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# Transcription-Coupled DNA Double-Strand Break Repair: Active Genes Need Special Care

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## Abstract

For decades, it has been speculated that specific loci on eukaryotic chromosomes are inherently susceptible to breakage. The advent of high-throughput genomic technologies has now paved the way to their identification. A wealth of data suggests that transcriptionally active loci are particularly fragile and that a specific DNA damage response is activated and dedicated to their repair. Here, we review current understanding of the crosstalk between transcription and double-strand break repair, from the reasons underlying the intrinsic fragility of genes to the mechanisms that restore the integrity of damaged transcription units.

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## Introduction

Chromosomes are exceptionally long molecules that must be faithfully replicated and segregated during each cell cycle to provide genetic information to daughter cells. A large body of evidence supports the idea that the DNA double helix is irregular: it can form non-canonical structures such as R-loops (three-stranded structures composed of RNA:DNA hybrids and single-stranded DNA), hairpins, G-quadruplex (G4), and underwound or over-twisted DNA helices that are further translated into negative and positive supercoiling [1]. Conversely, negative supercoiling destabilizes the DNA helix, favoring the formation of these atypical DNA structures [2]. In eukaryotes, DNA associates with over half a thousand of proteins to form chromatin, which adopts multiple conformational states from the linear “beads on a string” nucleosomal fiber to more complex structures such as chromatin loops and topologically associated domains [2]. The transcription, replication, and repair machineries must cope with this great variety of secondary and tertiary structures if they are to accurately execute transcriptional programs and maintain genome integrity.

It has been known for decades that some genomic loci are particularly prone to breakage and instability, but it is only very recently that, owing to high-throughput genomic techniques, their positions have been determined at near-nucleotide resolution. Importantly, these

studies identified active genes as particularly fragile and thus the theater of dedicated repair events. In this review, we discuss our current understanding of the crosstalk between transcription and double-strand break (DSB) repair, with a particular focus on recent evidence that (i) points to transcriptional activity as a major threat to the genome and (ii) sheds light on the DNA damage response at active gene.

## Transcription as a Threat to Genome Stability

### Genome-wide mapping of fragile sites and DSB-prone loci

In the 70s, cytological studies of metaphase chromosome spreads indicated that dividing cells experience recurrent DNA breakage events at certain positions [3]. These “common fragile sites” (CFS), which arise upon mild replication stress, were first defined as chromosomal bands with significantly elevated break/gap frequencies on mitotic chromosomes. They were later mapped with higher resolution using fluorescence *in situ* hybridization. CFS have been subjected to intense investigation, as they coincide with translocation break points and are hotspots for gross chromosomal rearrangements in

cancer cells (for a review, see Ref. [4]). It has long been supposed that their fragility results from incomplete replication before segregation. Repli-Seq and ChIP-seq analyses demonstrated that CFS are devoid of replication origins, are replicated late, overlap with very long genes (>300 kb) [5–8], and accumulate DSB repair proteins [9]. Moreover, it also became clear that CFS expression (i.e., frequency of breakage) is tissue-specific [10,11]. This indicates that CFS are epigenetically defined rather than just sequences inherently difficult to replicate. Accordingly, CFS instability coincides with the expression of the underlying gene [7]. Since these very long genes need more than one cell cycle to be entirely transcribed, it has been proposed that the collision between the replication and transcription machineries could lead to the slowing or stalling of replication forks [7]. Nevertheless, since not all expressed long genes are prone to breakage [10,12], instability might rather result from secondary DNA structures and/or specific chromatin features assembled on a subset of these very long active genes.

ChIP-seq analyses of DNA-bound RPA,  $\gamma$ H2AX, and BRCA1 following hydroxyurea treatment led to the identification of another class of fragile sites, named early replicating fragile sites (ERFS) [13]. ERFS are replicated early, and like CFS, their instability (detected as gaps on mitotic chromosomes) is cell-type-specific. Notably, these regions are highly transcribed, and it is this transcriptional activity rather than the timing of replication that promotes their instability [13]. Importantly, binding of BRCA1 and RPA at ERFS is also detected without hydroxyurea treatment, suggesting that breakage at these sites occurs to some extent during normal replication [13]. Although not directly linked to the occurrence of DNA damage, it has to be noted that other genome-wide profiles also revealed the accumulation of repair proteins at transcriptionally active loci (P-DNAPK, BRCA1, PALB2) [14–16].

Novel sequencing-based techniques that allow the direct mapping of DSBs on the genome at near-nucleotide resolution have recently provided insights in the recurrent DSB landscape in different cell types. The ability of endogenous DSBs to translocate to a “bait” DSB (introduced by either Cas9 or I-SceI endonucleases) is the basic principle of high-throughput genome-wide translocation sequencing (HTGTS) and translocation capture sequencing (TC-Seq) techniques. These techniques mapped recurrent DSBs in B-cells and neuronal stem progenitor cells [17–22]. Combined with global run-on experiments to assess the ongoing transcription at the whole-genome level, these studies showed that (i) upon mild replicative stress (and to a lesser extent in unchallenged cycling cells), clusters of DSBs occur in long active genes that replicate late [22], and (ii) DSB frequency is generally higher in nucleosome-depleted regions at transcriptional start sites of active genes and is proportional to transcription rate [17,18,21]. BLESS [23], Break-seq

[24], END-seq [25], and DSBCapture [26] have been further developed as more direct approaches to mapping DSBs at the genomic scale *in vivo*. Importantly, DSBCapture and BLESS recently allowed, for the first time, the visualization of DSB induction at active promoters [26,27]. Similarly, BLESS allowed the clear identification of long genes as preferential sites of DSB following replication stress [23].

Taken together, these studies support the existence of two classes of fragile sites that recurrently experience DSB: (1) long, active genes, which replicate late (CFS), and are particularly susceptible to replicative stress; and (2) promoters of transcriptionally active genes that replicate early.

