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Heterochromatin: Guardian of the Genome

Aniek Janssen,[1,2,*] Serafin U. Colmenares,[1,2,*] and Gary H. Karpen[1,2]

[1]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA; email: ghkarpen@lbl.gov

[2]Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA

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*These authors contributed equally.

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Abstract

Constitutive heterochromatin is a major component of the eukaryotic nucleus and is essential for the maintenance of genome stability. Highly concentrated at pericentromeric and telomeric domains, heterochromatin is riddled with repetitive sequences and has evolved specific ways to compartmentalize, silence, and repair repeats. The delicate balance between heterochromatin epigenetic maintenance and cellular processes such as mitosis and DNA repair and replication reveals a highly dynamic and plastic chromatin domain that can be perturbed by multiple mechanisms, with far-reaching consequences for genome integrity. Indeed, heterochromatin dysfunction provokes genetic turmoil by inducing aberrant repeat repair, chromosome segregation errors, transposon activation, and replication stress and is strongly implicated in aging and tumorigenesis. Here, we summarize the general principles of heterochromatin structure and function, discuss the importance of its maintenance for genome integrity, and propose that more comprehensive analyses of heterochromatin roles in tumorigenesis will be integral to future innovations in cancer treatment.

Contents

INTRODUCTION

The quest to cure cancer has uncovered many critical genetic programs that drive tumorigenesis, which results from mutation, loss, or overexpression of genes that regulate cell growth and genome integrity [e.g., oncogenes, tumor suppressors, and DNA repair factors (Hanahan & Weinberg 2011)]. However, recent studies demonstrate that epigenetic changes and mutations in chromatin proteins are also strongly correlated with cancer progression (Flavahan et al. 2017). Epigenetic changes can directly alter transcriptional programs, which could promote cancer progression by increasing cancer cell plasticity (Flavahan et al. 2017) or by directly silencing tumor suppressor genes, such as *BRCA1* or *CDKN2A* (Shen & Laird 2013).

However, what is much less explored is the impact of epigenetic changes on genome instability, a key hallmark of cancer cells and driver of tumor heterogeneity. Genome instability causes cancer genotypes to continuously change and evolve, either through loss and gain of whole chromosomes (aneuploidy) or through structural chromosomal changes (e.g., translocations) resulting from increased levels of DNA damage or faulty repair (Janssen & Medema 2013).

A crucial but poorly understood epigenetic component of eukaryotic genomes is constitutive heterochromatin (*c*-Het) (**Figure 1*a***). Although highly enriched for repeated DNA sequences and containing few protein-coding genes, the *c*-Het domain plays critical roles in safeguarding the genome, including chromosome segregation, telomere protection, suppression of transposon activity, and DNA repair. In this review, we first summarize the general principles of heterochromatin structure and function, most of which have been discovered in studies of model organisms (Allshire & Madhani 2018). We then discuss the importance of maintaining heterochromatin for mammalian genome integrity and what we know about molecular components and mechanisms. Finally, we evaluate the hypothesis that defective heterochromatin promotes human cancer progression by increasing genetic instability and discuss how this perspective impacts our views of cancer development.

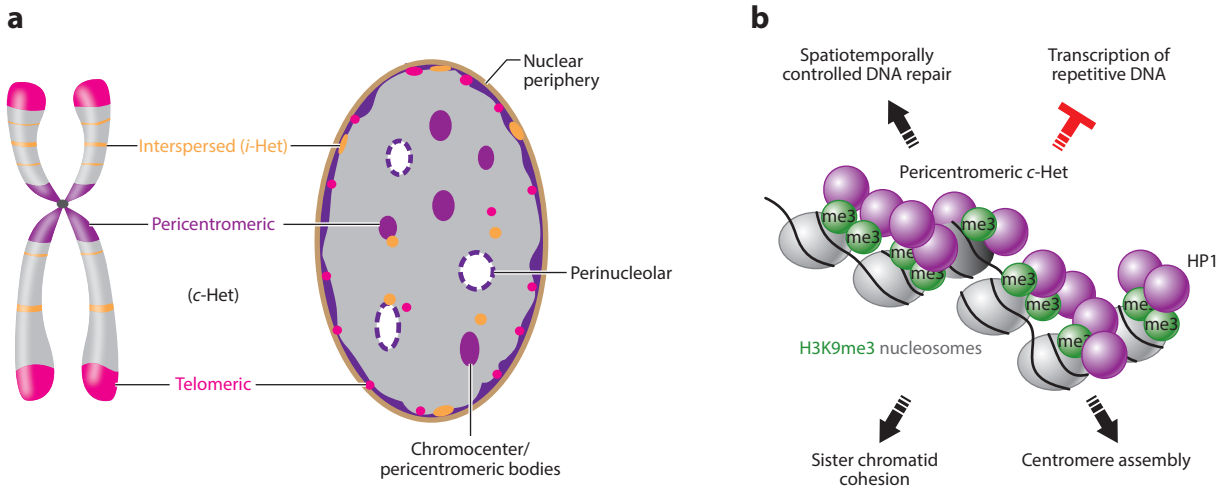

Figure 1

Localization and functions of heterochromatin. (*a*) (*Left*) Repetitive sequences such as satellite DNA and transposons are enriched at pericentromeric and telomeric regions of chromosomes to form constitutive heterochromatin (*c*-Het). Heterochromatin is also found at interspersed, sometimes unique (nonrepetitive), euchromatic sequences (*i*-Het). (*Right*) Heterochromatin localization patterns can differ extensively across cell types and can appear as perinucleolar domains, as pericentromeric bodies, as one or a few large nuclear bodies (chromocenters), and at the nuclear periphery. (*b*) Heterochromatin is marked with di- and trimethylated histone H3 lysine 9 (H3K9me2 and H3K9me3, respectively) and its reader protein heterochromatin protein 1 (HP1). Pericentromeric *c*-Het promotes centromere and cohesion assembly on mitotic chromosomes, silencing of repetitive sequence transcription, and spatiotemporally controlled DNA repair.

GENERAL PRINCIPLES OF HETEROCHROMATIN STRUCTURE AND FUNCTION

Heterochromatin Is a Distinct Nuclear Domain

The heterochromatin domain is a major, highly conserved, and structurally distinct element of eukaryotic genomes that is responsible for critical genome functions. Composing ~25% to 90% of multicellular eukaryotic genomes (Lander et al. 2001, Vicient & Casacuberta 2017), it is typically situated at pericentromeric and telomeric domains of chromosomes. Heterochromatin is also enriched for di- and trimethylated histone H3 lysine 9 (H3K9me2 and H3K9me3, respectively) and its binding protein, heterochromatin protein 1 (HP1) (James & Elgin 1986) (**Figure 1**).

