The Importance of Satellite Sequence Repression for Genome Stability

PETER ZELLER1,2 AND SUSAN M. GASSER1,2

1Friedrich Miescher Institute for Biomedical Research, CH-4058 Basel, Switzerland
2Faculty of Natural Sciences, University of Basel, CH-4056 Basel, Switzerland

Correspondence: susan.gasser@fmi.ch

Up to two-thirds of eukaryotic genomes consist of repetitive sequences, which include both transposable elements and tandemly arranged simple or satellite repeats. Whereas extensive progress has been made toward understanding the danger of and control over transposon expression, only recently has it been recognized that DNA damage can arise from satellite sequence transcription. Although the structural role of satellite repeats in kinetochore function and end protection has long been appreciated, it has now become clear that it is not only these functions that are compromised by elevated levels of transcription. RNA from simple repeat sequences can compromise replication fork stability and genome integrity, thus compromising germline viability. Here we summarize recent discoveries on how cells control the transcription of repeat sequence and the dangers that arise from their expression. We propose that the link between the DNA damage response and the transcriptional silencing machinery may help a cell or organism recognize foreign DNA insertions into an evolving genome.

In eukaryotic organisms, DNA is packaged by proteins into a structure called chromatin that influences genomic interactions with the transcription machinery. Chromatin structure is modulated by the accumulation of covalent modifications to the DNA itself, to histones and nonhistone chromatin factors, and by protein composition. Essentially it is the accessibility of a DNA sequence to the transcription machinery that regulates gene expression, although additional regulation can also be imposed co- and posttranscriptionally (Tippmann et al. 2012).

The main DNA modification in vertebrate genomes is methylation on the relatively underrepresented dinucleotide, CpG, producing 5mCpG or 5-methylcytosine. Although much of the mammalian genome carries this modification constitutively, it is regulated at promoters that carry CpG clusters (CpG islands), as well as over the gene bodies (Weber et al. 2005). At both sites, 5mCpG is usually associated with transcriptional inhibition (Razin and Riggs 1980). It silences either by recruiting 5mC-binding factors (Jones et al. 1998; Bird and Wolfe 1999) or by disabling transcription factor binding sites (e.g., at CpG islands [Watt and Molloy 1988; Bell and Felsenfeld 2000]).

In addition to DNA modification, posttranslational histone tail modifications are especially well-studied. Depending on the nature and position of the modification on the histone, they can either work on nucleosome–nucleosome or nucleosome–DNA interactions by changing the charge of the highly basic histone tail or by generating specific binding sites for proteins that recognize modified lysine or arginine residues. The range of characterized chemical modifications on histones has been expanding steadily, with the most common being lysine methylation, acetylation, ubiquitination, SUMOylation, and ribosylation or serine or threonine phosphorylation. Different chromatin states are defined by the combination of histone modifications that some considered to be an instructive “histone code” (Strahl and Allis 2000). The proteins that recognize covalent histone modifications often act in trans by promoting or inhibiting the recruitment of additional regulators either of transcription or chromatin compaction.

Here as well, there is a growing list of motifs that characterize the readers of specific histone modifications (Tavner et al. 2007).

HETEROCHROMATIN

Early microscopy experiments in moss by Heitz in 1928 distinguished two chromatin “states” in the interphase nucleus. Heterochromatic regions at the nuclear envelope and around the nucleolus stained strongly during the whole cell cycle, which was interpreted as a constant high level of compaction, whereas euchromatic regions stained strongly only during mitosis, suggesting that they “unfold” in interphase. This observation was the foundation for the model that the higher-order packaging in heterochromatic regions could be refractory for the binding of the transcription machinery. Later, heterochromatic regions were further separated into constitutive and facultative heterochromatin, often correlated with either methyl-K9 (Noma et al. 2001; Schotta et al. 2002) or methyl-K27 on histone H3, respectively (Bernstein et al. 2006; Kalantry et al. 2006). Detailed chromatin immunoprecipitation (ChIP) experiments mapped H3K9 methylation to both the centromere-flanking satellite repeats and interspersed repetitive elements (REs) throughout the genome in all cells, thus defining constitutive heterochromatin (Pimpinelli et al. 1995; Gerstein et al. 2010; Liu et al.

© 2017 Zeller and Gasser. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial License, which permits reuse and redistribution, except for commercial purposes, provided that the original author and source are credited.
Two main approaches have been used to examine if chromatin composition can indeed regulate access to the underlying DNA sequence. One method was to expose chromatin to exogenously added DNase1. In early experiments using isolated nuclei, DNaseI showed a preferential digestion of the actively transcribed albumin gene in liver tissue (Weintraub and Groudine 1976). Combining this approach with whole genome sequencing, it became clear that this method primarily identifies nucleosome-free regions, particularly at the enhancers and transcription start sites of active genes (Boyle et al. 2008). Outside of these two classes of elements, the sensitivity of this approach turned out to be rather limited, as it did not clearly distinguish euchromatin and heterochromatin. The second approach was based on the expression of an E. coli DNA methyl transferase (DAM) in intact cells, whose modification on adenine can be quantified as the degree of protection against the methyl-sensitive restriction enzyme DpnI. Once again, in a genome-wide study, Bell et al. (2010) were able to show a small reduction in DAM methylation over H3K27me3 regions, but no difference between H3K9-methylated regions and unexpressed euchromatic loci could be observed. One caveat of these techniques may be that the chromatin compaction induced by heterochromatin does not interfere strongly with the temporal interaction of a single protein but rather hinders the assembly of multiprotein complexes, such as the general transcription machinery. Indeed, these methods primarily mapped nucleosome density, which may not represent the organizational level affected by heterochromatin. Recent work suggests that nucleosome turnover rates may be the more important criterion that distinguishes euchromatin from heterochromatin (Taneja et al. 2017), given that local histone turnover is greatly reduced in heterochromatic domains (Aygün et al. 2013; Toyama et al. 2013). This could either reflect a reduced action of nucleosome remodelers or of demethylating enzymes that remove the repressive H3K9me mark, such as KDM4b (Tsurumi et al. 2013).