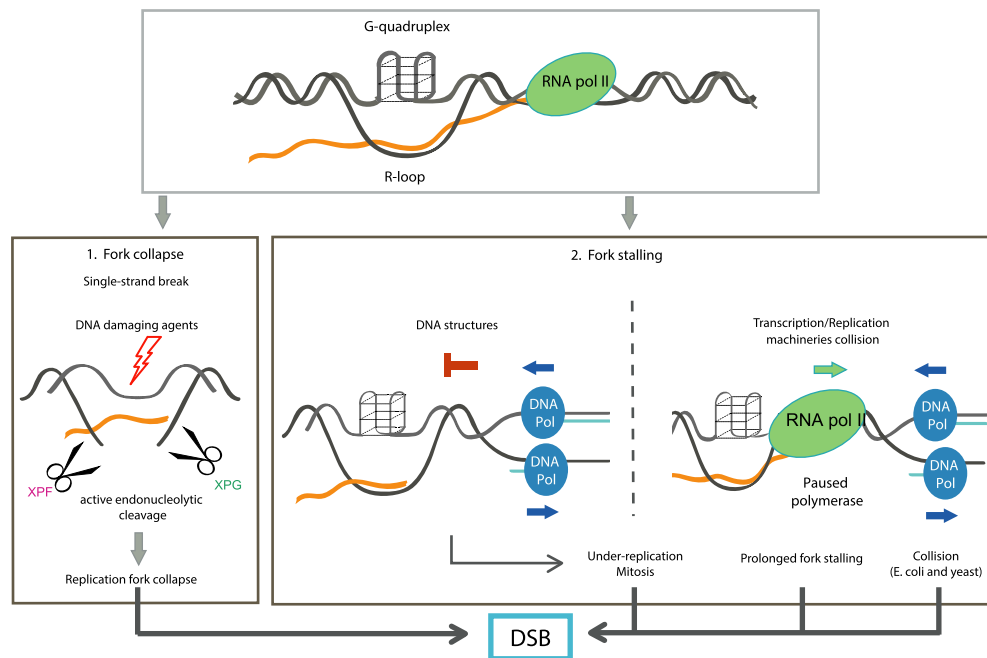
### Molecular mechanisms underlying the fragility of active genes: R-loop and G4-driven DSBs

The molecular mechanisms underlying ERFS and CFS fragility, and by extension, the susceptibility of active genes to breakage, are still not fully understood. At CFS, late firing and scarcity of replication origins have been proposed to generate under replicated DNA that would trigger a DSB at the next mitosis, in a manner that depends on the Mus81 structure-specific endonuclease [28–31]. However, this mechanism unlikely applies to ERFS that replicate early and so have more time to complete replication.

An alternative is suggested by recent work, which indicated that two potentially interlinked structures, R-loops and G4s, contribute to genomic instability (for reviews, see Refs. [32–34]). R-loops are three-stranded RNA:DNA hybrids that can form as RNA polymerases progress through duplex DNA, by hybridization of the nascent RNA to the template DNA strand [35,36] (for a review, see Ref. [32]). DNA:RNA hybrid immunoprecipitation (DRIP) using S9.6 antibody followed by next generation sequencing (DRIP-seq or DRIPc-seq) has recently allowed their genome wide profiling at a near-nucleotide resolution and in a strand-specific manner [37–39]. These studies revealed that R-loops form co-transcriptionally and accumulate at promoters that carry transcriptionally active marks. Most R-loops form at sites exhibiting GC-skew (increased G over C on the non-template strand) due to increased thermodynamic stability of G-rich RNA hybridized with C-rich DNA strands [37–39]. In parallel, the first genome-wide mapping of G4s, four-stranded non-canonical DNA structures, which arise at G-rich loci, revealed that they form at transcriptionally active promoters [40]. This raises the exciting possibility that G4 may arise on displaced G-rich DNA strand at R-loops. This structure termed G-loop has first been proposed to happen on the immunoglobulin locus and to contribute to class switch recombination [41] and later found to occur *in vitro* and on plasmid DNA in *Escherichia coli* [42]. One attractive hypothesis is that upon transcription activation at GC-skewed

promoters, DNA unwinding facilitates the formation of these coupled R-loop/G4 structures. On one hand, G-loops would favor transcription by stabilizing the initiation bubble. On the other hand, they could also trigger fragility if not removed appropriately. Genetic and biochemical analyses, cytogenetics of chromosome spreads, and staining or mapping of DSB markers in organisms ranging from bacteria to higher eukaryotes have provided a wealth of evidence that R-loop accumulation provokes DSB (for example, see Refs. [7,43–51]). The persistence of R-loops was also recently shown to impede replication at CFS [52], suggesting that R-loops may be an important determinant of CFS instability. Similarly, G4 structures impede replication fork progression and threaten genome integrity if not properly unfolded during replication (for reviews, see Refs. [33,34]). R-loop and/or G4-driven DSB could arise in a replication-dependent manner through several non-exclusive mechanisms (Fig. 1). First, R-loop and G4 formation result in an unannealed DNA strand, which is more sensitive to damaging agents. This could lead to a higher frequency of single-strand breakage (SSB), leading to fork collapse and DSB formation during subsequent replication (reviewed in Refs. [32,53,54]). In addition, such nicks may also be

produced by active endonucleolytic cleavage. Indeed, Stork *et al.* and Sollier *et al.* demonstrated that XPG and XPF, known to generate ssDNA gaps during transcription-coupled nucleotide excision repair (TC-NER), are required for DSB induction at R-loop-forming loci [49,50] (Fig. 1, left). This XPF/XPG-dependent R-loop processing may happen independently of replication but may also be coordinated with DNA replication as a mean to remove secondary structures counteracting DNA polymerase progression. Second, R-loop/G4 structures may interfere directly with the progression of the replication fork and/or cause RNA polymerase pausing, which itself can impede fork progression (Fig. 1, right). Fork deceleration or stalling upon encountering these roadblocks would give rise to under-replicated DNA, further triggering DNA breakage at the following mitosis in a nuclease-dependent manner [29–31]. Moreover, prolonged fork stalling can also itself trigger nuclease-induced breaks and fork collapse (for a review, see Ref. [55]). Notably, NER factors might also contribute to generate these breaks, as the replication machinery encounters R-loop structures [49,50]. Finally, collision between replication and transcription machineries in either head-on (converging) or co-directional orientations can directly generate DSBs in *E. coli* [56,57] (for reviews, see



**Fig. 1.** Possible mechanisms for R-loop/G4-driven DSB production. R-loops and G4 accumulate preferentially at transcriptionally active genes, mainly in nucleosome-depleted promoter regions. Both structures provoke replication-dependent DSB formation. Left panel: Displaced single-stranded DNA is more sensitive to DNA-damaging agents. In addition, R-loops can be targeted by the endonucleases XPG and XPF, components of the TC-NER machinery that generates a single-strand gap. In both cases, SSBs lead to fork collapse and DSB generation during replication. Right panel: G4 and/or R-loops can directly interfere with the progression of the replication fork, provoking DNA polymerase retardation or stalling. Under replicated DNA further experiences breakage during mitosis in a manner that depends on structure-specific endonucleases. These structures could also trigger RNA polymerase pausing that can collide with the replication machinery. Such collision can directly trigger DSB production as shown in *Escherichia coli*.