Unlike the gene-rich euchromatin domain, *c*-Het exhibits a dense organization throughout interphase, enrichment for repetitive DNA sequences, relatively low transcription levels, and distinct replication timing (Allshire & Madhani 2018). *c*-Het differs from Polycomb-based facultative heterochromatin, which is associated with transcriptional regulation of developmental genes and employs different histone marks and readers. Here, we focus on the genome stability roles of *c*-Het at pericentromeric and telomeric regions but also touch on the potential relevance of heterochromatic domains present in interspersed regions in euchromatin (*i*-Het) (Vogel et al. 2006) (**Figure 1**).

c-Het was first recognized by Emil Heitz in liverwort and *Drosophila* in the 1920s and 1930s as chromosomal regions that failed to decondense following mitosis (Heitz 1928). Today, *c*-Het is increasingly realized to be a nuclear domain integral to many cellular processes, including chromosome segregation, transcription, and transposon suppression, and is essential

for organismal health (Allshire & Madhani 2018). As the biological significance of these domains has become more apparent in recent decades, advances in microscopy now enable us to view these chromatin regions in nearly all studied eukaryotic species, from fission yeast to mammals. In humans, *c*-Het appears as perinucleolar domains, as pericentromeric bodies, and at the nuclear periphery in interphase cells (Horsley et al. 1996, Minc et al. 1999) (**Figure 1*a***). In mouse cells, some of these interior *c*-Het domains aggregate and materialize more prominently as large chromocenters, in addition to localizing to the nuclear periphery (Mayer et al. 2005). The nuclear pattern of *c*-Het can also vary and may have different roles depending on the cell type or tissue type. For example, the single large, central chromocenter and peripheral euchromatin in mouse rod photoreceptor nuclei are thought to be an adaptation that facilitates nocturnal vision (Solovei et al. 2009). Moreover, stem cells possess lower levels of heterochromatin marks and a more dispersed heterochromatin architecture relative to differentiated cells (Meshorer et al. 2006), which are thought to help maintain epigenetic plasticity in stem cells (Sridharan et al. 2013).

The dense packaging of *c*-Het—as evinced from various photometric techniques, enzyme inaccessibility assays, and biochemical centrifugation—has long been thought to form the basis for heterochromatin properties such as transcriptional repression and inaccessibility of DNA to various factors (**Figure 2**). Indeed, transcriptional silencing induced by targeting of HP1 to ectopic sites coincides with chromatin compaction and the formation of coalesced domains composed of multiple heterochromatic regions present on different chromosomes (Li et al. 2003, Verschure et al. 2005). However, chromatin compaction is not sufficient to explain the presence of both highly dynamic and stable HP1 populations within heterochromatin (Cheutin et al. 2004). Recent findings identify a liquid-like HP1 population that generates a phase transition compartment surrounding the less-mobile chromatin-bound fraction (Larson et al. 2017, Strom et al. 2017). Such a biophysical compartmentalization mechanism may facilitate the enrichment and rapid exchange of heterochromatin proteins necessary for the dynamic structural changes that heterochromatin undergoes during cell cycle progression and in response to stress or DNA damage (Chiolo et al. 2011). Thus, unlike chromatin compaction, phase separation provides a mechanism for selective accessibility to heterochromatin, enriching for phase-compatible proteins while also excluding proteins that may destabilize heterochromatin structure (**Figure 2**). Future challenges entail deconvolving the contributions of chromatin compaction and phase properties to *c*-Het structure and function, which could have important implications for understanding heterochromatin's maintenance of genome stability.

Heterochromatin Is Enriched for Diverse Repeated DNAs

Repetitive sequences, which consist primarily of satellite DNA (simple, short tandem repeats), transposons, and ribosomal DNA (rDNA) (Padeken et al. 2015), are usually marked with H3K9me2/3 catalyzed by SUV39 methyltransferases. Multiple copies of a transgene are sufficient to acquire heterochromatin characteristics (Dorer & Henikoff 1994), indicating that the presence of repetitive sequences alone can drive heterochromatin nucleation. Clusters of repetitive DNAs compose pericentromeric and telomeric regions of chromosomes in most organisms, resulting in the enrichment of H3K9me2/3 at these domains (**Figure 1**). The DNA sequence varies widely between different types of repeats, highlighting the ability of heterochromatin to form independently of sequence.

In humans, functional centromeres are composed predominantly of pure tandem repeats of the 177-bp α-satellite, whereas the pericentromeric heterochromatin domains are populated by α-satellite and different satellite subfamilies (i.e., HSATII, HSATIII, sn5, and β- and γ-satellite) (Miga 2015). Meanwhile, human telomeres comprise tandem TTAGGG repeats that help protect the ends of linear chromosomes. Even the highly transcribed rDNA from five human chromosomes

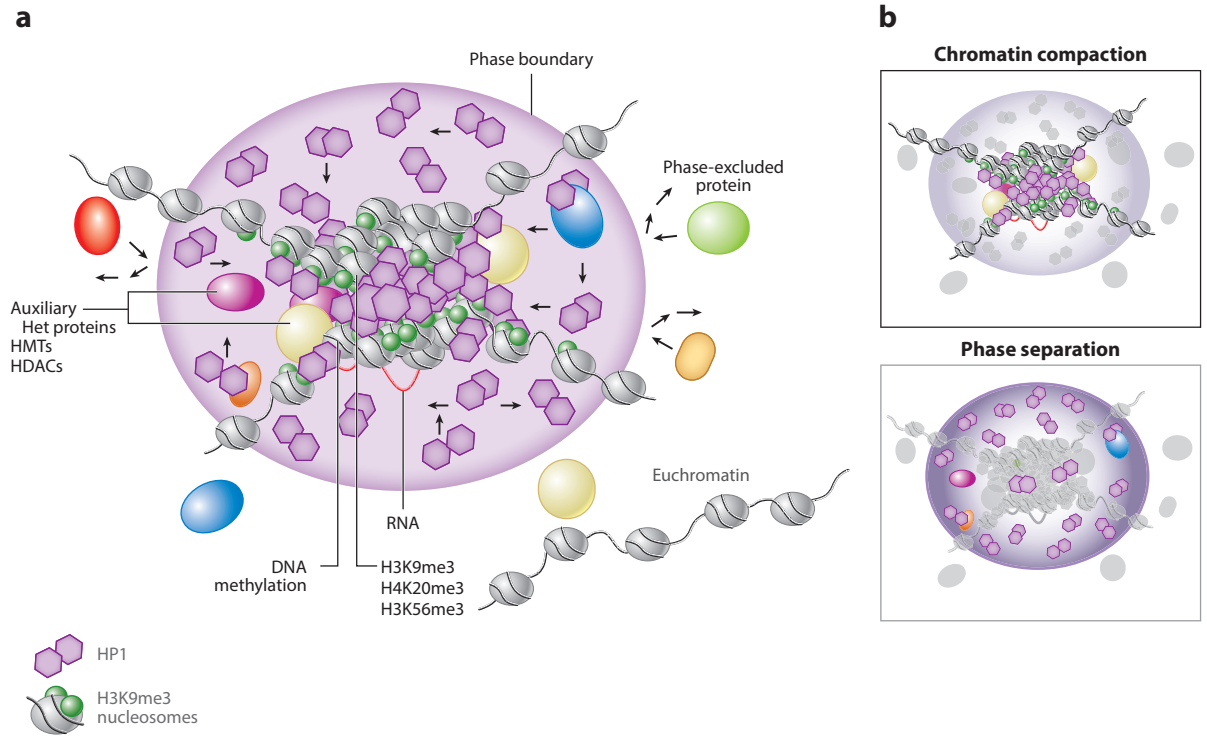

Figure 2

Heterochromatin composition and compartmentalization. (*a*) Constitutive heterochromatin (*c*-Het) is enriched for nucleosomes containing di- and trimethylated histone H3 lysine 9 (H3K9me2 and H3K9me3, respectively) and its binding protein, heterochromatin protein 1 (HP1). In addition to these core components, DNA methylation, other histone methylation marks, and a plethora of HP1-binding proteins [auxiliary heterochromatin proteins, histone deacetylases (HDACs), and histone methyltransferases (HMTs)] promote heterochromatin formation and maintenance that create a biophysically distinct compartment from euchromatin. Arrows denote intraheterochromatin movements of phase-compatible heterochromatin proteins and exclusion of phase-incompatible euchromatin proteins. HP1 molecules are present in euchromatin as well, although at lower concentrations relative to heterochromatin. (*b*) Two compartmentalization mechanisms proposed to form the *c*-Het domain and to mediate its functions are illustrated: chromatin compaction (*upper panel*) and phase separation (*lower panel*) with the relevant heterochromatin components in panel *a* highlighted. Although the compaction model explains heterochromatin properties through steric hindrance of certain protein complexes, phase separation explains spectrometric evidence of both static and highly dynamic pools of heterochromatin proteins and the selective permeability of the domain to different proteins. We propose that *c*-Het is a highly dynamic domain in which both compaction and phase separation contribute to its biological functions (Larson et al. 2017, Strom et al. 2017).