THE TWO-FACED ROLE OF HETEROCHROMATIN IN DNA DAMAGE AND REPAIR

Not only transcription, but DNA damage as well, occurs in the context of chromatin. Intriguingly, during the DNA damage response, heterochromatin seems to play both positive and negative roles. On one hand, nucleosomes are thought to be an obstacle for the DNA damage repair machinery and thus must be removed. Indeed, following UV damage ubiquitination-mediated histone mobilization has been reported (Wang et al. 2006; Lan et al. 2012; Adam et al. 2013) and in the case of DNA double-strand breaks (DSBs) in yeast, histone ChIP experiments showed a local depletion around an induced DSB (van Attikum et al. 2004, 2007), as well as a global loss of histones at high levels of Zeocin- or γIR-induced DNA damage (Hauer et al. 2017). Local histone release was shown to depend on the activity of nucleosome remodelers BRG1/RSC and/or INO80 (van Attikum et al. 2004, 2007; Zhao et al. 2009; Jiang et al. 2010). On the other hand, multiple repressive factors, including HP1 (Luijsterburg et al. 2009), Polycomb (Hong et al. 2008), and HDAC1/2 (Miller et al. 2010), were reported to be recruited to sites of DNA damage. Consistently, animals lacking HP1 (Luijsterburg et al. 2009) or Polycomb components (Hong et al. 2008) are hypersensitive to genotoxic stress. Besides a potential role of these factors in the recruitment of the repair machinery, they are also thought to promote silencing around sites of damage to prevent conflicts or interference between the repair and transcription machineries (Ui et al. 2015; Vissers et al. 2012) reviewed in Polo (2017).

An interesting twist to this role was nicely shown in Drosophila. In the absence of the fly heterochromatin components Su(var)3-9 and HP1α, an increase of spontaneous RAD51 foci was scored specifically in DAPI-dense regions of the nucleus, suggesting that heterochromatin itself might prevent spontaneous DNA DSBs (Peng and Karpen 2009; Chiolo et al. 2011). This effect is thought to stem from the loss of transcriptional repression coincident with loss of H3K9me and its reader. One of the main sources of DSBs in eukaryotic cells in the absence of exogenous insult is the stalling of replication forks. Moreover, it has been clear for many years that transcription itself can be a major impediment to replication fork progression (Brewer 1988; French 1992; Liu and Alberts 1995). Thus, to avoid collisions of the replication fork with the transcription machinery, cells coordinate these two nuclear events. This is achieved in part by delaying the firing of certain origins of replication; transcriptionally active sites were shown to replicate early in S phase, whereas transcriptional silent sites were replicated late (Schübeler et al. 2002; Rivera et al. 2014).

The importance of this coordination of transcription and replication was elegantly shown in a study that identified DNA breaks by ligating sequencing adapters onto the open ends of unfragmented DNA (Break-seq). The authors could show that fragile sites occurred most frequently at sites where replication and transcription coincide. The shift of collision points by perturbing replication timing or by inducing unscheduled transcription resulted in a corresponding change in position or intensity of the breaks (Hoffman et al. 2015). At the longest human genes, such conflicts seem to be impossible to avoid, given that a full-length transcript takes more than one cell cycle in some cases, and this in turn leads to the formation of fragile sites that break in a transcription-dependent manner (Helmrich et al. 2011).

RNA:DNA HYBRIDS: TRANSCRIPTION INTERMEDIATES IMPAIR REPLICATION FORK PROGRESSION

One characteristic feature that identifies sites of conflict between the replication and transcription machineries is...
the formation of RNA:DNA hybrids, also called R-loops. These hybrid nucleic acid structures result from the displacement of the second DNA strand by a transcribed RNA. Studies in yeast and mammalian cells have mapped RNA:DNA hybrid accumulation to highly transcribed genes (Wahba et al. 2016). Studies performed to elucidate the mechanisms that protect cells from RNA:DNA hybrid accumulation led to the identification of factors involved in transcriptional processivity, as well as factors known to be involved in the restart of collapsed forks (Santos-Pereira and Aguilera 2015).

The dangers posed by RNA:DNA hybrid formation was first shown in cells depleted for certain RNA biogenesis and processing factors, such as the THO complex in yeast (Huertas and Aguilera 2003) and *Caenorhabditis elegans* (Castellano-Pozo et al. 2012), or the serine/arginine-rich splicing factor 1 (SRSF1; previously known as ASF and SF2) in vertebrates (Li and Manley 2005). Additionally, there is evidence for a replication independent role of R-loops in generating DNA breaks, as the nucleotide excision repair (NER) nucleases XPG and XPF were shown to be able to process R-loops into DSBs in some situations (Sollier et al. 2014). Recently, our laboratory found that the absence of H3K9me leads to an accumulation of RNA:DNA hybrids in *C. elegans* on repetitive elements (Zeller et al. 2016), and that R-loop occurrence correlated strikingly with the mapping of genomic mutations in REs.

The presence of DSBs in repetitive regions of the genome constitutes a particular challenge for repair mechanisms. Repair by homologous recombination (HR) can result in translocations between chromosomes that bear common repeats, rather than restoring intact chromatinids, and single-strand annealing can lead to small inserts and deletions or copy-number variation. It was proposed that the methylation of H3K9 in these repetitive regions might sequester damage away from the HR machinery, thus reducing the risk of inappropriate repair. Consistently, the groups of Karpen (Chiolo et al. 2011; Janssen et al. 2016), Chiolo (Ryu et al. 2015), and Soutoglou (Tsouroula et al. 2016) found that breaks within heterochromatic sequences were repaired differently than breaks in euchromatin, at least when it came to HR. They described an HP1α-dependent pathway for repair in heterochromatin (Chiolo et al. 2011) that allows early steps of the DNA damage response to occur, but then ensures that later steps (i.e., Rad51 binding) occurred only after the break site had been relocated outside of the heterochromatic domain. Nonhomologous end joining, on the other hand, occurred at normal rates within heterochromatin, given that end joining requires no template (Janssen et al. 2016). The suppression of HR within heterochromatin depends on both the H3K9 methytransferase Su(var)3-9 and its reader HP1α.

Although it is still unclear exactly how heterochromatin factors impact the repair pathway (Janssen et al. 2016), in the absence of H3K9me there is an increase in repeat-specific DNA damage. Indeed, H3K9 methylation can both reduce damage by suppressing the frequency of replication fork collisions with the transcription machinery and repress homology-driven recombination in a repeat-rich domain.

**REPETITIVE ELEMENTS AND THE DANGER OF THEIR EXPRESSION**

In constitutive heterochromatin, the main sequence classes are repetitive elements, which make up a large fraction of the human genome (de Koning et al. 2011). Repetitive elements can be subdivided into tandem repeats and transposable elements, based on their sequence characteristics. Tandem repeats are head-to-tail repetitions of 2–200 bp long sequence elements, present either as micro- and minisatellites of 5–150 bp in length, dispersed around the genome, or as megabase long stretches of major satellite sequence around centromeres. There is no protein encoded by tandem repeats and they do not contain canonical RNA-Pol promoters, even though transcription factors occasionally bind in repeats. In contrast to tandem repeats, complete transposable elements do harbor promoter regions and encode for proteins that facilitate their transposition in the genome. Based on the intermediates formed during transposition, these elements can be further subdivided into RNA (copy–paste) and DNA (cut–paste) transposons. Among both RNA and DNA transposon classes are some that are autonomous (encoding all the proteins necessary for transposition) and others that are nonautonomous. These depend on autonomous family members for transposition, as they contain coding regions that are incomplete or mutated.