Refs. [54,58,59]), providing an additional means of generating DSBs during the replication of R-loop/G4-forming loci.

In addition to this replication-dependent R-loop/G4-driven DSB induction, these structures can also trigger DSB in non-dividing cells by less well-characterized mechanisms in *E. coli* [47] and mammalian post-mitotic cells [44,60].

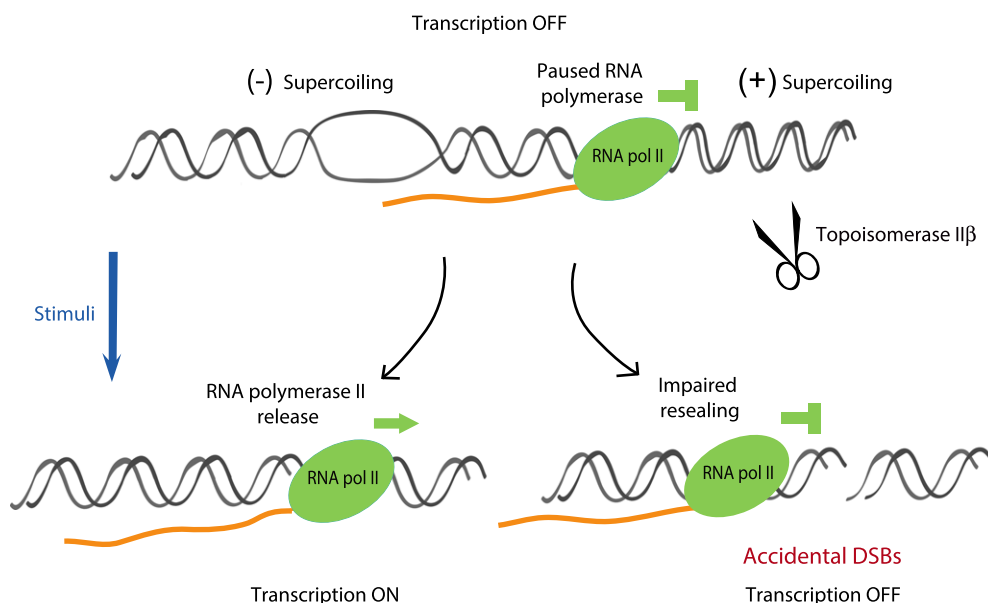
### Molecular mechanisms that underlie the fragility of active genes: Topoisomerase-II-driven DSB

Beyond the contribution of R-loops and G4 to DSB production at active genes, there is evidence that transcription activation itself can trigger DSB formation (Fig. 2). Both estrogen and androgen stimuli trigger the appearance of DSBs at early responsive genes [49,61–64]. Similarly, stimulation of neuronal activity leads to DSB induction not only in a subset of immediate responsive genes in primary cultured neurons but also in normal mouse brains following fear conditioning or novel environment exploration [65,66]. Finally, heat shock or serum stimulation also rapidly triggers DSB in early responsive genes [16]. While estrogen-induced DSBs appear as cells progress into S phase and may be replication-dependent [49,64], serum-induced DSBs appear within the first 15 min after reentry into the cell cycle [16]. Together with the fact that stimulus-induced DSBs can occur in adult mouse brains (in post-replicative cells) [66], this observation strongly

suggests that damage induction following stimulation can also occur in a replication-independent manner. Notably, these breakage events depend on topoisomerase II $\beta$  (TOP2B) activity [16,61,62,64,65]. Given that DNA torsional stress impedes RNA polymerase elongation, a current model proposes that TOP2B-mediated DNA breakage would release topological constraints, thereby allowing RNA Polymerase II (RNA Pol II) to escape from its pause site and engage in an elongation mode (Fig. 2) (for a review, see Ref. [54]). It is likely that DSBs arise at these genes following impaired resealing of TopoII intermediates. Application of technologies such as HTGTS, BLESS, End-Seq, or DSBCapture to mapping DSBs in different cell types in response to various stimuli should soon allow the determination of the frequency of active promoter-associated DSBs and their exact positioning relative to R-loop/G4 structures and to TOP2B and RNA Pol II binding sites.

### Towards “Transcription-Coupled DSB Repair”: Specific DSB Repair Pathways Take Place at Active Genes

While further investigation is required to determine whether DSB production is an accidental by-product of transcription activation or a necessity for RNA Pol II release following stimulation, the aforementioned studies clearly show that the DSB landscape is strongly biased toward active genes. This raises the



**Fig. 2.** Topoisomerase-II-dependent DSB induction upon transcription stimulation. A large number of genes exhibit RNA polymerase pausing that contributes to transcription regulation. Upon certain stimuli, the release of paused RNA polymerase II into the gene body may require topoisomerase II $\beta$  (TOP2B) activity. DSBs might accidentally arise following impaired resealing of TOP2B intermediates.

question of how cells cope with breaks that would be particularly detrimental since they occur in what can be regarded as the most important parts of the genome.

Investigating how the transcriptional status of a damaged locus influences the repair reaction and how transcription is regulated at genes that experience a DSB have proved to be extremely challenging since it requires the induction of DSBs at specific, known loci on the genome. During the past decade, most studies have been performed using irradiation ( $\gamma$ -rays, X-rays, heavy ions) or drugs (topoisomerase poisons, intercalating agents...), which induce (i) uncharacterized damage beyond the DSB, including SSBs and various DNA adducts; (ii) damage at random, unknown positions on the genome; and (iii) damage at various stages of the cell cycle. This wide variety of DSB-inducing methods has blurred the overall picture and precluded any analysis of repair pathway preference throughout the genome and of transcription regulation at damaged genes. Consequently, much effort has been made to develop systems where sequence-specific and annotated DSBs can be induced on the genome. In yeast, this was already possible in the 80s, owing to the use of HO endonuclease combined with the possibility of engineering the genome [67,68]. In higher eukaryotes, the first accurate and controlled method of inducing a single DSB at a specific locus was devised by the laboratory of Maria Jasin in 1994. To accurately quantify homologous recombination (HR) events, they introduced into the mouse genome a transgene that carries the recognition site for the endonuclease I-SceI within a GFP reporter system [69]. This methodology has been superseded by the use of restriction enzymes, Zinc-finger nucleases, and, more recently, CRISPR/Cas9 technology [70–73], which allow the induction of sequence-specific DSBs at endogenous locations and anywhere in the genome.

