation. Science, 2015 347(6225): p. 1017–21. [PubMed: 25722416]
33. Grewal SI and Klar AJ, A recombinationally repressed region between mat2 and mat3 loci shares homology to centromeric repeats and regulates directionality of mating-type switching in fission yeast. Genetics, 1997 146(4): p. 1221–38. [PubMed: 9258669]
34. Bernard P, et al., Requirement of heterochromatin for cohesion at centromeres. Science, 2001 294(5551): p. 2539–42. [PubMed: 11598266]
35. Saksouk N, Simboeck E, and Dejardin J, Constitutive heterochromatin formation and transcription in mammals. Epigenetics Chromatin, 2015 8: p. 3. [PubMed: 25788984]
36. Kanoh J, et al., Telomere binding protein Taz1 establishes Swi6 heterochromatin independently of RNAi at telomeres. Current biology : CB, 2005 15(20): p. 1808–19. [PubMed: 16243027]
37. Hansen KR, Ibarra PT, and Thon G, Evolutionary-conserved telomere-linked helicase genes of fission yeast are repressed by silencing factors, RNAi components and the telomere-binding protein Tazl. Nucleic Acids Res, 2006 34(1): p. 78–88. [PubMed: 16407326]

38. Nimmo ER, Cranston G, and Allshire RC, Telomere-associated chromosome breakage in fission yeast results in variegated expression of adjacent genes. EMBO J, 1994 13(16): p. 3801–11. [PubMed: 8070408]

39. Cooper JP, et al., Regulation of telomere length and function by a Myb-domain protein in fission yeast. Nature, 1997 385(6618): p. 744–7. [PubMed: 9034194]

40. Nimmo ER, et al., Defective meiosis in telomere-silencing mutants of Schizosaccharomyces pombe. Nature, 1998 392(6678): p. 825–8. [PubMed: 9572142]

41. Kelly M, et al., Four mating-type genes control sexual differentiation in the fission yeast. Embo j, 1988 7(5): p. 1537–47. [PubMed: 2900761]

42. D'Urso A and Brickner JH, Epigenetic transcriptional memory. Curr Genet, 2017 63(3): p. 435–439. [PubMed: 27807647]

43. Chen SY, Action de la température sur trois mutants à panachure de Drosophilia melanogastor, w258-18, wm5 et z. Bull Biol Fr Belg, 1948 82(2-3): p. 114–29. [PubMed: 18099940]

44. Woolcock KJ, et al., RNAi keeps Atf1-bound stress response genes in check at nuclear pores. Genes Dev, 2012 26(7): p. 683–92. [PubMed: 22431512]