DNA transposons make up the largest fraction of repetitive elements in the human genome and are the most active transposon class. They either do or do not contain long terminal repeats (LTRs; retrovirus-like transposons). Of special importance is the non-LTR transposon LINE-1, as it makes up ~17% of our genomes and is one of the most transpositionally active transposons in the human genome (Lander et al. 2001; Boissinot and Furano 2005). A variety of heterochromatin-generating pathways mediate transcriptional or posttranscriptional silencing of these sequences, with the implementation and choice depending on the sequence composition of the repeat, the organism, the cell type concerned, stage of development, and other biological features of the repeat (Grewal and Rice 2004; Martens et al. 2005; Padeken et al. 2015; Nishibuchi and Dejardin 2017; Papin et al. 2017). Several of the repression mechanisms are mentioned below, although here we focus primarily on the repercussions of repeat expression and not on the range of mechanisms that lead to their repression.

The existence and transcriptional activity of these repetitive elements were shown to pose a serious threat to the genome’s integrity (summarized in Fig. 1). First of all, the de novo integration of transposable elements into coding regions was found at the origin of multiple inheritable diseases. To date 124 genetic diseases have been identified that are caused by transposon insertions (Hancks and Kazazian 2016). The first of these identified was a form of hemophilia A characterized in 1988 (Kazazian et al. 1988). With respect to cancer, most studies have focused on the most abundant repeat elements in man, the autonomous LINE-1 and the nonautonomous Alu RNA transposons. A large genome sequencing study of 244 cancer
patients bearing 12 different cancer types came to the surprising discovery that in 50% of these cases somatic transposition had occurred (Tubio et al. 2014). In a few cases of colorectal cancer the transposition was considered causative for tumorigenesis, as it disrupted the APC tumor-suppressor gene (Miki et al. 1992). An interesting observation arising from these studies was that nearly one-fourth of the observed transposition events also copied additional nonrepetitive sequence pieces, because of transposon transcription continuing after the 3′ end of the transposable element (Tubio et al. 2014). When the original transposon was situated upstream of a coding exon, this led to a phenomenon called exon shuffling.

In most cases, however, a causal link between transposon hopping and oncogenic transformation is unlikely, and transposon expression is more likely a reflection of the general loss of heterochromatic silencing that accompanies oncogenic transformation (Burns 2017). Indeed, studies have linked LINE-1 promoter DNA hypomethylation, the expression and transposition of the transposon and a loss of genome integrity, with poor overall prognosis in non–small cell lung cancer (Daskalos et al. 2009; Saito et al. 2010). This can be extended to generally poor outcomes in many other cancer types as well (Burns 2017). Although it is rarely the initial trigger for oncogenesis, the increased mutation rate caused by the transposon activity may enhance tumor progression and contribute to drug resistance. It is important to note that all repetitive elements can serve as a basis for homology-driven chromosome rearrangements.

Recently, we found that the unscheduled transcription of repetitive elements alone can compromise genome integrity (Zeller et al. 2016). Using a mutant of the nematode C. elegans that lacks all histone H3K9 methylation, we could specifically detect the accumulation of insertions and deletions in derepressed RE, which correlates with a repeat-specific increase in RNA:DNA hybrids (Fig. 2A). Although some genes are also derepressed (234 genes in embryos lacking H3K9me at 20°C are derepressed more than twofold), there was a striking increase in transcripts from all three RE classes (Zeller et al. 2016). Moreover, a DRIP-seq analysis to map RNA:DNA hybrids genome-
wide showed their accumulation on DNA transposons and tandem repeats, specifically in the H3K9me-deficient worms. We also scored a detectable level of R-loops in wild-type worms on a subset of highly transcribed genes, as earlier reported (Wahba et al. 2016), yet these do not increase in the absence of H3K9me. It is not clear if replication is necessary for RNA:DNA hybrid formation or whether this stems from the specific character of the repeat RNA.

Different sequences may be differentially prone to form RNA:DNA hybrids when transcribed, as previously suggested from studies in yeast and mammalian cells. Whereas the accumulation of RNA:DNA hybrids generally correlates with the strength of gene expression (Wahba et al. 2016), sequence features that facilitate hybrid formation, such as GC content and poly(A) tracks, were also found (Ginno et al. 2012; Wahba et al. 2016). Of particular note is the accumulation of RNA:DNA hybrids on telomeres and Thy1 transposons (Chan et al. 2014), despite the relatively low expression level of those sequences.

In recent years several studies have tried to identify the mechanisms that protect cells from RNA:DNA hybrid accumulation. These efforts identified a role for many factors involved in transcriptional processivity and RNA degradation (Santos-Pereira and Aguilera 2015). A screen for synthetic sterility with the loss of H3K9me in C. elegans, identified many of the same factors, as well as factors involved in replication fork stability (J Padeken, P Zeller, and SM Gasser, pers. comm.). Based on such screens we postulate that four parameters regulate RNA:DNA hybrid formation: first is the affinity of the transcribed RNA for the homologous DNA, a feature dictated by sequence; second, the nature of the transcript itself with respect to introns, secondary structures and the nuclear RNA degradation machinery; third, the factors that bind, process and protect the RNA; and finally, the processivity and/or abundance of the RNA polymerase and its coordination with the replication fork.

It is intriguing that under the conditions that generate the strongest increase in the level of RNA:DNA hybrids, that is, higher temperatures and the loss of histone H3K9 methylation, the major transcriptional difference is a derepression of tandem repeats (Zeller et al. 2016) (J Padeken, P Zeller, and SM Gasser, pers. comm.). In C. elegans, this class of repetitive elements is composed of linear repeats of 2–5-bp sequence units, which in theory allows for RNA:DNA pairing in multiple positions. As mentioned above, they neither possess promoter sequences, open reading frames, nor the sequence elements that promote the recruitment of RNA processing factors. These characteristics therefore facilitate the formation of RNA:DNA hybrids upon transcription. A pyrimidine versus purine preference in RNA:DNA hybrid formation could not be detected in REs.

Interestingly, tandem repeats have previously been shown to be very fragile if transcribed, particularly if transcription is bidirectional (Wierdl et al. 1996; Lin et al. 2006; Nakamori et al. 2011). This was mostly attributed to their potential to form higher-order structures such as stem-loop structures (Pearson et al. 2005), given that the torsional stress produced by bidirectional transcription can favor stem loops and G-quadruplex formation. These factors indeed favor RNA:DNA hybrid accumulation, as well (Roy and Lieber 2009; El Hage et al. 2010; Hamperl and
Cimprich 2014). Because RNA:DNA pairing is more stable thermodynamically than DNA:DNA pairing, RNA:DNA hybrids are an obstacle for DNA replication fork progression (Thomas et al. 1976). It is still unclear, however, exactly what the harmful structures that form at transcribed tandem repeats are. Experiments that combine systems for induced tandem repeat expression with a depletion of RNA:DNA hybrids through RNaseH overexpression, or specific targeting of a RNaseH fusion protein, may be able to answer this question.