### Transcription extinction and recovery at damaged active genes

One of the consequences of DSB production in active genes is the rapid extinction of transcription at sites of damage (recently reviewed in Ref. [74]). Transcription inhibition occurs at the damaged gene [70,71,75–80] and can also spread a few kilobases away from the DSB [71,80], although not over the entire megabase-wide  $\gamma$ H2AX domain [70,79,81,82]. Transcriptional repression is an active process that relies on ATM signaling, ubiquitination of H2A lysine K119, which is a well-known repressive histone mark established by Polycomb group proteins, and histone deacetylation by the NuRD complex [71,77,83,84] (Fig. 3, right panel). Importantly, this transcriptional shut-down is tightly linked to the completion of repair of damaged genes [77,83–85], suggesting that it may help “clean” the damaged locus to enable repair activity. This proposal agrees with the finding that transcriptional extinction is correlated with the disap-

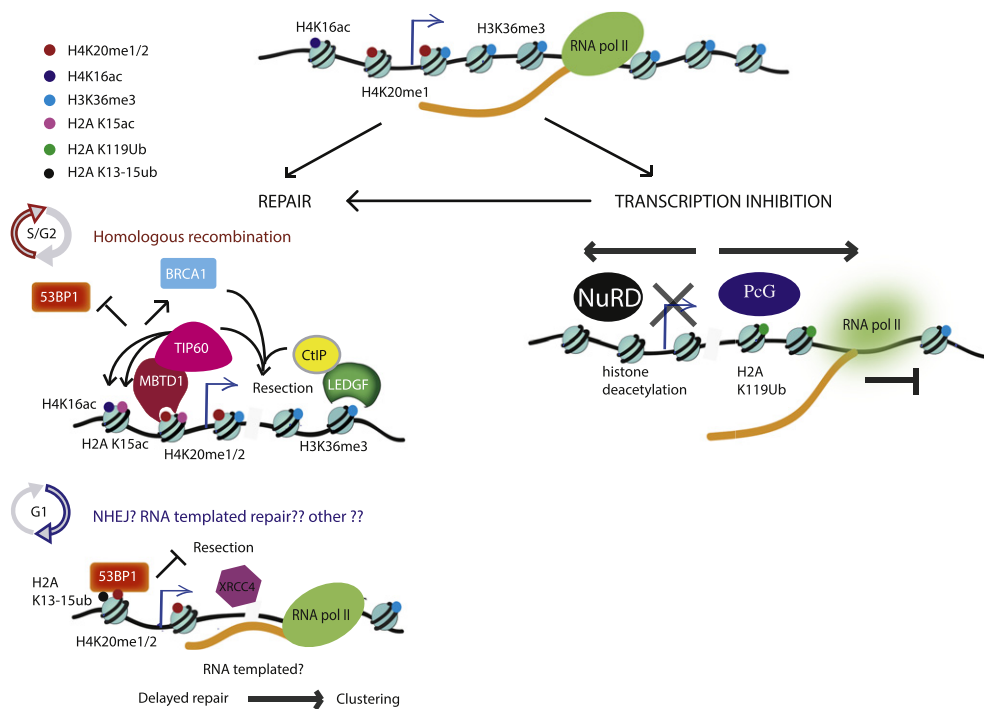
pearance of RNA Pol II from the broken gene, which may rely on proteasome activity [75]. It is also consistent with the observation that in yeast, transcription inhibition spreads over flanking genes as resection proceeds [78–80], even if this might not be the case for mammalian cells [71,77].

Once repair has been achieved, transcription must be suitably revived to maintain cell fate. A recent study indicates that transcription of the repaired gene recovers normally in non-dividing cells, indicating that cell cycle progression is not required for the restoration of the epigenetic information and for the resumption of transcription [82]. Transcription recovery at DSB-flanking genes was shown to take place within 2 h after the termination of DSB induction and to require the deubiquitinylation of H2A-K119 by USP16 [71]. At present, little is known about these essential steps, probably because investigating transcription recovery at damaged genes has been challenging. The recent development of tools that not only permit DSB induction at controlled loci but also repair completion, owing to the availability of degradable or reversible enzymes [71,86,87], should now enable more rapid advance.

### Repair pathways influence faithful sequence recovery at damaged genes

Beyond the transcriptional regulation of damaged active genes, the cell's most important challenge is to accurately recover genetic information at these loci.

Multiple, partly redundant, and extremely well-conserved repair pathways exist in eukaryotes (reviewed in Ref. [88]). They are usually classified into two major groups: non-homologous end joining (NHEJ) and Homologous Recombination (HR). NHEJ promotes the direct ligation of the two DNA ends with minimal processing, while HR accomplishes repair using an intact copy of the broken locus (mostly the sister chromatid) as a template. To this end, HR relies on a process called resection, during which endo- and exonucleases generate single-strand DNA required for D-loop formation with the template DNA. D-Loop establishment is followed by DNA synthesis, tightly coupled with dissolution/resolution mechanisms, which determine the output of HR and give rise to crossover or non-crossover products. Since this initial classification, several alternative mechanisms have been discovered, which soften the distinction between these two major pathways. Single-strand annealing initially resembles HR, resecting to form single strands, but then uses an illegitimate homologous copy *in cis* to anneal and directly reseal the two resected ends. Similarly, alternative NHEJ relies on short-range resection to expose microhomologies, which are further used to synapse and ligate the break (therefore also known as microhomology-mediated end joining). In yeast, break-induced replication initiates a non-canonical replication fork, which can proceed to the end of the



**Fig. 3.** Transcription-coupled DSB repair. Left panel: Transcriptionally active genes are subjected to specific repair. In G<sub>2</sub>, homologous recombination is specifically targeted at transcribing genes, in a manner that largely depends on chromatin features associated with active transcription. Trimethylation of histone H3 on lysine 36 (H3K36me<sub>3</sub>), which is correlated with elongating RNA Pol II, recruits CtIP via LEDGF, allowing the initiation of resection and HR repair. In addition, acetyl H4K16, a histone mark enriched in active regions, interferes with the binding of 53BP1 to H4 methylated on lysine 20, hence promoting BRCA1 binding and resection. Finally MBTD1, part of the TIP60 complex, competes with 53BP1 for binding to H4K20me. DSB-recruited TIP60 acetylates H2A on lysine 15 (H2AK15ac) in G<sub>2</sub>, counteracting its ubiquitination and thereby further destabilizing 53BP1. Together, these chromatin marks favor resection, RAD51 loading, and HR repair. In G<sub>1</sub>, specific repair events that occur on active gene are still under characterization. Tip60 does not acetylate H2AK15, allowing its ubiquitination that, together with H4K20me, stabilizes 53BP1 and inhibits resection. Although XRCC4 is recruited, these DSBs exhibit a strong repair delay and undergo clustering. RNA might also participate in the repair reaction as a patch to synapse the two DNA ends or as a template for Rad52-dependent repair. Right panel: Gene transcription is inhibited locally following DSB induction, while transcription is maintained farther away within the  $\gamma$ H2AX domains. Transcriptional repression is achieved by the eviction of RNA Pol II from the damaged gene and possibly via its degradation, as well as multiple chromatin modifications, including histone deacetylation by the NuRD complex and Polycomb dependent ubiquitination of H2A at lysine 119.