(13, 14, 15, 21, and 22) (McStay & Grummt 2008) recruits heterochromatin and transcriptionally represses approximately half of the 300–400 rDNA repeats at any given time.

Finally, transposons are coding DNA sequences closely related to viruses that can change positions and/or propagate in the genome. Transposons are enriched in *c*-Het and are predominantly inactive and fragmented. In addition, more than 50% of the assembled human euchromatic genome consists of repeats, including transposon-related elements such as LINEs (de Koning et al. 2011, Lander et al. 2001). These euchromatic repeats are similarly targeted for heterochromatin formation and silencing. The high levels of repetitive sequences in human euchromatin (that likely represent *i*-Het) suggest that heterochromatin components also protect euchromatin from genome instability caused by repetitive DNA.

Heterochromatin Contains Distinct Histone and DNA Modifications and Associated Proteins

In mammalian cells, H3K9me2/3 is catalyzed by five SET domain–containing methyltransferases: G9a and GLP promote mono- and di-methylation, whereas SETDB1, SUV39H1, and SUV39H2 catalyze di- and trimethylated forms of H3K9. The generation of transgenic mouse models with mutations or deletions of these H3K9-directed methyltransferases has revealed the importance of heterochromatin formation and maintenance during organismal development (Cho et al. 2012; Eymery et al. 2016; O'Carroll et al. 2000; Tachibana et al. 2002, 2005). For example, mice mutated for either SUV39H1 or SUV39H2 are viable, but double-knockout mice display late embryonic defects and lethality (Peters et al. 2001), indicating that SUV39H1 and SUV39H2 play redundant roles during development.

Mammalian genomes contain three HP1 homologs that localize to heterochromatin domains (HP1α, HP1β, and HP1γ). HP1 homologs contain a chromodomain (Paro & Hogness 1991) responsible for binding to H3K9me2/3, a hinge region associated with nucleic acid binding, and a chromoshadow domain (Aasland & Stewart 1995) essential for protein-protein interactions and dimerization. HP1 self-interaction enables the formation of higher-order chromatin structures thought to mediate the compaction of the heterochromatin domain (Canzio et al. 2013), but HP1 proteins also act as a platform to recruit and regulate many diverse protein complexes within heterochromatin (Eskeland et al. 2007, Swenson et al. 2016).

In addition to H3K9me2/3, other histone modifications enriched in mammalian heterochromatin include H4K20me3, H3K64me3, and H3K56me3. These histone modifications have been implicated in modulating heterochromatin structure and are thought to be dependent on H3K9 methylation or directly catalyzed by SUV39 (Jack et al. 2013; Lange et al. 2013; Schotta et al. 2004, 2008). More recently, H3K23me3 was also identified as a heterochromatin component in *Tetrahymena* and *Caenorhabditis elegans* meiosis (Papazyan et al. 2014).

A less-conserved but closely associated feature of human *c*-Het is DNA methylation. In several organisms, H3K9 methylation has been shown to drive DNA methylation at heterochromatin (Jackson et al. 2002, Tamaru & Selker 2001), which may further stabilize heterochromatin domains. In mammals, loss of either the H3K9 or DNA methylation pathway produces only partial defects in the other pathway (Lehnertz et al. 2003), indicative of overlapping but independent mechanisms of assembly. Both DNA methylation and H3K9me3 inhibit the recruitment of Polycomb facultative heterochromatin to *c*-Het (Peters et al. 2003, Saksouk et al. 2014), consistent with the semidependent relationship between DNA and H3K9 methylation.

Heterochromatin Establishment, Spreading, and Maintenance

Heterochromatin formation can be initiated through noncoding RNA–dependent mechanisms prevalent in yeast and plants (for detailed reviews, see Allshire & Madhani 2018, Martienssen et al. 2008). Briefly, this mechanism requires the assembly of RNA interference (RNAi) transcriptional silencing components on repetitive transcripts, which recruit heterochromatin proteins, like SUV39, to repetitive DNA. Whether this mechanism is conserved in mammalian cells remains largely unresolved. Mouse heterochromatin exhibits sensitivity to RNase A treatment (Maison et al. 2002) and requires transcription of heterochromatin repeats (Probst et al. 2010). In addition, mouse SUV39H1/H2 is stably recruited to pericentromeric sequences through RNA-dependent mechanisms (Johnson et al. 2017, Shirai et al. 2017, Velazquez Camacho et al. 2017). Mouse heterochromatin may also include alternative modes of heterochromatin formation, such as the binding of the HP1 interactor Pax3 and related transcription factors to a consensus sequence in heterochromatin repeats (Bulut-Karslioglu et al. 2012).

Following establishment, heterochromatin needs to be maintained and propagated. One major maintenance mechanism is the binding of HP1 to SUV39 (Aagaard et al. 1999) and histone deacetylases (Fischer et al. 2009, Motamedi et al. 2008). Hypoacetylation of H3K9 is a prerequisite for methylation of H3K9 at pericentric heterochromatin, and treatment with deacetylase inhibitors disperses HP1α (Taddei et al. 2001). However, deacetylation of histone residues other than H3K9 is also important for heterochromatin maintenance (Alper et al. 2013, Contrepois et al. 2012, Yamada et al. 2005). Iterative cycles of deacetylation, methylation, HP1 binding, and recruitment of deacetylases and SUV39 enable a sequence-independent, stochastic spreading of heterochromatin.

This stochastic propagation is often dependent on the doses of regulatory proteins. Loss of one copy of HP1 or SUV39 results in partial transcriptional derepression, whereas extra copies of HP1 or SUV39 generate even higher levels of silencing (Elgin & Reuter 2013). Excessive heterochromatin spreading could also prove deleterious to neighboring euchromatic gene functions. Therefore, various mechanisms exist to limit heterochromatin propagation into euchromatin. Boundary elements such as tRNA genes as well as other actively transcribed regions restrict this spreading of heterochromatin onto euchromatic genes, which is mediated by factors involved in transcription, histone modification, and nucleosome turnover (Ahmad & Henikoff 2002, Allshire & Madhani 2018). Alternative inhibitory mechanisms independent of DNA boundary elements are also observed. For example, spreading of heterochromatin can be limited by the putative histone demethylase Epe1 (Trewick et al. 2007), which removes H3K9me2 in fission yeast, or by the kinase JIL1, which phosphorylates H3S10 in euchromatin and thereby inhibits HP1 binding and further H3K9 methylation (Zhang et al. 2006).

Heterochromatin maintenance and reestablishment regularly occur following disruptive cellular processes such as mitosis and DNA replication. Upon mitotic entry, HP1 proteins largely disperse from chromosomes due to phosphorylation of H3S10 by Aurora B, which blocks HP1 binding to H3K9me2/3 (Hirota et al. 2005), and reassembly occurs in anaphase/telophase (Wurzenberger & Gerlich 2011). Various other HP1-associated heterochromatin factors similarly disperse and reassemble during mitosis (Swenson et al. 2016). Heterochromatin structure is therefore maintained through cycles of HP1 assembly and disassembly during every mitotic cell division.