The only other repeat elements that are enriched for H3K9me2 over me3 and enriched for RNA:DNA hybrids are DNA transposons. The most prominent group of DNA transposons in C. elegans works by a cut-and-paste mechanism leaving a double-strand break behind upon relocation (Vos et al. 1996; Bessereau 2006). Thus, in C. elegans, the two classes of repeat elements that are preferentially modified by H3K9me2 rather than H3K9me3 (i.e., DNA transposons and tandem repeats) can potentially generate a DSB if expressed. The former may activate a transposition pathway, whereas the latter provokes replication fork collapse.

Whereas RNA transposons are also derepressed in the absence of H3K9 methylation, they do not accumulate RNA:DNA hybrids based on DRIP-seq in C. elegans embryos (Zeller et al. 2016). Moreover, although C. elegans contains full-length RNA transposons of the LTR class (Ganko et al. 2001), their transposition has not been reported (Bessereau 2006). There may be a link between the absence of RNA:DNA hybrid formation on RNA transposons and their lack of transposition. Indeed, cotranscriptional RNA:DNA hybrid formation (Huertas and Aguilera 2003) may be prevented by cotranscriptional RNA degradation. A likely player in this is the 5′–3′ exonuclease XRN-2, which was shown to be involved in the cotranscriptional degradation of nascent RNA as part of the transcriptional termination process (West et al. 2004). More recent studies additionally showed that XRN-2 is involved in the degradation of many endogenous transcripts when transcriptional processivity is impaired (Davidson et al. 2012). Given that the RNA transposons in C. elegans are considered to be evolutionary very young (Ganko et al. 2001), their transcripts may not be efficiently processed for export and thus be preferred targets for XRN-2 mediated degradation. Given that retrotransposons are particularly enriched for H3K9me3, a mark that frequently co-occurs with H3K27me3 in worms, it is conceivable that the transcripts from RNA transposons are specifically targeted for degradation by one or the other mark. This hypothesis could be tested by analyzing sequence-specific RNA:DNA hybrid accumulation in worms lacking both H3K27me and H3K9me3.

**DOES INSTABILITY INITIATE TRANSCRIPTIONAL SILENCING?**

For both transposable elements and genes, the mechanisms that are known to recruit H3K9 histone methyltransferases (HMTs) include small RNA pathways like the PIWI pathway in the germline (Haynes et al. 2006; Sienski et al. 2012), dsRNA transcripts at satellite repeats in *Schizosaccharomyces pombe* (Keller et al. 2012), and transcription factors, including zinc-finger proteins or the orphan nuclear receptor SHP (Fang et al. 2007; Garcia-Bassets et al. 2007; Bulut-Karslioglu et al. 2012). Considering the risk of genome instability that arises from the transcription of tandem repeats, it is relevant to ask how cells ensure that this class of RE is efficiently modified with H3K9me2.

One intriguing possibility is the existence of a feedback loop from the DNA damage caused by the transcription of tandem repeats, which could recruit HMTs to deposit H3K9me. Several arguments support such an idea. First, repressive factors, including HP1 (Luijsterburg et al. 2009), Polycomb (Hong et al. 2008) and HDAC1/2 (Miller et al. 2010), are recruited to sites of DNA damage and the MBT-domain containing protein LIN-61 was shown to be crucial for repair of DSBs by homologous recombination (Fig. 2B; Johnson et al. 2013). Besides a role for heterochromatin factors in repair enzyme recruitment, they might also be implicated in the local transcriptional silencing around damage sites to prevent conflicts between the repair and the transcription machineries (Vissers et al. 2012; Ui et al. 2015). In this context, the Almouzni group showed that a transient transcriptional silencing occurs during DNA damage repair (Adam et al. 2013). In their study, they identified the histone chaperone HIRA as a crucial component that ensures transcriptional reactivation after UVC-induced DNA damage repair. It remains to be seen what would happen in a region that does not contain a strong and specific transcription factor that recruits HIRA. Would the heterochromatic state persist, marking the region as a potentially unstable domain? Would this prevent future breaks from happening or target it for a specific pathway of repair? UV damage and replication-fork-associated damage are repaired by distinct mechanisms, but it is possible that fork-associated damage also recruits heterochromatin components to silence and protect regions that have a bias for fork collapse.

The histone acetyltransferase TIP-60 truly is another example how heterochromatin and the DNA repair machinery are linked. A crucial step during the DNA damage response is the activation of the DNA damage checkpoint protein ATM through acetylation by TIP-60 (Fig. 2C). Sun et al. (2009) could show that the enzymatic activity of TIP-60 depends on its binding to H3K9me. They further showed that under damaging conditions histone H3K9me levels are not increasing globally, but rather the mark is made more accessible by the release of HP1β. Tying the activity of TIP-60-mediated ATM activation to the presence of ligand-free H3K9me could in principle allow the chromatin state to stimulate selectively one repair pathway over another.

**REPAIR FACTORS WITH A ROLE IN TRANSCRIPTIONAL SILENCING**

The cross talk between DNA damage and heterochromatin formation, would predict that loss of repair factors might cause defects in de novo silencing, particularly with respect
to tandem repeats. Recent studies on the human breast-cancer susceptibility gene 1 (BRCA1) support such a functional connection. The BRCA1 protein is a tumor suppressor with E3 ubiquitin ligase activity that contributes to DNA repair by homologous recombination (Scully et al. 1996; Anderson et al. 1998). Mutations in BRCA1 give a strong predisposition toward breast and ovarian cancers. C. elegans BRCA-1, as well as mammalian BRCA-1, forms a heterodimer with BRD-1/BARD1, respectively (Boulton et al. 2004), and its dimerization is essential both for preserving genome integrity and for depositing histone H2A ubiquitination (Polanowska et al. 2006).

New evidence suggests that the repressive function of BRCA1 is of central importance for the preservation of genome integrity (Fig. 2D). Zhu et al. (2011) could show that loss of BRCA1 in mice leads to the derepression of tandemly repeated satellite DNA but not of other heterochromatic sequences, such as transposable elements. The function of BRCA1 in repeat silencing is largely mediated by its ability to monoubiquitinate H2A, because the expression of a constitutively ubiquitinated histone H2A could restore satellite expression to wild-type levels. Strikingly, artificial expression of these tandem repeats from a transgene can phenocopy the defects arising from BRCA1 mutation, including centrosome amplification, cell cycle checkpoint defects, DNA damage, and genomic instability (Zhu et al. 2011). This argues that transcriptional control of tandem repeats is one of the main functions of this central and well-studied tumor suppressor and DNA damage response factor. In addition to its role in the DNA damage response and in transcriptional silencing, BRCA1 was also found to directly initiate RNA:DNA hybrid removal by the recruitment of the RNA helicase Senataxin (Hatchi et al. 2015).