chromosome and generate complex chromosome rearrangements. More recently, it has been proposed that RNA-templated repair also occurs in yeast and possibly in higher eukaryotes (reviewed in Ref. [89]). All these repair mechanisms coexist and can give rise to a multitude of genomic scars, such as point mutations, translocations, and even chromothripsis, a massive chromosomal reshuffling observed in cancer. The choice between these pathways at DSBs induced in active genes is therefore critical, as it will clearly determine the quality of the repair event and thus the subsequent functionality of the damaged gene.

### A role for HR in repairing active genes

In the late 80s, a study from the Thomas and Rothstein established that spontaneous recombination between duplicated sequences inserted in the yeast *GAL10* gene was strongly enhanced when *GAL10* was

transcribed. The authors had already discussed the “avant-garde” possibility that chromatin status may contribute to this elevated recombination rate [90]. Similarly, mammalian cell studies led to the conclusion that transcription enhances recombination rate by a process termed “transcription-associated recombination” (TAR) [91,92]. In yeast and bacteria, an increase of mutation rate was also observed in active genes (also known as TAM or transcription-associated mutagenesis; for a review, see Ref. [93]); hence, TAR was attributed to higher damage frequency in genes (for a review, see Ref. [94]). However, recent work, described below, also raises the possibility that TAR may be related to repair mechanisms specifically set up at active genes.

Analysis of the fate of an HO break introduced at dedicated position on the yeast genome revealed that an active gene exhibits faster repair than an inactive gene, supporting the existence of a

“transcription-coupled DSB repair” pathway [95]. Analyses of repair in higher eukaryotes at an I-SceI site close to inducible promoters showed that transcription activity generally did not strongly affect HR and NHEJ pathway usage or outcome [92,96,97]. However, the inherently transgenic nature of this single I-SceI-targeted locus called for further work to compare repair events that take place at different endogenous loci on the genome. ChIP-seq analyses of the distribution of HR and NHEJ proteins at DSBs introduced by the AsiSI restriction enzyme throughout the genome [70,98] revealed that among AsiSI-induced DSBs, mostly those lying within or close to transcriptionally active loci (e.g., promoters) could recruit the HR factor RAD51 [86]. This result clearly demonstrated that damaged genes that are active exhibit a preference for HR repair compared to other sequences located in euchromatin (AsiSI activity being inhibited by DNA methylation, these studies did not allow the investigation of repair at heterochromatin loci). Preference of transcribed loci for HR was further confirmed by inducing damage with Killer Red (a DNA-damaging agent activated by visible light) at a transcriptionally active or repressed cassette inserted in the genome [99].

### Chromatin-driven HR targeting to damaged active genes

Transcription itself does not seem to be responsible for HR recruitment at damaged active genes. It is rather the trimethylation of histone H3 on lysine 36 (H3K36me3), well known to be correlated with elongating RNA Pol II, that acts as a critical determinant for resection and RAD51 loading [86,100]. Accordingly, depletion of SetD2, the main histone methyltransferase responsible for H3K36me3 in human cells, strongly impedes HR repair [86,100,101]. H3K36me3 has been reported to be directly recognized by the PWWP domain of LEDGF, a protein that interacts with the CtIP resection factor [102]. Hence, these studies point toward a model in which H3K36me3 recruits CtIP at least in part via LEDGF, allowing the initiation of resection and the use of HR pathway at active genes (Fig. 3, left panel). Following another line of investigation, Tang *et al.* discovered that histone acetylation also regulates the repair outcome. Acetylation of histone H4 lysine16, a histone mark correlated with active genomic regions, counteracts the recruitment of 53BP1, an anti-resection factor, to H4 methylated on lysine 20 and favors the binding of BRCA1, a pro-resection factor [87]. Accordingly, Tip60 and hMof, two histone acetyltransferases with known activity on K16, have been shown to favor HR [87,103]. More recently, Tip60 was shown to acetylate H2A lysine K15 during G2, which precludes H2A ubiquitination on the same residue and so destabilizes 53BP1 binding at the break site [104,105] (Fig. 3, left panel). Taken together,

these studies reveal a complex regulatory network of multiple chromatin marks that fine-tunes repair outcomes by influencing the recruitment and stabilization of repair factors and identified chromatin status as central in HR recruitment at active genes (for a review, see Ref. [106]; Fig. 3, left panel).

### Other pathways are involved in active gene repair in G1

An additional layer of complexity in pathway usage is cell cycle dependence. Indeed, HR is strongly suppressed during the G1 phase [107], and HR preference at active genes only occurs during G2 [86]. This raises the question of how DSBs in active genes are repaired during G1 or in non-dividing cells (Fig. 3, left panel). NHEJ proteins are clearly recruited at DSBs induced in active genes throughout the cell cycle [84,86,99]. Accordingly, NHEJ repair proteins are associated with RNA Pol II (for example, see Refs. [108,109]). However, XRCC4 depletion does not affect repair kinetics at transcriptionally active regions [86], and Ku70 dissociates rapidly after its recruitment at a damaged transcribed locus, while HR proteins persist much longer [99]. This raises the possibility that although recruited, canonical NHEJ might not be entirely efficient at these loci. In agreement, a recent genome-wide analysis by BLESS of repair kinetics revealed that transcriptionally active genes are not fully proficient for repair in G1, in contrast to non-transcribed sequences on the genome [110]. Moreover, these delayed DSBs exhibit an increased ability to cluster (i.e., coalesce) as shown by high-resolution mapping of long-range contact using Capture Hi-C [110]. These unrepaired, damaged active genes assemble within foci that are reminiscent of the 53BP1/OPT bodies. Indeed, following breakage in mitosis, CFS, which occur in long and active genes, assemble within 53BP1 bodies. These bodies persist throughout the G1 phase and might be cleared during the following replication phase [111]. These data suggest that in G1, a suboptimal efficacy of NHEJ at active genes, combined with the unavailability of HR, could result in persistent DSBs that cluster and await the arrival of S phase to undergo repair by a still uncharacterized mechanism.