Passage of the replication machinery also likely disrupts heterochromatin structure. Postreplicative restoration of heterochromatin structure is facilitated by the random retention of parental H3K9me2/3 histones on both daughter strands behind replication forks and may also involve the recruitment of HP1 by the CAF-1 complex responsible for nucleosome reassembly (Quivy et al. 2004, 2008). The disruption of higher-order heterochromatin structure during replication, however, may also reestablish RNA-dependent heterochromatin formation. In fission yeast, a burst of repeat transcription during S phase is required for restoration of heterochromatin levels (Kloc et al. 2008). In mammals, satellite transcription has also been demonstrated during replication and may serve a similar function (Lu & Gilbert 2007).

In summary, *c*-Het composes a large portion of the eukaryotic genome that packages underlying repetitive sequences and provides structural organization to eukaryotic chromosomes (**Figure 1**). It functions by establishing a spatially distinct, dynamic chromatin domain that helps maintain genome stability through various mechanisms, which we outline in detail below.

HETEROCHROMATIN IS REQUIRED TO ENSURE GENOME INTEGRITY

Many studies have implicated dysfunctional heterochromatin in disease and, in particular, cancer progression. Loss of large blocks of H3K9 di- and tri-methylation correlates with gene expression

changes in cancer cells and has been proposed to contribute to their phenotypic plasticity (Feinberg et al. 2016). Cancer progression and metastasis are associated with changes in the distribution of H3K9me2/me3 and HP1 expression levels (De Koning et al. 2009, Dialynas et al. 2008, Feinberg et al. 2016, Rondinelli et al. 2015, Slee et al. 2012, Vad-Nielsen et al. 2016), and loss of H3K9 di- and tri-methylation results in an increased rate of tumorigenesis in mouse models (Braig et al. 2005, Peters et al. 2001).

We hypothesize that such cancer-associated heterochromatic changes provide cancer cells with an evolutionary advantage, not only by directly changing transcriptional programs (Feinberg et al. 2016) but also by increasing the level of genetic instability. For example, changes in heterochromatin components can alter the nuclear compaction of DNA sequences, thereby increasing susceptibility to DNA damage. Changes in heterochromatic histone modifications can also directly affect DNA damage repair efficiency since many histone modifications have been implicated in promoting or inhibiting the recruitment of specific repair proteins (Price & D'Andrea 2013).

Here we discuss the evidence that supports these hypotheses and interrogate the mechanisms by which heterochromatin dysfunction can result in increased genome instability.

Heterochromatin Safeguards Mitotic Fidelity

The most evident role for *c*-Het in genome stability can be inferred from its enrichment at pericentromeric repeats surrounding centromeres (**Figure 1**). Centromeric chromatin, the site of kinetochore assembly in mitosis, is distinct from both euchromatin and heterochromatin (Sullivan & Karpen 2004) and contains the centromere-specific H3 variant CENP-A required for centromere assembly to allow for proper chromosome segregation in mitosis.

Pericentromeric heterochromatin components are essential for de novo CENP-A assembly at centromeres in fission yeast (Folco et al. 2008) and promote proper kinetochore-microtubule attachments in mitosis and subsequent equal chromosome segregation to both daughter cells (Ekwall et al. 1995, 1996). In addition, fission yeast HP1 (Swi6) is enriched in pericentromeric heterochromatin and is required for sister cohesion (Bernard et al. 2001, Nonaka et al. 2002, Yamagishi et al. 2008).

In mammalian cells, HP1α helps mediate the assembly and maintenance of cohesion complexes (Kang et al. 2011, Perera & Taylor 2010). However, mammalian HP1α also recruits and binds the chromosomal passenger complex protein INCENP (Abe et al. 2016, Ainsztein et al. 1998, Kang et al. 2011, Perera & Taylor 2010), which corrects aberrant kinetochore-microtubule interactions in mitosis, indicating that the mitotic role of HP1α in mammalian cells extends beyond cohesion maintenance (Abe et al. 2016, Ainsztein et al. 1998).

In line with a role for pericentromeric heterochromatin in the proper assembly of centromeres and cohesion, loss of H3K9 methyltransferases or HP1 homologs results in an increase in chromosome segregation errors in mice, *Drosophila*, and fission yeast (Ekwall et al. 1995, Ekwall et al. 1996, Peng & Karpen 2009, Peters et al. 2001) (**Figure 3**). These missegregation events can cause a plethora of defects in the daughter cells, including the formation of replication stress–prone micronuclei (Crasta et al. 2012); chromosome breaks during cytokinesis (Janssen et al. 2011); and an unequal distribution of chromosomes between daughter cells, termed aneuploidy—all of which are phenomena strongly connected with cancer progression.

The increased rate of chromosome segregation errors could be responsible for the enhanced tumor incidence observed in SUV39H1/2-deficient mice (Braig et al. 2005, Peters et al. 2001) (**Figure 3**). However, a direct correlation between loss of HP1 or H3K9me2/3 and increased chromosome segregation errors in human tumor tissue has not been investigated. Such studies in