If a cell accumulates too much DNA damage or if certain oncogenic pathways become hyperactive, the last resort a cell has to protect the organism from the onset of cancer is to enter the nonproliferative state of senescence. This process depends on the DNA damage checkpoint proteins Rb and p53, which silence the target genes controlled by E2F, as their expression drives the cell division cycle. These genes accumulate H3K9 methylation and HP1 binding upon repression (Beausejour et al. 2003; Narita et al. 2003). On a cellular level the accumulation of heterochromatin can also be seen as the appearance of senescence-associated heterochromatic foci (SAHFs), although it is still a matter of debate how crucial SAHF formation is for the state of cellular senescence (Kosar et al. 2011). On the other hand, this would be an example of how the DNA damage checkpoint can use heterochromatin to prevent the proliferation of transformed cells.

**OPEN QUESTIONS: SILENCING AND SEQUESTRATION**

Many questions remain to be resolved concerning the role of repeat elements and their repression in genome stability and in disease. The first unanswered question is how either H3K9me2 or H3K9me3 is specifically targeted to repeats. In C. elegans, it is clear that the H3K9me2 HMT, MET-2, can methylate sites independently of the second HMT, SET-25 (J Padeken, P Zeller, and SM Gasser, pers. comm.). A small but specific subset of genomic loci depends on the H3K9me3 mark deposited by SET-25 for repression, and among these are genes whose silent state is stably inherited across generations, following repression by the addition of exogenous RNAi (Ashe et al. 2012; Buckley et al. 2012). Although the authors did not prove that the MET-2 HMT has no role in this process, they showed the ability of the H3K9me3 machinery to target sequences de novo for methylation, independent of their position in the genome. This pathway may be crucial for the survival of a species in an environment where new retrotransposons can infect at any time, and require de novo repression.

This interplay of two parallel methylation pathways is not unlike that of DNA methylation in mammalian cells, where one CpG methyltransferase, DNMT1, is dedicated to maintenance of preexisting methylation (Gruber et al. 1982; Bestor and Ingram 1983), whereas DNMT3a and DNMT3b exist to methylate de novo sequences (Okano et al. 1998; Lyko et al. 1999). Links between DNA methylation and histone H3K9 methylation exist, but it is poorly understood how they cooperate and how this depends on chromosomal context.

A final open question concerns the role of spatial sequestration of heterochromatin at the nuclear envelope for facilitating transcriptional repression. Peripheral localization of genomic regions in general correlates with low-level transcription (Pickersgill et al. 2006) and silencing factors such as HDAC3 (Somech et al. 2005) and HP1 (Ye and Worman 1996) have been shown to interact with components of the nuclear envelope. In worms the level of de-repression of satellite or simple repeat sequences provoked by loss of H3K9me2 correlates with the proximity of the sequence to the nuclear periphery, which is not true for sequences bearing H3K9me3 (J Padeken, P Zeller, and SM Gasser, pers. comm.). Indeed, not every sequence tethered to the nuclear periphery is silenced (Finlan et al. 2008; Reddy et al. 2008; Ruault et al. 2008). Consistently, the loss of heterochromatin anchoring in C. elegans embryos did not alter the transcription of genes or repeats dramatically (Gonzalez-Sandoval et al. 2015). Thus the role of heterochromatin sequestration in genome stability remains an open question. It may be that the proper control of replication timing and origin usage requires association with the nuclear envelope (Guelen et al. 2008; Hansen et al. 2010). Moreover, deletion of the origin of replication-associated protein (ORCA) was shown to interfere with H3K9me propagation (Wang et al. 2017). Thus, the link between repeat element repression, replication timing, and the spatial organization of chromatin in the nucleus remains an active area of research.

**ACKNOWLEDGMENTS**

We thank Jan Padeken, Robin van Schendel, Marcel Tijsterman, and the FMI Genomics and Microscopy facilities...
for advice and discussion and Jan Padeken for communicating unpublished results. S.M.G. thanks the Swiss National Science Foundation, the European Research Council, and the Novartis Research Foundation for support.

REFERENCES

Adam S, Polo SE, Almouzni G. 2013. Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperoneHIRA. Cell 155: 94–106.

Anderson SF, Schlegel BP, Nakajima T, Wolpin ES, Parvin JD. 1998. BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. Nat Genet 19: 254–256.

Ashe A, Sapetschnig A, Weick E-M, Mitchell J, Bagijn MP, Cording AC, Doebely A-L, Goldstein LD, Lehrbach NJ, Le Pen J. 2012. piRNAs can trigger a multigenerational epigenetic memory in the germline of C. elegans. Cell 150: 88–99.

Aygiön O, Mehta S, Grewal SIS. 2013. HDAC-mediated suppression of histone turnover promotes epigenetic stability of heterochromatin. Nat Struct Mol Biol 20: 547–554.

Beaumage JM, Krützli A, Galimi F, Narita M, Lowe SW, Yasswen P, Campisi J. 2003. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. EMBO J 22: 4212–4222.

Bell AC, Felsenfeld G. 2000. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 405: 482–485.

Bell O, Schweiger M, Oakeley EJ, Lienert F, Beisel C, Stadler MB, Schubeler D. 2010. Accessibility of the Drosophila genome discriminates PCr repression, H4K16 acetylation and replication timing. Nat Struct Mol Biol 17: 894–900.

Bernstein E, Duncan EM, Masui O, Gil J, Heard E, Allis CD. 2006. Mouse polycomb proteins bind differentially to methylated histone H3 and RNA and are enriched in facultative heterochromatin. Mol Cell Biol 26: 2560–2569.

Bessereau JL. 2006. Transposons in C. elegans. WormBook: 1–13.

Bestor TH, Ingram VM. 1983. Two DNA methyltransferases from murine erythroleukemia cells: Purification, sequence specificity, and mode of interaction with DNA. Proc Natl Acad Sci 80: 5559–5563.

Bird AP, Wolfe AP. 1999. Methylation-induced repression: Belts, braces, and chromatin. Cell 99: 451–454.

Boissinot S. 2005. The recent evolution of the human L1 retrotransposons. Cyto-genet Genome Res 110: 402–406.

Boulton SJ, Martin JS, Polanowska J, Hill DE, Gartner A, Vidal M. 2004. BRCA1/BARD1 orthologs required for DNA repair in Caenorhabditis elegans. Curr Biol 14: 33–39.