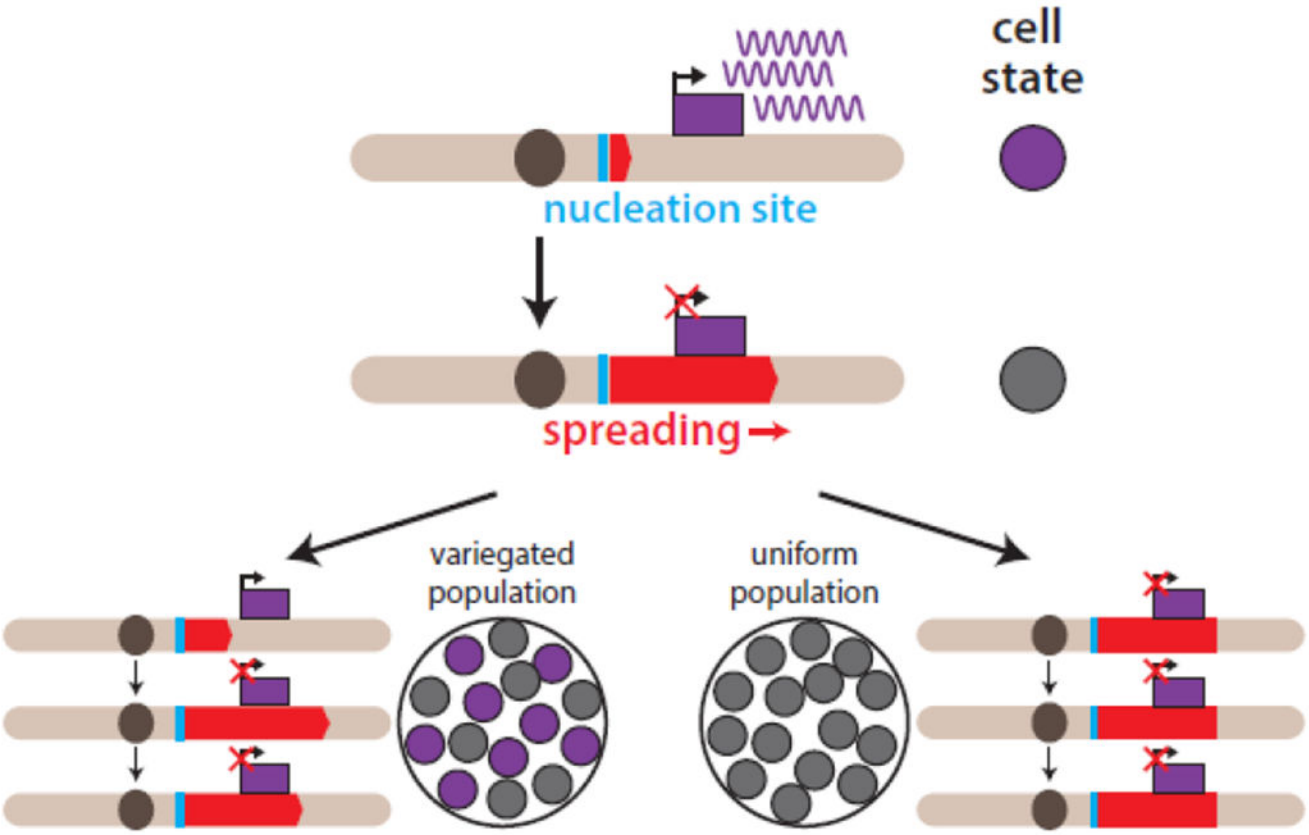

Figure 1: Variegation and inheritance of heterochromatin spreading generate mixed or uniform cell states respectively.

Heterochromatin is formed by a sequential process whereby first small heterochromatin domains (red) are formed proximal to nucleation sites (blue) by short range spreading. Nucleation-distal genes remain expressed (purple state). Long-range spreading then propagates the repressed state along the chromosome, silencing information orthogonal to the cellular state (grey state). The resultant heterochromatin domain can be either inherited with high fidelity, leading to a robust recapitulation of the repressed state and a uniform population (right) or with low fidelity, leading to variegated expression across a population (left).

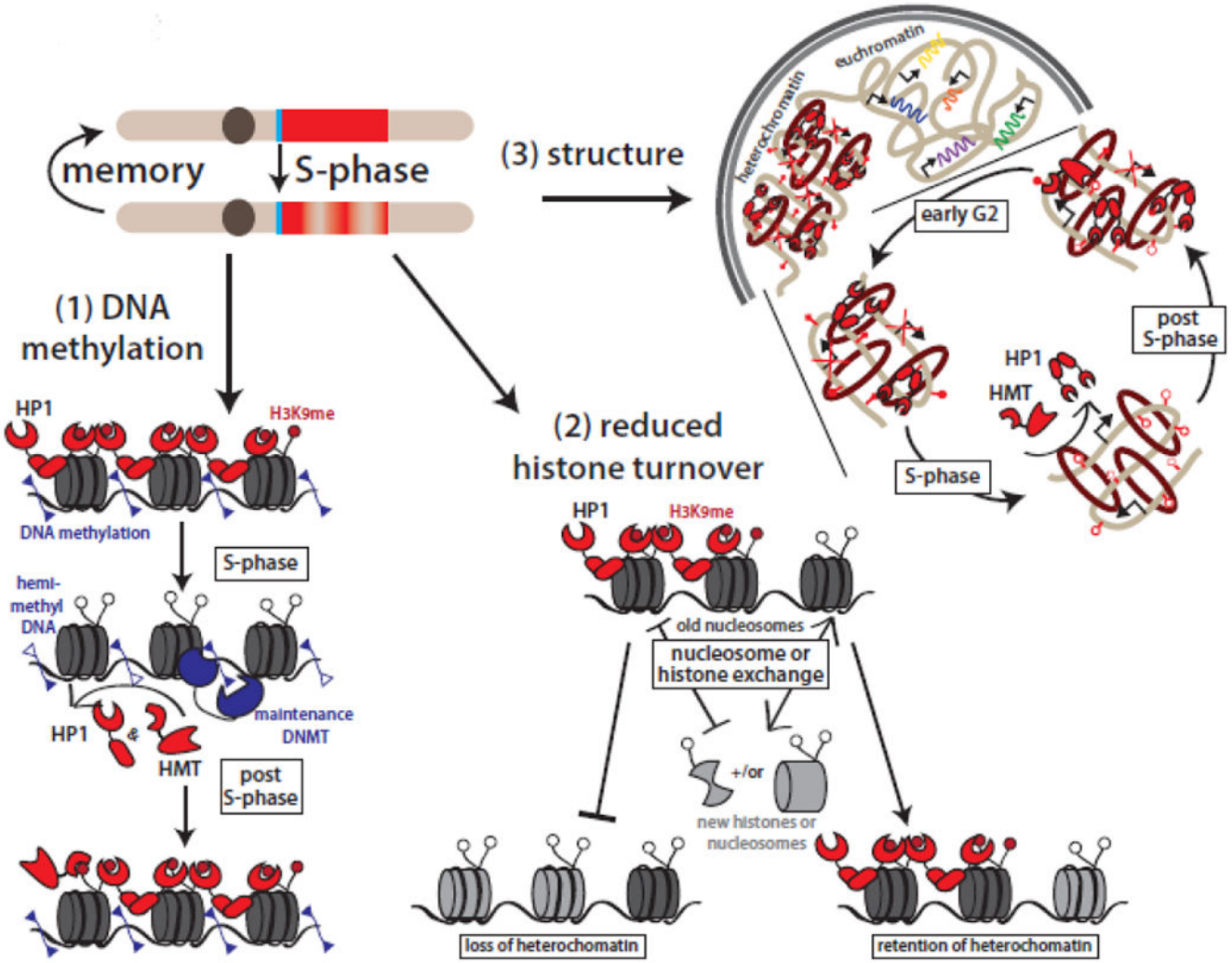

Figure 2: Systems of memory retention for nucleation-distal heterochromatin regions.