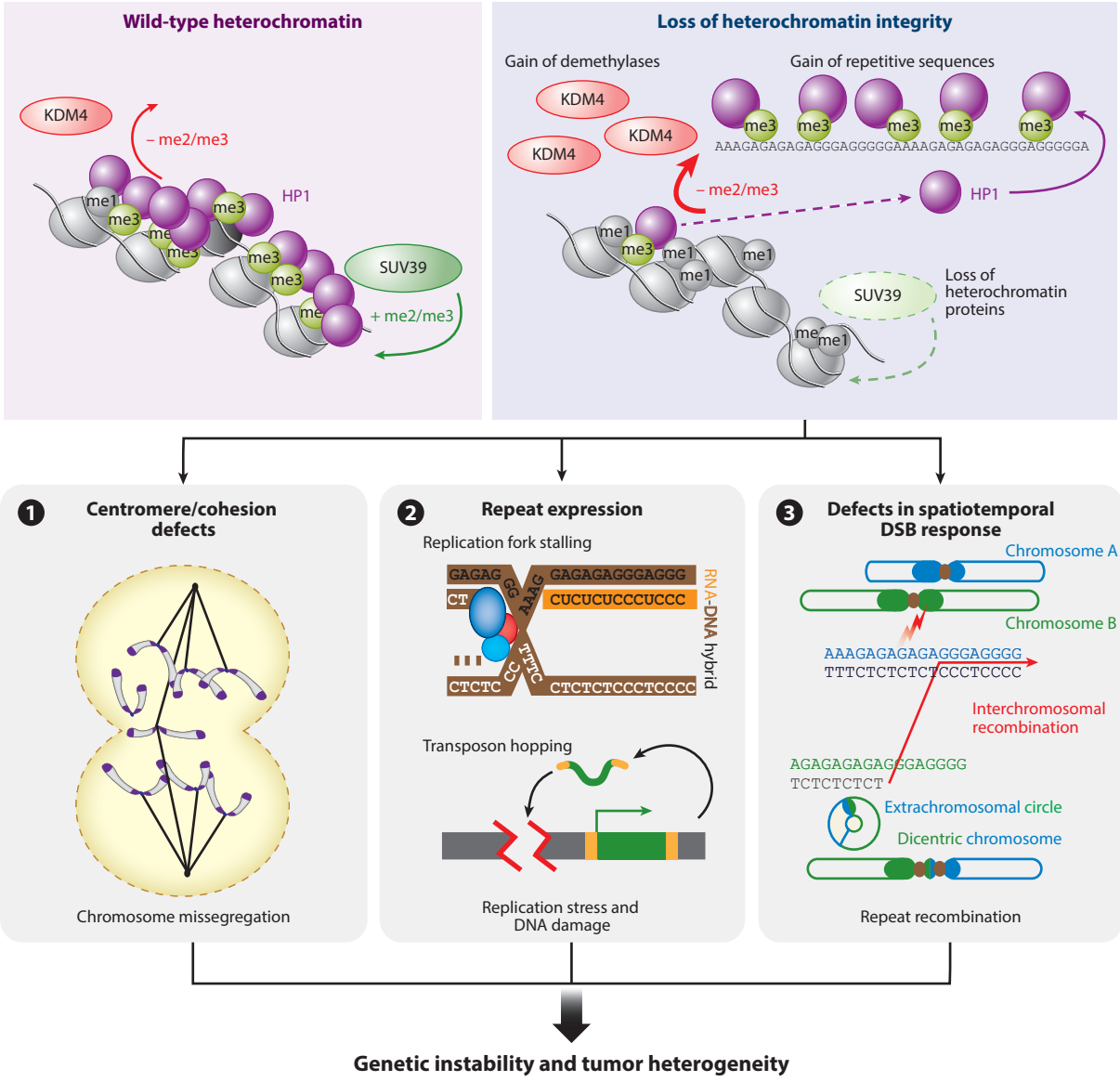

Figure 3

(*Top left panel*) Wild-type heterochromatin structure is dynamically regulated by SUV39H1/H2 methyltransferase and KDM4 demethylase activities. HP1 denotes heterochromatin protein 1. (*Top right panel*) Model for increased genome instability through loss of heterochromatin integrity. Loss of heterochromatin integrity can be achieved through a variety of mechanisms. KDM4A–D demethylase overexpression or loss of SUV39H1/H2 histone methyltransferase activity results in decreased *c*-Het H3K9 methylation. Alternatively, gain of repetitive sequences, e.g., due to aneuploidy, can titrate the limited pool of *c*-Het components like HP1, resulting in inefficient heterochromatin maintenance at repetitive sequences. Loss of heterochromatin integrity can generate a variety of genome stability defects, including ❶ chromosome missegregation events through aberrant centromere or cohesion assembly; ❷ increased DNA damage from replication problems due to RNA:DNA hybrid formation or from transposon hopping; and ❸ aberrant recombination between repeats on nonhomologous chromosomes, causing translocations and abnormal chromosome structures due to defects in spatiotemporal regulation of double-strand break (DSB) repair. Together these defects contribute to genome instability and can thereby promote tumor heterogeneity and evolution.

cancer tissues could give insights into direct links between *c*-Het defects, chromosomal instability, and tumorigenesis, which would have important implications for understanding cancer progression and treatment.

Heterochromatin Prevents Aberrant Repeat Recombination

c-Het not only provides a structural platform to protect centromere and kinetochore integrity in mitosis but also forms a compact, silenced, phase-separated environment (Larson et al. 2017, Strom et al. 2017) that protects the integrity of the multitude of repetitive sequences present within this domain.

The repetitive nature of heterochromatic sequences makes them extremely vulnerable to improper DNA damage repair. DNA double-strand breaks (DSBs), in which both strands of the DNA helix are broken, are especially dangerous in repetitive sequences. DSBs can be fixed by a variety of repair pathways, but the two major mechanisms are nonhomologous end joining (NHEJ) and homologous recombination (HR) (Ciccia & Elledge 2010). NHEJ repairs DNA by ligating both ends of the DSB together, often resulting in small insertions and deletions at the break site, and is therefore considered more error prone. HR repair involves more extensive processing of the DSB site; 5′-to-3′ end resection of the DSB ends by specialized enzymes results in a single-stranded DNA sequence, which invades and perfectly copies homologous sequences on the sister chromatid or homologous chromosome to repair the DSB (Ciccia & Elledge 2010). HR is usually considered the safest, error-free choice for DSB repair in single-copy sequences; however, the presence of up to millions of homologous repetitive sequences from different chromosomes, all concentrated within *c*-Het domains, poses a major challenge for safe HR repair. Recombination between a DSB and homologous repeats in *cis*, or on nonhomologous chromosomes, can result in lethal dicentric chromosomes, as well as insertions, deletions, and other chromosomal translocations (**Figure 3**).

Indeed, loss of H3K9 methylation or HP1a in *Drosophila* results in aberrant intrachromosomal recombination among repeats and in significantly elevated levels of extrachromosomal repeated DNA sequences (Peng & Karpen 2007). The maintenance of pericentromeric heterochromatin is therefore essential for preserving repetitive sequences and preventing aberrant chromosomal structures (**Figure 3**). Furthermore, cytological as well as genome sequence analyses of cancer tissues have shown that pericentromeric heterochromatin regions are more prone to translocations and copy number changes (Cramer et al. 2016, Hermsen et al. 1996, Jin et al. 2000), potentially reflecting the vulnerability of heterochromatic repeats to improper DNA damage repair.

Heterochromatin Utilizes Dynamic Responses to Repair Double-Strand Breaks

To prevent potentially dangerous HR repair between repeats, heterochromatic regions have evolved specialized temporal and spatial responses to safely repair DSBs. Initially found in budding yeast and *Drosophila* (Chiolo et al. 2011, Torres-Rosell et al. 2007) and later demonstrated to also occur in mammals (Tsouroula et al. 2016), these dynamic responses include the relocalization of DSBs to the heterochromatin or nuclear periphery, away from the regions where homologous sequences are most highly concentrated (reviewed in Caridi et al. 2017). In *Drosophila*, these specific dynamics require HP1 and its interactors, the SMC5/6 complex (related to condensins and cohesins), myosin and nuclear actin, and the histone demethylase dKDM4A, as well as the SUMOylation pathway (Caridi et al. 2018, Chiolo et al. 2011, Colmenares et al. 2017, Ryu et al. 2015), and promote safe recombination between sister chromatids or homologs (Janssen et al. 2016). Depletion of these proteins results in retention of DSBs within the heterochromatin domain and in increased recombination defects in heterochromatic regions of *Drosophila* cells (Chiolo et al. 2011). Loss of heterochromatin proteins did not lead to *c*-Het retention of

CRISPR-induced DSBs in mammalian cells (Tsouroula et al. 2016), indicating that mammals could have acquired redundant pathways to promote the movement of DSBs. However, this study induced thousands of breaks simultaneously in satellite repeats, which could have masked local DSB retention relative to more modest break induction in heterochromatin using irradiation (Chiolo et al. 2011) or single DSB systems (Janssen et al. 2016). Interestingly, abolishing DSB end resection also results in retention of breaks within the *c*-Het domain in both *Drosophila* and mouse cells, indicating that early steps of the HR repair pathway are required to initiate DSB relocalization (Chiolo et al. 2011, Tsouroula et al. 2016).

In addition to the specific movement of resected DSBs to the *c*-Het periphery, another layer of heterochromatin DSB protection has evolved. Rad51, the major HR protein that executes homology search and recombination, associates with resected heterochromatic DSBs only upon their relocalization outside the heterochromatin domain (Chiolo et al. 2011). Rad51 exclusion from *c*-Het DSBs is thought to prevent premature and erroneous recombination between heterochromatic repeats (Chiolo et al. 2011).