Boyle AP, Davis S, Shulha HP, Meltzer P, Margulies EH, Weng Z, Furey TS, Crawford GE. 2008. High-resolution mapping and characterization of open chromatin across the genome. Cell 132: 311–322.

Brewer BJ. 1988. When polymers collide: Replication and the transcriptional. Cell 53: 679–686.

Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, Kimble J, Fire A, Kennedy S. 2012. A nuclear Argonaute promotes multigenic epigenetic inheritance and germline immortality. Nature 489: 447–451.

Bulut-Karslioglu A, Perren V, Scarano M, de la Rosa-Velazquez IA, van de Nobelen S, Shuker N, Popow J, Gerle B, Opravil S, Pagani M, et al. 2012. A transcription factor–based mechanism for mouse heterochromatin formation. Nat Struct Mol Biol 19: 1023–1030.

Burns KH. 2017. Transposable elements in cancer. Nat Rev Cancer 17: 415–424.

Castellano-Pozo M, Garcia-Muse T, Aguilera A. 2012. R-loops cause replication impairment and genome instability during meiosis. EMBO Rep 13: 923–929.

Chan YA, Aristizabal MJ, Lu PY, Luo Z, Hamza A, Kobor MS, Stirling PC, Hieter P. 2014. Genome-wide profiling of yeast DNA:RNA hybrid prone sites with DRIP-chip. PLoS Genet 10: e1004288.

Chapman MS, Verma IM. 1996. Transcriptional activation by BRCA1. Nature 382: 678–679.

Chiolo I, Minoda A, Colmenares SU, Suvrav P, Cassidy A, Zakopoulou R, Kotsinas A, Gorgoulis V, Field JK, Liloglou T. 2009. Hypomethylation of retrotransposon elements correlates with genomic instability in non-small cell lung cancer. Int J Cancer 124: 81–87.

Davidson L, Kerr A, West S. 2012. Co-transcriptional degrada-tion of aberrant pre-mRNA by Xrn2. EMBO J 31: 2566–2578.

de Koning AJ, Gu W, Castoe TA, Batzer MA, Pollock DD. 2011. Repetitive elements may comprise over-two-thirds of the hu-man genome. PLoS Genet 7: e1002384.

El Hage A, French SL, Beyer AL, Tollervey D. 2010. Loss of topoiso-merase 1 leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis. Genes Dev 24: 1546–1558.

Fang S, Miao J, Xiang L, Ponguoti B, Treuter E, Kemper JK. 2007. Coordinated recruitment of histone methyltransferase G9a and other chromatin-modifying enzymes in SH-Prerotransposon-mediated regulation of hepatic bile acid metabolism. Mol Cell Biol 27: 1407–1424.

Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, Ylstra B, Chubb JR, Bickmore WA. 2008. Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet 4: e1000039.

French S. 1992. Consequences of replication fork movement through transcription units in vivo. Science 258: 1362–1365.

Ganko EW, Fielman KT, McDonald JF. 2001. Evolutionary his-tery of Ccr elements and their impact on the C. elegans genome. Genome Res 11: 2066–2074.

Garcia-Bassets I, Kwon Y-S, Telese F, Prefontaine GG, Hutt KR, Cheng CS, Ju B-G, Ohgi KA, Wang J, Escoubet-Lozach L. 2007. Histone methylation-dependent mechanisms impose li-gand dependency for gene activation by nuclear receptors. Cell 128: 505–518.

Gerstein MB, Lu ZJ, Van Nostrand EL, Cheng C, Arshinoff K, et al. 2010. Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. Science 330: 1775–1787.

Grewal SI, Lott PL, Christensen HC, Korf I, Chédin F. 2012. The R-loop formation is a distinctive characteristic of unmethylated human CpG island promoters. Mol Cell 45: 814–825.

Gonzalez-Sandoval A, Towbin BD, Kalick V, Cabianca DS, Gai-datzis D, Hauer MH, Geng L, Wang L, Yang T, Wang X, et al. 2015. Perinuclear anchoring of H3K9-methylated chromatin stabilizes induced cell fate in C. elegans embryos. Cell 163: 1333–1347.

Grewal SI, Rice JC. 2004. Regulation of heterochromatin by histone methylation and small RNAs. Curr Opin Cell Biol 16: 230–238.

Gruenbaum Y, Cedar H, Razin A. 1982. Substrate and sequence specificity of a eukaryotic DNA methylase. Proc Natl Acad Sci 79: 620–622.

Hamperl S, Cimprich KA. 2014. The contribution of co-transcriptional RNA:DNA hybrid structures to DNA damage and genome instability. DNA Repair (Amst) 19: 84–94.

Hancks DC, Kazazian HH Jr. 2016. Roles for retrotransposon insertions in human disease. Mob DNA 7: 9.

Hansen RS, Thomas S, Sandstrom R, Canfield TK, Thurman RE, Weaver M, Dorschnier MO, Gartler SM, Stamatoyannopoulos JA. 2010. Sequencing newly replicated DNA reveals widespread plasticity in human replication timing. Proc Natl Acad Sci 107: 139–144.
GENOME INSTABILITY ARISING FROM SIMPLE REPEAT EXPRESSION

Hatchi E, Skourtii-Thatchaki K, Ventz S, Pinello L, Yen A, Kamieniarz-Gudela K, Dimitrov S, Pathanitsa S, McKinney KM, Eaton ML. 2010. BRCA1 recruitment to transcriptional pause sites is required for R-loop-driven DNA damage repair. Mol Cell 37: 636–647.

Hauer MH, Seebier A, Singh V, Thierry R, Sack R, Amitai A, Kryzanovska M, Eglinger J, Holcman D, Owen-Hughes T, et al. 2017. Histone degradation in response to DNA damage enhances chromatin dynamics and recombination rates. Nat Struct Mol Biol 24: 99–107.

Haynes KA, Caudy AA, Collins L, Elgin SC. 2006. Element 1360 and RNA components contribute to H1-dependent silencing of a pericentric reporter. Curr Biol 16: 2222–2227.

Helmrich A, Ballarino M, Tora L. 2011. Collisions between replication and transcription complexes cause common fragile site instability at the longest human genes. Mol Cell 44: 966–977.

Hoffman EA, McCulley A, Haarer B, Arnak R, Feng W. 2015. Break-seq reveals hydroxymethylation-induced chromosome fragility as a result of unscheduled conflict between DNA replication and transcription. Genome Res 25: 402–412.

Hong Z, Jiang J, Lan L, Nakajima S, Kanno S-I, Koseki H, Yasui A. 2008. A polycomb group protein, PHF1, is involved in the response to DNA double-strand breaks in human cell. Nucleic Acids Res 36: 2939–2947.

Huertas P, Aguilera A. 2003. Cotranscriptionally formed DNA: RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol Cell 12: 711–721.