Thus, while HR seems to be preferentially used to repair active loci in G2, the contribution of other less well-characterized repair pathways at DSBs arising in active genes, especially during G1, needs further investigation. Among these elusive pathway(s), RNA-templated repair, which bypasses the need for a homologous DNA template and thus may occur in G1, is further discussed below.

### A direct function for RNA as template at active genes?

In addition to exhibiting specific chromatin patterns, active genes are distinguished from the rest of the



genome by their ability to produce RNA. It has recently emerged that RNA could serve as a template for HR repair (recently reviewed in Ref. [89]). In budding yeast, RNA oligonucleotides can be used as direct templates for a synthesis-dependent repair, probably involving the pol $\delta$  replicative polymerase [112]. Moreover, using a very elegant reporter system, the Keskin *et al.* demonstrated that a transcript produced *in cis* to the gene experiencing a DSB can aid repair not only indirectly via the formation of cDNA but also directly by providing a template for a Rad52-dependent repair mechanism [113]. However, the latter process was observed only in mutant yeast strains devoid of RNaseH1 and RNaseH2 and thus deficient in DNA:RNA hybrid processing, raising doubt as to whether this RNA-templated repair pathway occurs in normal cells. How RNA-templated DNA repair functions in yeast remains speculative. It could involve the use of the RNA molecule as a patch, which maintains the DNA ends in close proximity to facilitate their ligation or the RNA molecule could be used to extend the 3' end of the DSB by an as yet unknown polymerase. These findings challenge the traditional view that HR requires a DNA template for repair and highlight the notion that RNA, which is available at transcriptionally active genes, could be used to template DNA repair. RNA-dependent repair might also take place in higher eukaryotes: quiescent human cells use a RNA and RAD52-dependent mechanism for DSB repair [99], and NHEJ has been proposed to operate at active genes through an RNA-templated mechanism [114]. However, the massive amount of damage induced in the latter study (1 DSB/10 kb, based on the failure to PCR amplify 10-kb amplicons following drug treatment) means that confirmation of these findings following milder or more relevant genotoxic insults will be required. While direct evidence demonstrating the use of RNA as a template for repair in higher eukaryotes is still lacking, these studies open the possibility that RNA-templated repair is a conserved alternative pathway for repairing regions of the genome in the course of transcription (Fig. 3). Interestingly, small non-coding double-stranded RNAs have also been discovered at or in the vicinity of DSBs in plant, *Drosophila*, and human cells (recently reviewed in Refs. [115–117]). Whether these DSB-induced small RNAs (diRNAs) arise only at DSBs induced in active genes is still under debate. Indeed, in *Drosophila*, diRNAs are produced to much higher levels from a plasmid linearized by the induction of DSBs in a highly active gene than from DSBs in a weakly transcribed one [118]. Yet, in mammalian cells, diRNAs were also detected at an I-SceI-induced break in an inactive locus, indicating that the induction of diRNAs at DSB might not be specific to active genes [119]. Future work is clearly needed to determine whether diRNAs arise by *de novo* transcription in both sense and antisense orientations or whether they require a preexisting RNA precursor.

While the function of these diRNAs is still being characterized, it is interesting that under conditions of no DNA damage, double-stranded RNAs have been proposed to function in terminating transcription and assembling repressive chromatin [120], raising the possibility that diRNAs could be similarly involved in transcriptional extinction at damaged genes. Finally, a very recent study by Ohle *et al.* [121] demonstrated that RNA:DNA hybrids accumulate at resected ends in fission yeast. Importantly, these hybrids were found to control single-strand annealing usage and to exert strong regulation of rDNA copy number following damage in rDNA, suggesting that this mechanism might be particularly relevant in transcribed regions.

## Concluding Remarks

The existence of a transcription-coupled repair process for DNA damage such as chemical adducts or UV-induced pyrimidine dimers, called transcription-coupled NER (TC-NER, has emerged since the 90s (for a review, see Ref. [122]). Notably, mutations in TC-NER proteins are associated with diseases that provoke premature aging (for a review, see Ref. [123]). The fact that specific processes are involved to maintain gene integrity may now be extended to DSB repair since the evidence discussed clearly establishes that active genes are the theater of complex and tightly regulated DSB repair events that together could define a “transcription-coupled DSB repair” pathway.

These transcription-coupled DSB repair mechanisms may reveal to be important, given that normal transcription activity is now emerging as a potent DSB-inducing agent. We shall soon see whether transcription-coupled DSB repair deficiency underlies particular diseases, as does the impairment of TC-NER. Mutations of some DSB repair factors are associated with progeria or segmental premature aging phenotypes (such as Werner disease) and neurodegenerative diseases (Ataxia Telangiectasia, Nijmegen breakage syndrome...). Whether these disorders impede the correct repair of DSB induced in active regions of the genome is a possibility that deserves attention in the near future.

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**Abbreviations used:**

G4, G-quadruplex; DSB, double-strand break; CFS, common fragile site; ERFS, early replicating fragile site; DRIP, DNA:RNA hybrid immunoprecipitation; SSB, single-strand break; NER, nucleotide excision repair; TC-NER, transcription-coupled nucleotide excision repair; HR, homologous recombination; NHEJ, non-homologous end joining; TAR, transcription-associated recombination; H3K36me3, trimethylation of histone H3 on lysine 36; diRNA, DSB-induced small RNA; TOP2B, topoisomerase II $\beta$ ; RNA Pol II, RNA polymerase II.