These dynamic heterochromatin responses are dependent on the DNA damage kinases ATM and ATR. Inhibition of ATR in *Drosophila* and of ATM in mammalian cells results in defects in heterochromatin expansion and in peripheral DSB movement (Ayoub et al. 2008, Chiolo et al. 2011, Tsouroula et al. 2016). The involvement of these canonical damage response kinases suggests that DNA damage–specific posttranslational modifications of heterochromatin proteins regulate the intrinsic movement of heterochromatic DSBs. Indeed, Kap1 and HP1β, two proteins associated with mammalian heterochromatin domains, are phosphorylated upon damage by ATM and casein kinase 2, respectively (Ayoub et al. 2008, Goodarzi et al. 2008), and unphosphorylatable mutants of these proteins impair repair. These findings suggest that heterochromatin imposes additional physical constraints on the DNA damage response machinery that are alleviated through either relocalization outside the domain or reorganization of local chromatin structure. However, HP1 proteins and phosphorylated Kap1 have been observed to be increased at euchromatic DSB sites as well, concomitant with a transient deposition of the H3K9me3 mark (Ayrapetov et al. 2014, Baldeyron et al. 2011). In addition, all human HP1 homologs localize to sites of UV and oxidative DNA damage (Dinant & Luijsterburg 2009), and HP1γ helps recruit cohesin and the BRCA1 complex (important for HR) to DSBs (Oka et al. 2011, Wu et al. 2015), making it difficult to conclude that modifications on Kap1 or HP1 proteins are specific to heterochromatic breaks.

It is tempting to speculate that all the local changes that occur at heterochromatic DSBs, such as posttranslational modification of HP1, SUMOylation of specific substrates, and removal of nucleosomes upon DSB end resection, promote the movement of DSBs to the heterochromatin periphery through a biophysical incompatibility of these complexes with heterochromatin phase properties (Strom et al. 2017). Moreover, the biophysical differences between euchromatin and heterochromatin may cause the exclusion of Rad51 recruitment from heterochromatin DSBs. Indeed, loss of HP1, which drives heterochromatin phase properties (Larson et al. 2017, Strom et al. 2017), results in accumulation of Rad51 within heterochromatin in *Drosophila* cells (Chiolo et al. 2011). This observation indicates that loss of biophysical or other distinctions between euchromatin and heterochromatin allows for aberrant Rad51 recruitment to DSBs within the heterochromatin domain and promotes erroneous recombination.

Together, these studies indicate that loss of *c*-Het structure and function significantly increases genomic instability through illegitimate recombination between heterochromatic repeats (**Figure 3**), resulting in sequence gains and losses, as well as aberrant chromosome structures (e.g., translocations, dicentric chromosomes). However, whether loss of heterochromatin components also promotes the formation of aberrant chromosomal structures in human cancers is currently unknown.

H3K9me3-Enriched Regions Are Associated with Elevated Mutation Rates in Cancer

Although heterochromatin has evolved specific ways to protect repetitive sequences from forming extrachromosomal DNA (Peng & Karpen 2007) and undergoing erroneous recombination (Chiolo et al. 2011), regions enriched for the heterochromatic H3K9me3 mark in cancer cells are highly prone to accumulating single-nucleotide variants as well as copy number alterations (Cramer et al. 2016, Lim et al. 2017, Nair et al. 2017, Woo & Li 2012). In fact, H3K9me3 distributions can predict up to 40% of the total mutational load in cancer cells, whereas active chromatin marks actually anticorrelate with mutation load (Polak et al. 2015, Schuster-Bockler & Lehner 2012). The H3K9me3-enriched regions analyzed do not include the highly repetitive sequences present in *c*-Het regions and are thus likely composed of repeat-flanking unique sequences or H3K9me3-regulated sites in euchromatin (*i*-Het). Nevertheless, these studies can potentially reveal valuable insights into both the role of H3K9me3 in maintaining genetic fidelity and its influence on mutational load in cancer.

Both genic and nongenic H3K9me3-enriched regions reflect a higher mutation rate relative to their counterparts with low H3K9me3 levels (Schuster-Bockler & Lehner 2012). Thus, the increased mutation rate in H3K9me3 regions is unlikely to result from reduced selection against mutations in domains that are more transcriptionally inert than is actively transcribed euchromatin. The increased mutational load of H3K9me3-enriched regions is observed in a variety of cancer types and is largely irrespective of mutation type, such as transition mutations (purine-to-purine mutations, e.g., A → G) or transversion mutations (purine-to-pyrimidine mutations, e.g., G → T, or vice versa) (Polak et al. 2015, Schuster-Bockler & Lehner 2012). Several studies have provided insights into the role of heterochromatin signatures in DNA damage susceptibility, and below we highlight and discuss different hypotheses that could explain the link between H3K9me3 enrichment and increased mutational load.

Differential mismatch repair and nucleotide excision repair between chromatin regions. Interestingly, cancers associated with defective DNA mismatch repair (MMR), a pathway that corrects base mismatches between DNA strands, do not display the mutational disparity between high- and low-H3K9me3 regions. This loss of mutational disparity in MMR-deficient cancers is due to increased mutation rates in regions lacking H3K9me3 (Supek & Lehner 2015). Thus, the higher recovery of mutations in H3K9me3 domains observed in MMR-competent cancer cells is likely caused by reduced MMR repair rates in high-H3K9me3 regions.

MMR is highly active during DNA replication (Edelbrock et al. 2009). Since H3K9me3-enriched regions replicate late in S phase, they may have reduced opportunity to efficiently repair DNA mismatches using MMR. Increased transcriptional activity has also been associated with enhanced MMR efficiency (Li et al. 2013), suggesting that MMR is normally excluded from regions with low transcriptional activity, such as heterochromatin.

In addition to mutations associated with MMR deficiency (Supek & Lehner 2015), other types of mutations are increased in H3K9me3-enriched regions (Schuster-Bockler & Lehner 2012). Thus, other repair pathways besides reduced MMR could play a role in the increased mutational load in heterochromatic regions. Indeed, abolishing global genome nucleotide excision repair (NER), a pathway that repairs DNA base adducts and cross-links, also removes the disparity in mutational load between high- and low-H3K9me3 regions (Zheng et al. 2014). This result suggests that NER may also be less efficient in H3K9me3-enriched regions, which is supported by links between repressive chromatin states and delayed NER repair of UV- and cisplatin-induced damage (Adar et al. 2016, Hu et al. 2016).

Together, these studies indicate that differences in MMR and NER repair efficiency between heterochromatic and more open, actively transcribed chromatin regions could play a causal role in the differential recovery of mutations between these two chromatin states (Supek & Lehner 2015, Zheng et al. 2014).

DNA damage susceptibility of heterochromatic sequences. The organizational and sequence differences between heterochromatin and euchromatin are also important factors in their susceptibility to DNA damage. In fact, heterochromatin regions are thought to be more susceptible to replication stress and formation of fragile sites. Common fragile sites (CFSs), regions in the genome that show DNA breakage or rearrangements in the presence of replication stress, are associated with early stages of tumorigenesis (Gorgoulis et al. 2005). Chromatin surrounding CFSs is generally hypoacetylated, and treatment of cells with deacetylase inhibitors results in decreased breakage at CFSs, indicating that CFSs are associated with more compact chromatin regions (Jiang et al. 2009). This predisposition to replication stress could stem from the higher repeat content of heterochromatic regions, resulting in stable secondary structures inhibitory to fork progression (Branzei & Foiani 2010, Pearson et al. 2005, Zhao et al. 2010) that require additional mechanisms to be properly duplicated (Miller et al. 2006).