Janssen A, Breuer GA, Brinkman EK, van der Meulen AI, Borden SV, van Steensel B, Bindra RS, LaRocque JR, Karpen GH. 2016. A single double-strand break system reveals repair dynamics and mechanisms in heterochromatin and euchromatin. Genes Dev 30: 1645–1657.

Jiang Y, Wang X, Bao S, Guo R, Johnson DG, Shen X, Li L. 2010. INO80 chromatin remodeling complex promotes the removal of UV lesions by the nucleotide excision repair pathway. Proc Natl Acad Sci 107: 17274–17279.

Johnson NM, Lehmann BB, Tijsserman M. 2013. A role for the malignant brain tumour (MBT) domain protein LIN-61 in mammalian (cytosine-5) methyltransferases cause genomic DNA methylation and lethality in Drosophila. Nat Genet 35: 363–366.

Jorgensen M, Nakamura Y. 1992. Disruption of the APC gene by a retrotranspositional insertion of L1 sequence in a colon cancer. Cancer Res 52: 643–645.

Miller KM, Tijerres JV, Coates J, Legube G, Polo SE, Britton S, Jackson SP. 2010. Human HDAC1 and HDAC2 function in the DNA damage-response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol 17: 1144–1151.

Moynan ME, Chiu JW, Koller BH, Jasins M. 1999. Brcal controls homology-directed DNA repair. Mol Cell 4: 511–518.

Nakamori M, Pearson CE, Thornton CA. 2011. Bidirectional transcription stimulates expansion and contraction of expanded (CTG)3(CAG) repeats. Hum Mol Genet 20: 580–588.

Narita M, Nunez S, Heard E, Narita M, Lin A W, Hearn SA, Spector DL, Hannon GJ, Lowe SW. 2010. Human HDAC1 and HDAC2 function in the DNA damage-response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol 17: 1144–1151.

Noma K, Allis CD, Grewal SI. 2001. Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Science 293: 1130–1135.

Okano M, Xie S, Li E. 1999. Chromatin remodeling and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet 19: 219–220.

Pakdek J, Zeller P, Gasser SM. 2015. Repeat DNA in genome organization and stability. Curr Opin Genet Dev 31: 12–19.

Papin C, Ibrahim A, Gart SL, Velt A, Stoll J, Jost B, Menini H, Bronner C, Dimitrov S, Hamiche A. 2015. Combinatorial DNA methylation codes at repetitive elements. Genome Res 25: 934–946.

Pearson CE, Nichol Edamura K, Cleary JD. 2005. The molecular basis of the organization of repetitive DNA-containing constitutive heterochromatin in mammals. Chromosome Res 25: 77–87.

Pجيلي H, Baner J, Proctor SL, Sugiuira R, Zhang R, Jakett M, et al. 2013. Switching genes to silent mode near DNA double-strand breaks. EMBO J 22: 198–207.

Lin Y, Dion V, Wilson JH. 2006. Transcription promotes contraction of CAG repeat tracts in human cells. Nat Struct Mol Biol 13: 179–180.

Liu B, Alberts BM. 1995. Head-on collision between a DNA replication apparatus and RNA polymerase transcription complex. Science 267: 1131–1137.

Liu T, Rechtsteiner A, Gelelhofer TA, Vieille A, Latorre I, Cheung MS, Ercan S, Ikegami K, Jensen M, Kolasinska-Zwierz P, et al. 2011. Broad chromosomal domains of histone modification patterns in C. elegans. Genome Res 21: 236–246.

Luijsterburg MS, Dinant C, Lans H, Staj J, Wierman E, Lagerwerf S, Warmerdam DO, Lindh M, Brink MC, Dobrucki JW. 2009. Heterochromatin protein 1 is recruited to various types of DNA damage. J Cell Biol 185: 577–586.

Lyko F, Ramsahoye BH, Kashevsky H, Tudor M, Mastrandelo MA, Orr-Weaver TL, Jaenisch R. 1999. Mammalian (cytosine-5) methyltransferases cause genomic DNA methylation and lethality in Drosophila. Nat Genet 23: 363–366.

Martsens JH, O’Sullivan RJ, Braunschweig U, Oprival S, Radolf M, Steinlein P, Jenuwein T. 2005. The profile of repeat-associated histone lysine methylation states in the mouse epigenome. EMBO J 24: 800–812.

Miki Y, Nishishio H, Horii A, Miyoshi Y, Utsunomiya J, Kinzler KW, Vogelstein B, Nakamura Y. 1992. Disruption of the APC gene by a retrotranspositional insertion of L1 sequence in a colon cancer. Cancer Res 52: 643–645.

Miller KM, Tijetters JV, Coates J, Legube G, Polo SE, Britton S, Jackson SP. 2010. Human HDAC1 and HDAC2 function in the DNA damage-response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol 17: 1144–1151.

Moynan ME, Chiu JW, Koller BH, Jasins M. 1999. Brcal controls homology-directed DNA repair. Mol Cell 4: 511–518.

Nakamori M, Pearson CE, Thornton CA. 2011. Bidirectional transcription stimulates expansion and contraction of expanded (CTG)3(CAG) repeats. Hum Mol Genet 20: 580–588.

Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. 2010. Human HDAC1 and HDAC2 function in the DNA damage-response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol 17: 1144–1151.
Toyama BH, Riggs AD. 1980. DNA methylation and gene function. Science 210: 604–610.

Rigby K, Lin HY, Tsouroula K, Tongino E, Singh H. 2008. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature 452: 243–247.

Rivera C, Gurard-Levin ZA, Almouzni G, Loyola A. 2014. Histone lysine methylation and chromatin replication. Biochim Biophys Acta 1839: 1433–1439.

Roy D, Lieber MR. 2009. G clustering is important for the initiation of transcription-induced R-loops in vitro, whereas high G density without clustering is sufficient thereafter. Mol Cell Biol 29: 3124–3133.

Ruault M, Dubarry M, Taddei A. 2008. Re-positioning genes to the nuclear envelope in mammalian cells: Impact on transcription. Trends Genet 24: 574–581.

Ryu T, Spatola B, Delabaere L, Bowlin K, Hopp H, Kunitake R, Razin A, Riggs AD. 1980. DNA methylation and gene function. Cell 154: 312–313.

Tippmann SC, Ivanek R, Gaidatzis D, Scholer A, Hoerner L, van Schübel D, Scalzo D, Kooperberg C, van Steensel B, Delrow J, Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. 2007. Histone lysine methylation is a marker of poor prognosis in stage IA non-small cell lung cancer. Clin Cancer Res 13: 5409–5418.

Santos-Pereira JM, Aguilera A. 2015. R loops: New modulators of genome dynamics and function. Nat Rev Genet 16: 383–397.

Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G. 2002. Central role of Drosophila H3K9 methylation and heterochromatic gene silencing. EMBO J 21: 1121–1131.