**References**

- [1] G. Wang, K.M. Vasquez, Impact of alternative DNA structures on DNA damage, DNA repair, and genetic instability, *DNA Repair (Amst)* 19 (2014) 143–151.
- [2] S. Corless, N. Gilbert, Effects of DNA supercoiling on chromatin architecture, *Biophys. Rev.* 8 (2016) 245–258.
- [3] G.R. Sutherland, Heritable fragile sites on human chromosomes II. Distribution, phenotypic effects, and cytogenetics, *Am. J. Hum. Genet.* 31 (1979) 136–148.
- [4] D. Sarni, B. Kerem, The complex nature of fragile site plasticity and its importance in cancer, *Curr. Opin. Cell Biol.* 40 (2016) 131–136.
- [5] A. Helmrich, K. Stout-Weider, K. Hermann, E. Schrock, T. Heiden, Common fragile sites are conserved features of human and mouse chromosomes and relate to large active genes, *Genome Res.* 16 (2006) 1222–1230.
- [6] B. Le Tallec, B. Dutrillaux, A.M. Lachages, G.A. Millot, O. Brison, M. Debatisse, Molecular profiling of common fragile sites in human fibroblasts, *Nat. Struct. Mol. Biol.* 18 (2011) 1421–1423.
- [7] A. Helmrich, M. Ballarino, L. Tora, Collisions between replication and transcription complexes cause common fragile site instability at the longest human genes, *Mol. Cell* 44 (2011) 966–977.
- [8] D.I. Smith, S. McAvoy, Y. Zhu, D.S. Perez, Large common fragile site genes and cancer, *Semin. Cancer Biol.* 17 (2007) 31–41.
- [9] J.A. Harrigan, R. Belotserkovskaya, J. Coates, D.S. Dimitrova, S.E. Polo, C.R. Bradshaw, P. Fraser, S.P. Jackson, Replication stress induces 53BP1-containing OPT domains in G1 cells, *J. Cell Biol.* 193 (2011) 97–108.
- [10] B. Le Tallec, G.A. Millot, M.E. Blin, O. Brison, B. Dutrillaux, M. Debatisse, Common fragile site profiling in epithelial and erythroid cells reveals that most recurrent cancer deletions lie in fragile sites hosting large genes, *Cell Rep.* 4 (2013) 420–428.
- [11] A. Letessier, G.A. Millot, S. Koundrioukoff, A.M. Lachages, N. Vogt, R.S. Hansen, B. Malfoy, O. Brison, M. Debatisse, Cell-type-specific replication initiation programs set fragility of the FRA3B fragile site, *Nature* 470 (2011) 120–123.
- [12] K. Miron, T. Golan-Lev, R. Dvir, E. Ben-David, B. Kerem, Oncogenes create a unique landscape of fragile sites, *Nat. Commun.* 6 (2015) 7094.
- [13] J.H. Barlow, R.B. Faryabi, E. Callen, N. Wong, A. Malhowski, H.T. Chen, G. Gutierrez-Cruz, H.W. Sun, P. McKinnon, G. Wright, R. Casellas, D.F. Robbani, L. Staudt, O. Fernandez-Capetillo, A. Nussenzweig, Identification of early replicating fragile sites that contribute to genome instability, *Cell* 152 (2013) 620–632.
- [14] H. Chandler, H. Patel, R. Palermo, S. Brookes, N. Matthews, G. Peters, Role of polycomb group proteins in the DNA damage response—a reassessment, *PLoS One* 9 (2014) e102968.
- [15] A. Gardini, D. Baillat, M. Cesaroni, R. Shiekhattar, Genome-wide analysis reveals a role for BRCA1 and PALB2 in transcriptional co-activation, *EMBO J.* 33 (2014) 890–905.
- [16] H. Bunch, B.P. Lawney, Y.F. Lin, A. Asaithamby, A. Murshid, Y.E. Wang, B.P. Chen, S.K. Calderwood, Transcriptional elongation requires DNA break-induced signaling, *Nat. Commun.* 6 (2015) 10,191.
- [17] I.A. Klein, W. Resch, M. Jankovic, T. Oliveira, A. Yamane, H. Nakahashi, M. Di Virgilio, A. Bothmer, A. Nussenzweig, D.F. Robbani, R. Casellas, M.C. Nussenzweig, Translocation-capture sequencing reveals the extent and nature of chromosomal rearrangements in B lymphocytes, *Cell* 147 (2011) 95–106.
- [18] R. Chiarle, Y. Zhang, R.L. Frock, S.M. Lewis, B. Molinie, Y.J. Ho, D.R. Myers, V.W. Choi, M. Compagno, D.J. Malkin, D. Neuberg, S. Monti, C.C. Giallourakis, M. Gostissa, F.W. Alt, Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells, *Cell* 147 (2011) 107–119.
- [19] R.L. Frock, J. Hu, R.M. Meyers, Y.J. Ho, E. Kii, F.W. Alt, Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases, *Nat. Biotechnol.* 33 (2015) 179–186.
- [20] J. Hu, R.M. Meyers, J. Dong, R.A. Panchakshari, F.W. Alt, R.L. Frock, Detecting DNA double-stranded breaks in mammalian genomes by linear amplification-mediated high-throughput genome-wide translocation sequencing, *Nat. Protoc.* 11 (2016) 853–871.
- [21] B. Schwer, P.C. Wei, A.N. Chang, J. Kao, Z. Du, R.M. Meyers, F.W. Alt, Transcription-associated processes cause DNA double-strand breaks and translocations in neural stem/progenitor cells, *Proc. Natl. Acad. Sci. U. S. A.* 113 (2016) 2258–2263.
- [22] P.C. Wei, A.N. Chang, J. Kao, Z. Du, R.M. Meyers, F.W. Alt, B. Schwer, Long neural genes harbor recurrent DNA break clusters in neural stem/progenitor cells, *Cell* 164 (2016) 644–655.
- [23] N. Crosetto, A. Mitra, M.J. Silva, M. Bienko, N. Dojer, Q. Wang, E. Karaca, R. Chiarle, M. Skrzypczak, K. Ginalski, P. Pasero, M. Rowicka, I. Dikic, Nucleotide-resolution DNA double-strand break mapping by next-generation sequencing, *Nat. Methods* 10 (2013) 361–365.
- [24] E.A. Hoffman, A. McCulley, B. Haarer, R. Arnak, W. Feng, Break-seq reveals hydroxyurea-induced chromosome fragility as a result of unscheduled conflict between DNA replication and transcription, *Genome Res.* 25 (2015) 402–412.