The increased sensitivity of repetitive chromatin domains to replication stress may be the reason for the temporal separation of heterochromatin and euchromatin replication during S phase. In most organisms, heterochromatin is late replicating (Lima-de-Faria & Jaworska 1968), but the biological significance of delaying replication has thus far remained largely elusive. From a genome stability standpoint, the separation of replication timing may help ensure the effective assembly of replication and chromatin remodeling complexes to the less accessible, highly repetitive, heterochromatic substrates that are prone to the formation of secondary structures and that require efficient restoration of heterochromatin packaging. Together, these studies indicate that *c*-Het may be more sensitive to replication stress–associated DNA damage, due to the preponderance of repetitive sequences.

The increased mutational susceptibility of H3K9me3 domains in human cells (Schuster-Bockler & Lehner 2012) has also been attributed to the spatial distribution of heterochromatin in the nucleus. In differentiated human cells, H3K9me3 regions are often associated with the nuclear lamina, termed lamina-associated domains (LADs) (Guelen et al. 2008). LADs, due to their peripheral positioning around the nucleus, encounter greater UV exposure than do other parts of the genome (Garcia-Nieto et al. 2017). However, the sensitivity of LADs to UV damage does not necessarily explain the mutational load in H3K9me3 domains observed in many types of cancers arising from internal tissues, which are unlikely to encounter UV exposure. However, the peripheral location of LADs, and therefore a major portion of H3K9me3 domains in differentiated cells, may make them more sensitive to other types of exogenous DNA damage sources.

In conclusion, many studies point toward an increased vulnerability of heterochromatic regions to DNA damage through increased susceptibility to erroneous recombination, replication stress at repeats, or decreased MMR or NER repair efficiency. This hypothesis is in line with the clear correlation that exists between increased mutation load in cancer genomes and H3K9me3 distributions in the cancer's cell type of origin (Polak et al. 2015, Schuster-Bockler & Lehner 2012). However, since chromatin data from cultured cell lines were used for all these correlation studies, the actual chromatin signatures of the sequenced cancer samples remain unknown. Chromatin states are likely altered in cancer genomes, which in turn could give rise to the differential mutational signatures. Therefore, to be able to identify direct links between heterochromatin features and genome instability in cancer, future studies need to focus on directly combining cancer genome sequencing analyses with cancer chromatin signatures.

In addition, we do not know when these disparate mutational signatures arise during tumorigenesis and whether they have a causal role in cancer initiation and/or progression. Performing cancer genome mutational analysis in mouse tumor models or organoids at different stages during tumor development could provide answers to these questions. Finally, we currently lack cancer genome data on the stability and presence of mutations, translocations, or other aberrations in *c*-Het repetitive sequences, and such studies could reveal important insights into the role of these sequences in cancer progression.

Heterochromatin Silences Expression of Repetitive DNA Sequences

Another important function of heterochromatin in maintaining genome stability is the transcriptional silencing of repetitive DNA sequences, such as transposons and satellite DNA (Bulut-Karslioglu et al. 2014, Peng & Karpen 2007, Zeller et al. 2016). Although repeat RNAs play important physiological roles in heterochromatin formation (Eymery et al. 2009), aberrant overexpression of such RNA has been associated with misregulation of several cellular processes that could result in increased genome instability (**Figure 3**). Loss of the two *C. elegans* H3K9 methyltransferases *met-2* and *set-25* results in upregulated expression of a subset of repetitive elements. This increased repetitive element expression results in RNA:DNA loop formation and higher rates of insertions and deletions specifically at repeats normally enriched for H3K9me2/me3 (Zeller et al. 2016). RNA:DNA hybrids can produce DNA damage by obstructing the progression of the replication machinery, leading to fork collapse and DSB formation (Aguilera & Garcia-Muse 2013). Increased satellite repeat expression in mammalian cells also compromises centromere structure and induces mitotic spindle defects and chromosome missegregation in mitosis (Bouzinba-Segard et al. 2006, Zhu et al. 2011). Together, these studies indicate that repetitive transcripts are inherently prone to inducing DNA damage due to a propensity to interfere with replication and centromere assembly and that heterochromatin formation at repeats promotes genome stability in part by suppressing their transcription (**Figures 1** and **3**).

Heterochromatin and telomeric repeat silencing. Heterochromatin also maintains the integrity of telomeric domains. Human telomeres consist of several kilobases of tandemly repeated telomeric repeats, as well as distinct subtelomeric repeat sequences. An important factor in the maintenance of telomeric repeat silencing is ATRX, which, together with the histone chaperone DAXX, loads the histone variant H3.3 at telomeres (Lewis et al. 2010). ATRX interacts with H3K9me3 and HP1 (Eustermann et al. 2011, Iwase et al. 2011). Consistent with a role for heterochromatic silencing in telomere integrity, telomere maintenance is also dependent on SUV39h and DNA methyltransferases (Garcia-Cao et al. 2004, Gonzalo et al. 2006). Loss of telomeric repeat silencing through loss of H3.3 or ATRX can result in aberrant recombination as well as increased RNA:DNA loop formation at telomeres that can cause DNA damage (Arora et al. 2014, Lovejoy et al. 2012).

Replication-dependent telomere shortening is often counteracted by expression of telomerase or, in ~10% of cancers, by alternative lengthening of telomeres (ALT), a recombination-based mechanism (Dunham et al. 2000). Maintaining or increasing telomere length allows cancer cells to continue to divide without entering replicative senescence. The increased DNA damage induced by RNA:DNA loops in the absence of telomere silencing has been hypothesized to promote the onset of ALT (Arora et al. 2014, Lovejoy et al. 2012). These studies indicate that heterochromatin maintenance is essential for telomere integrity and that loss of telomeric silencing can result in ALT, which in turn could promote tumorigenesis.

Heterochromatin and LINE-1 silencing. In addition to the prevention of damaging RNA:DNA structures (Zeller et al. 2016), heterochromatin silencing of transposons also suppresses the deleterious hopping of these mobile elements into other genomic regions, which could otherwise result in disruption of coding regions and rearrangements (Slotkin & Martienssen 2007) (**Figure 3**). A particularly abundant transposon in mammalian cells is LINE-1 (long interspersed repeat element-1). LINE-1 elements are part of the retrotransposon family, and their sequences account for approximately 17% of the assembled human genome (Cordaux & Batzer 2009), which excludes elements located in pericentric heterochromatin. Most LINE-1 sequences are inactive due to disruptive mutations that accumulated during evolution. However, it is estimated that approximately 100 LINE-1 elements are completely intact and can retrotranspose to other parts of the genome (Burns 2017, Cordaux & Batzer 2009). Repressive chromatin marks, such as DNA methylation, Kap1, and H3K9me3, usually silence transcription and transposition of these elements (Bulut-Karslioglu et al. 2014, Garcia-Perez et al. 2010, Rowe et al. 2010). Thus, loss of heterochromatin components is likely to increase retrotransposition of LINE-1 and other *c*-Het and *i*-Het elements; however, this potential impact on genome instability needs direct assessment.

High levels of RNA repeat and transposon expression, including LINE-1 retrotransposition events, have been identified in different types of cancers (Burns 2017). This observation suggests that heterochromatin-dependent silencing of LINE-1 transcription is disrupted in these cancers. In one study, up to 53% of patients had LINE-1 retrotransposition events, of which half included 3′ transduction events in which neighboring sequences were also mobilized (Tubio et al. 2014). Although most of the LINE-1-induced genomic disruptions were mainly passenger mutations, some of the LINE-1 insertions disrupted tumor suppressor genes (Lee et al. 2012, Solyom et al. 2012). Therefore, increased expression and hopping of LINE-1 sequences in cancer, and of potentially other transposons, represent another mechanism by which loss of epigenetic silencing contributes to genome instability, providing cancer cells with genetic diversity and an evolutionary advantage. Interestingly, in contrast to the hypothesis that increased LINE-1 expression could drive genome instability, LINE-1 expression is also suppressed in a variety of cancer types (see sidebar titled Desilencing Retrotransposons Elicits an Antitumor Response).