Schübeler D. 2004. Genome-wide DNA replication profile for Drosophila melanogaster: A link between transcription and replication timing. Nat Genet 32: 438–445.

Scully R, Chen J, Plag A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM. 1997. Association of BRCA1 with Rad51 replication timing. EMBO J 16: 2137–2148.

Sienk G, Dörentas D, Brennecké J. 2012. Transcriptional silencing of trimotors by Pw1 and maelstrom and its impact on chromatin state and gene expression. Cell 151: 946–980.

Sollier J, Stork CT, García-Rubio ML, Paulsen RD, Aguilera A, Cimprich KA. 2014. Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability. Mol Cell 56: 777–785.

Somech R, Shalaki S, Geller O, Amargilio N, Simon AJ, Rechavi G, Gal-Yam EN. 2005. The nuclear-envelope protein and transcriptional complex L1BP interferes with HDAC3 at the nuclear periphery, inducing histone H4 deacetylation. J Cell Sci 118: 4017–4027.

Strahl BD, Allis CD. 2000. The language of covalent histone modifications. Nature 403: 41–45.

Sun Y, Jiang X, Xu Y, Ayrapetov MK, Moreau LA, Whetstine JR, Price BD. 2009. Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. Nat Cell Biol 11: 1376–1382.

Tanajeva N, Zofall M, Balachandran V, Thillainadesan G, Sugiyama T, Wheeler D, Zhou M, Grewal SI. 2017. SNF2 Family protein Frh3 suppresses nucleosome turnover to promote epigenetic inheritance and proper replication. Mol Cell 66: 50–62 e56.

Taverna SD, Li H, Runhenburg AJ, Allis CD, Patel DJ. 2007. How chromatin-binding modules interpret histone modifications: Lessons from professional pocket pickers. Nat Struct Mol Biol 14: 1025–1040.

Thomas M, White RL, Davis RW. 1976. Hybridization of RNA to double-stranded DNA: Formation of R-loops. Proc Natl Acad Sci USA 73: 2294–2298.

Tippmann SC, Ivanek R, Gaidatzis D, Scholer A, Hoerner L, van Nimwegen E, Stadler PF, Stadler MB, Schübeler D. 2012. Chromatin measurements reveal contributions of synthesis and decay to steady-state mRNA levels. Mol Syst Biol 8: 593.

Toyama BH, Savas JN, Park SK, Harris MS, Ingolia NT, Yates JR 3rd, Hetzer MW. 2013. Identification of long-lived proteins reveals exceptional stability of essential cellular structures. Cell 154: 971–982.

Tsouroula K, Furst A, Rogier M, Heyer V, Maglott-Roth A, Ferrand A, Reina-San-Martin B, Soutoglou E. 2016. Temporal and spatial uncoupling of DNA double-strand break repair pathways within mammalian heterochromatin. Mol Cell 63: 293–305.

Tsurumi A, Dutta P, Yan S-J, Shang R, Li WX. 2013. Drosophila Kdm4 demethylases in histone H3 lysine 9 demethylation and ecdysteroid signaling. Sci Rep 3: 2894.

Turbo JM, Li Y, Ju YS, Martincenova I, Cooke SL, Tojo M, Gundem G, Pipnikas CP, Zamora J, Raine K, et al. 2014. Mobile DNA in cancer. Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes. Science 345: 1251343.

Ui A, Nagaura Y, Yasui A. 2015. Transcriptional elongation factor ENL phosphorylated by ATM recruits polycomb and switches off transcription for DSB repair. Mol Cell 58: 468–482.

van Attikum H, Fritsch O, Hohn B, Gasser SM. 2004. Recruitment of the INO10 complex by I2A phosphorylation links AT-dependent chromatin remodeling with DNA double-strand break repair. Cell 119: 777–788.

van Attikum H, Fritsch O, Gasser SM. 2007. Distinct roles for SWR1 and INO10 chromatin remodeling complexes at chromosomal double-strand breaks. EMBO J 26: 4113–4125.

Vissers JH, van Lohuizen M, Citterio E. 2012. The emerging role of Polycomb repressors in the response to DNA damage. J Cell Sci 125: 3939–3956.

Vos JC, De Baere I, Plasterk RH. 1996. Transposase is the only nematode protein required for in vitro transcription of Tcl. Genes Dev 10: 755–761.

Wahl L, Costantino L, Tan LJ, Zimmer A, Koshland D. 2016. S1-DRIP-seq identifies high expression and polyA tracts as major contributors to R-loop formation. Genes Dev 30: 1327–1338.

Wang H, Zhai L, Xu J, Liu HY, Jackson S, Erdjument-Bromage H, Tempst P, Xiong Y, Zhang Y. 2006. Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. Mol Cell 22: 383–394.

Wang Y, Khan A, Marks AB, Smith OK, Giri S, Creager M, MacAlpine DM, Prasanth KV, Aladjem MI, et al. 2017. Temporal association of ORCA/LRDW1 to late-firing origins during G1 dictates heterochromatin replication and organization. Nucleic Acids Res 45: 2490–2502.

Watt F, Molloy PL. 1988. Cytosine methylation prevents binding to DNA of a HeLa cell transcription factor required for optimal expression of the adenovirus major late promoter. Genes Dev 2: 1136–1143.

Weber M, Davies JJ, Wittig D, Oakley EJ, Haase M, Lam WL, Schubeler D. 2005. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 37: 853–862.

Weintraub H, Groudine M. 1976. Chromosomal subunits in active genes have an altered conformation. Science 193: 848–856.

West S, Gromak N, Proudfoot NJ. 2004. Human 5′→3′exonuclease Xrn2 promotes transcription termination at co-transcriptional cleavage sites. Nature 432: 522–525.

Wierdl M, Greene CN, Datta A, Jinks-Robertson S, Petes TD. 1996. Destabilization of simple repetitive DNA sequences by transcription in yeast. Genetics 143: 713–721.

Ye Q, Worman HJ. 1996. Interaction between an integral protein of the nuclear envelope inner membrane and human chromo-domain proteins homologous to Drosophila HP1. J Biol Chem 271: 14653–14656.

Zeller P, Padenek J, van Schendel R, Kalck V, Tijsterman M, Gasser SM. 2016. Histone H3K9 methylation is dispensable for Caenorhabditis elegans development but suppresses RNA: DNA hybrid-associated repeat instability. Nat Genet 48: 1385–1395.

Zhao Q, Wang QE, Ray A, Wani G, Han C, Milum K, Wani AA. 2009. Modulation of nucleotide excision repair by mammalian SWI/SNF chromatin-remodeling complex. J Biol Chem 284: 30424–30432.

Zhu Q, Pao GM, Huyhn AM, Suh H, Tommu N, Nederlof PM, Gage FH, Verma IM. 2011. BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. Nature 477: 179–184.