Since heterochromatin PTMs and factors are at least partially lost during S-phase, epigenetic memory requires mechanisms to quickly and reliably regain the repressed state. These may include one or more of the following: (1) DNA methylation – methylation of DNA, which is mainly found in higher eukaryotes and is concurrent with heterochromatic histone PTMs, is linked to the high fidelity process of DNA replication. Following S-phase, hemi-methyl DNA is recognized by DNA methyltransferases (DNMT), which restore DNA methylation to both strands. This leads to the recruitment of heterochromatin writers and effectors by both DNMTs and DNAme reader proteins and restoration of the heterochromatin state (2) reduced histone turnover – compared to euchromatin, certain heterochromatin regions may experience less turnover of nucleosomes and/or histone proteins. This prevents loss of epigenetic information via the incorporation of un-marked nucleosomes and promotes the inheritance of the heterochromatic state by the retention of modified nucleosomes. (3) local chromatin structure – a higher order structure present at heterochromatin regions could be either directly maintained or quickly re-established post-replication. This structure either allows for the retention of epigenetic information or enhances the reformation of

heterochromatin (shown), perhaps by facilitating a local environment favorable to spreading or by recruiting the enzyme complexes required for it.

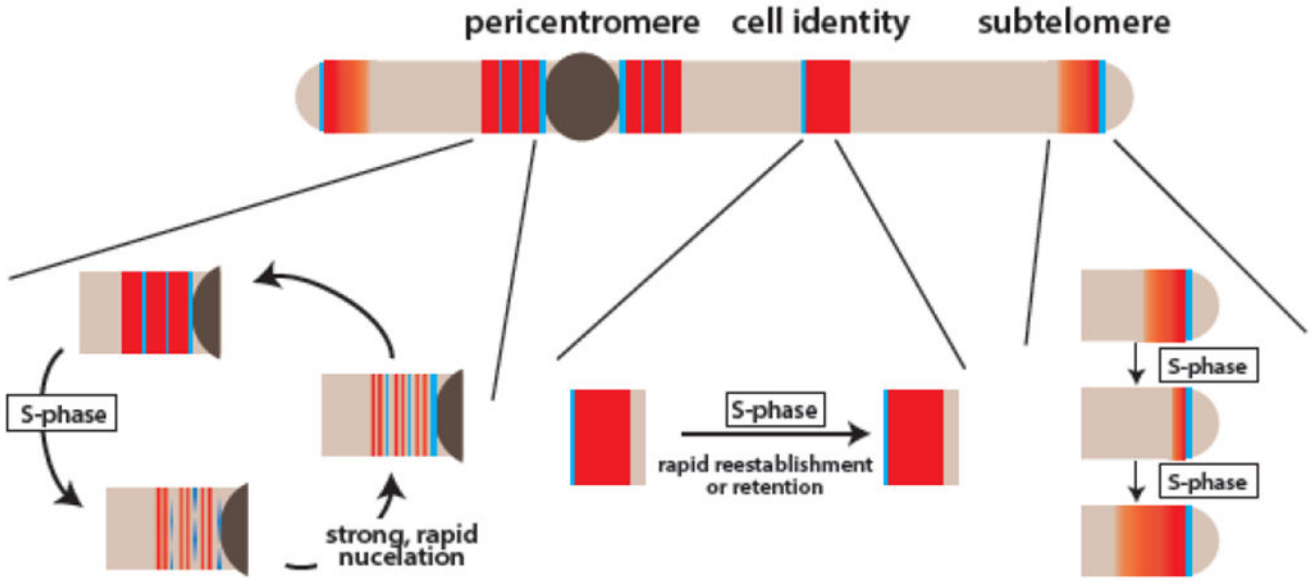

Figure 3: Different heterochromatin loci experience stochastic or stable inheritance of heterochromatin spreading.

Heterochromatin regions with different functions, and possibly different requirements for epigenetic memory, exist within the same genome. Pericentromeric heterochromatin (left) has a primarily structural role in centromere function and the suppression of repetitive elements. Multiple strong nucleators (blue) are present in these regions, likely facilitating the re-establishment of heterochromatic state and minimizing the requirement for long distance spreading following the weakening in S-phase. Subtelomeric heterochromatin (right) varies in its extent of spreading. At cell identity loci (middle), variability is not tolerated if the epigenetic state is to be maintained over many cell divisions. The presence of different classes of nucleators collaborating to regain the epigenetic state after S-phase, or the prevention of information loss in the first place, may ensure that cell identity information is robustly inherited.