CAUSAL FACTORS FOR HETEROCHROMATIN DEFECTS IN CANCER

As outlined above, heterochromatin defects can cause genome instability in diverse ways, but which genetic or epigenetic changes in cancer cells actually drive these heterochromatin defects? Point mutations, translocations, or deletions of coding regions for H3K9 methyltransferases,

DESILENCING RETROTRANSPOSONS ELICITS AN ANTITUMOR RESPONSE

Paradoxically, several lines of evidence recently showed that cancer cells heavily depend on epigenetic silencing of retrotransposons to evade antitumor immune responses and chemotherapy-induced cell death (Cuellar et al. 2017, Guler et al. 2017). Since retrotransposons originate from viral integrations, these sequences can be recognized as nonself by both the innate and adaptive immune systems and can thus trigger an immune response. Elevated expression of the H3K9 di- and trimethyltransferase SETDB1 results in increased H3K9 methylation of LINE-1 elements, which allows tumor cells to evade cell death (Cuellar et al. 2017, Guler et al. 2017). Indeed, loss of silencing by depletion of SETDB1 or other heterochromatic factors results in more effective cell death induction following chemotherapy treatment of cancer cells (Guler et al. 2017). These studies indicate that loss of epigenetic silencing could also have a potent antitumor effect. Inhibition of H3K9 methyltransferases and histone deacetylases is currently being tested in clinical trials (Pfister & Ashworth 2017).

HP1, and other heterochromatin components could result in reduced expression or activity of these proteins and loss of heterochromatin structure and function (**Figure 3**). Importantly, loss of heterochromatic silencing is dose dependent, and desilencing of heterochromatic sequences can occur with only a 50% reduction in expression of heterochromatin proteins, such as single-copy loss of HP1.

However, there are more indirect ways through which loss of heterochromatin marks could occur during tumor development. First, karyotype changes resulting from chromosome missegregation events could progressively sequester a limited pool of heterochromatin proteins. The presence of extra copies of repetitive sequences upon gain of a chromosome can result in titration of limited heterochromatin components, thereby relieving silencing at regions that are normally heavily heterochromatinized (**Figure 3**). This was first revealed in *Drosophila*, in which addition of an extra copy of the Y chromosome results in global loss of heterochromatin-dependent silencing (Gowen & Gay 1934).

Loss of heterochromatin structure and function has also been associated with aging in various organisms. Humans with diseases associated with early aging, such as Hutchinson-Gilford progeria syndrome and atypical Werner syndrome mutations, have germline mutations in lamins and show loss of *c*-Het structures (Shumaker et al. 2006, Zhang et al. 2015). Indeed, cells from healthy aging individuals reveal similar lamina defects and a concomitant loss of heterochromatin-associated H3K9me3 (Scaffidi & Misteli 2006). Together, these studies show that physiological aging, which is the major risk factor for cancer onset (de Magalhaes 2013), could contribute to progressive loss of *c*-Het structure, or vice versa. We hypothesize that this loss of heterochromatin integrity could cause the increased genome instability levels as well as cancer susceptibility of aging cells (Scaffidi & Misteli 2006).

Another way to achieve reduced H3K9 methylation is through increased H3K9me3 demethylase activity. Many histone demethylases are overexpressed in cancer, which could lead to local or global losses of histone methylation marks in the genome (Black et al. 2012). Overexpression of the H3K9me3- and H3K36me3-specific demethylase KDM4A protein has been found in different types of cancer (Black et al. 2013, Kim et al. 2012) and results in loss of H3K9me3 (Fodor et al. 2006).

Other described genetic defects in cancer that can cause loss of heterochromatin marks are deficiencies in the tumor suppressors JARID1C and BRCA1; loss of either protein leads to disruption of heterochromatin and to derepression of repetitive sequence transcription (Filipponi et al. 2013, Zhu et al. 2011). The interdependence between DNA methylation and H3K9 methylation in human cells (Saksouk et al. 2014) also indicates that changes in DNA methylation could affect H3K9 methylation and thereby disrupt heterochromatin formation. Since loss of and changes in DNA methylation patterns are strongly associated with cancer progression (Timp et al. 2014), its impact on overall heterochromatin integrity must be considered a potential tumorigenic factor.

SUMMARY AND FUTURE DIRECTIONS

A preponderance of studies now indicate that the delicate balance between H3K9 methylation and demethylation in heterochromatin strongly contributes to genome stability. Loss of heterochromatic factors can result in chromosome segregation defects, aberrant repeat recombination, and desilencing of repetitive sequences (**Figure 3**). These defects promote genome instability, a major hallmark of cancer that can increase genetic heterogeneity and tumor evolution. In addition, the complex phenotypes associated with *c*-Het loss are closely intertwined, involve similar or interacting protein effectors, and can act as a negative feedback loop. For example, the induction of chromosomal aberrations or replication defects could give rise to aberrant chromosome

segregation in mitosis, and vice versa (Burrell et al. 2013, Crasta et al. 2012). Second, cohesin is recruited by *c*-Het and is required for chromosome segregation but also functions in transcriptional regulation, DNA repair, and nuclear architecture. Therefore, disruption of heterochromatin structure has far-reaching effects on different aspects of genome function and maintenance.

This review highlights the important roles of *c*-Het in maintaining genome stability and reveals the need for advancements in understanding the roles of this chromatin domain in the prevention of cancer initiation and progression. We hypothesize that restoring heterochromatin homeostasis could be a potential preventative or therapeutic strategy for anticancer treatments. An alternative approach could be to exploit heterochromatin defects in tumors. Combining inhibitors for SUV39H1/H2 or histone deacetylases with DNA-damaging agents could be a useful anticancer strategy to increase the DNA damage sensitivity of tumor cells (Pfister & Ashworth 2017). However, given the potential side effects of increasing genetic instability and promoting tumorigenesis upon loss of heterochromatin-dependent silencing, future research should carefully assess whether the use of heterochromatin targeting in anticancer treatments is a viable and safe option.

Besides the genome stability defects highlighted in this review, other cellular functions disrupted by heterochromatin dysfunction could potentially promote tumorigenesis. For example, heterochromatin loss can result in problems in maintaining quiescence and oncogene-induced senescence (Braig et al. 2005, Joh et al. 2016, Roche et al. 2016). Also, since heterochromatin proteins are important for DNA damage repair in euchromatic regions (Ayrapetov et al. 2014, Soria & Almouzni 2012), the efficiency of repair in otherwise nonheterochromatic regions could also be affected. Reduced H3K9 methylation could impact nuclear membrane stiffness and integrity (Stephens et al. 2018), which are important for metastasis, or could induce a stem cell–like undifferentiated state that promotes tumorigenesis (Becker et al. 2016).

In short, there is a pressing need to improve our understanding of *c*-Het defects in tumorigenesis and its impact on cancer evolution. Advancements in cancer genome sequencing, chromatin analyses, and annotation of human *c*-Het and other unmapped, repetitive sequences will be invaluable in elucidating the heterochromatin defects present in different cancer types and stages. This understanding will undoubtedly provide important insights into and innovative applications toward the prevention and treatment of cancer, as well as other diseases associated with genome instability.

DISCLOSURE STATEMENT

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