- [25] A. Canela, S. Sridharan, N. Sciascia, A. Tubbs, P. Meltzer, B.P. Sleckman, A. Nussenzweig, DNA breaks and end resection measured genome-wide by end sequencing, *Mol. Cell* 63 (2016) 898–911.
- [26] S.V. Lensing, G. Marsico, R. Hansel-Hertsch, E.Y. Lam, D. Tannahill, S. Balasubramanian, DSBCapture: in situ capture and sequencing of DNA breaks, *Nat. Methods* 13 (2016) 855–857.
- [27] F. Yang, C.J. Kemp, S. Henikoff, Anthracyclines induce double-strand DNA breaks at active gene promoters, *Mutat. Res.* 773 (2015) 9–15.
- [28] B. Le Tallec, S. Koundrioukoff, T. Wilhelm, A. Letessier, O. Brison, M. Debatisse, Updating the mechanisms of common fragile site instability: how to reconcile the different views? *Cell. Mol. Life Sci.* 71 (2014) 4489–4494.
- [29] H. Duda, M. Arter, J. Gloggnitzer, F. Teloni, P. Wild, M.G. Blanco, M. Altmeyer, J. Matos, A mechanism for controlled breakage of under-replicated chromosomes during mitosis, *Dev. Cell* 40 (2017) 421–422.
- [30] V. Naim, T. Wilhelm, M. Debatisse, F. Rosselli, ERCC1 and MUS81-EME1 promote sister chromatid separation by processing late replication intermediates at common fragile sites during mitosis, *Nat. Cell Biol.* 15 (2013) 1008–1015.
- [31] S. Ying, S. Minocherhomji, K.L. Chan, T. Palmai-Pallag, W.K. Chu, T. Wass, H.W. Mankouri, Y. Liu, I.D. Hickson, MUS81 promotes common fragile site expression, *Nat. Cell Biol.* 15 (2013) 1001–1007.
- [32] K. Skourti-Stathaki, N.J. Proudfoot, A double-edged sword: R loops as threats to genome integrity and powerful regulators of gene expression, *Genes Dev.* 28 (2014) 1384–1396.
- [33] A.L. Valton, M.N. Prioleau, G-Quadruplexes in DNA replication: a problem or a necessity? *Trends Genet.* 32 (2016) 697–706.
- [34] M.L. Bochman, K. Paeschke, V.A. Zakian, DNA secondary structures: stability and function of G-quadruplex structures, *Nat. Rev. Genet.* 13 (2012) 770–780.
- [35] P. Huertas, A. Aguilera, Cotranscriptionally formed DNA: RNA hybrids mediate transcription elongation impairment and transcription-associated recombination, *Mol. Cell* 12 (2003) 711–721.
- [36] K. Yu, F. Chedin, C.L. Hsieh, T.E. Wilson, M.R. Lieber, R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells, *Nat. Immunol.* 4 (2003) 442–451.
- [37] P.A. Ginno, Y.W. Lim, P.L. Lott, I. Korf, F. Chedin, GC skew at the 5' and 3' ends of human genes links R-loop formation to epigenetic regulation and transcription termination, *Genome Res.* 23 (2013) 1590–1600.
- [38] P.A. Ginno, P.L. Lott, H.C. Christensen, I. Korf, F. Chedin, R-loop formation is a distinctive characteristic of unmethylated human CpG island promoters, *Mol. Cell* 45 (2012) 814–825.
- [39] L.A. Sanz, S.R. Hartono, Y.W. Lim, S. Steyaert, A. Rajpurkar, P.A. Ginno, X. Xu, F. Chedin, Prevalent, dynamic, and conserved R-loop structures associate with specific epigenomic signatures in mammals, *Mol. Cell* 63 (2016) 167–178.
- [40] R. Hansel-Hertsch, D. Beraldi, S.V. Lensing, G. Marsico, K. Zyner, A. Parry, M. Di Antonio, J. Pike, H. Kimura, M. Narita, D. Tannahill, S. Balasubramanian, G-quadruplex structures mark human regulatory chromatin, *Nat. Genet.* 48 (2016) 1267–1272.
- [41] L.A. Dempsey, H. Sun, L.A. Hanakahi, N. Maizels, G4 DNA binding by LR1 and its subunits, nucleolin and hnRNP D, a role for G-G pairing in immunoglobulin switch recombination, *J. Biol. Chem.* 274 (1999) 1066–1071.
- [42] M.L. Duquette, P. Handa, J.A. Vincent, A.F. Taylor, N. Maizels, Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA, *Genes Dev.* 18 (2004) 1618–1629.
- [43] X. Li, J.L. Manley, Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability, *Cell* 122 (2005) 365–378.
- [44] O. Sordet, C.E. Redon, J. Guirouilh-Barbat, S. Smith, S. Solier, C. Douarre, C. Conti, A.J. Nakamura, B.B. Das, E. Nicolas, K.W. Kohn, W.M. Bonner, Y. Pommier, Ataxia telangiectasia mutated activation by transcription- and topoisomerase I-induced DNA double-strand breaks, *EMBO Rep.* 10 (2009) 887–893.
- [45] P.C. Stirling, Y.A. Chan, S.W. Minaker, M.J. Aristizabal, I. Barrett, P. Sipahimalani, M.S. Kobor, P. Hieter, R-loop-mediated genome instability in mRNA cleavage and polyadenylation mutants, *Genes Dev.* 26 (2012) 163–175.
- [46] S. Tuduri, L. Crabbe, C. Conti, H. Tourriere, H. Holtgreve-Grez, A. Jauch, V. Pantescio, J. De Vos, A. Thomas, C. Theillet, Y. Pommier, J. Tazi, A. Coquelle, P. Pasero, Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription, *Nat. Cell Biol.* 11 (2009) 1315–1324.
- [47] H. Wimberly, C. Shee, P.C. Thornton, P. Sivaramakrishnan, S.M. Rosenberg, P.J. Hastings, R-loops and nicks initiate DNA breakage and genome instability in non-growing *Escherichia coli*, *Nat. Commun.* 4 (2013) 2115.
- [48] M.L. Garcia-Rubio, C. Perez-Calero, S.I. Barroso, E. Tumini, E. Herrera-Moyano, I.V. Rosado, A. Aguilera, The Fanconi anemia pathway protects genome integrity from R-loops, *PLoS Genet.* 11 (2015) e1005674.
- [49] C.T. Stork, M. Bocek, M.P. Crossley, J. Sollier, L.A. Sanz, F. Chedin, T. Swigut, K.A. Cimprich, Co-transcriptional R-loops are the main cause of estrogen-induced DNA damage, *elife* 5 (2016) e17548.
- [50] J. Sollier, C.T. Stork, M.L. Garcia-Rubio, R.D. Paulsen, A. Aguilera, K.A. Cimprich, Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability, *Mol. Cell* 56 (2014) 777–785.
- [51] R.D. Paulsen, D.V. Soni, R. Wollman, A.T. Hahn, M.C. Yee, A. Guan, J.A. Hesley, S.C. Miller, E.F. Cromwell, D.E. Solow-Cordero, T. Meyer, K.A. Cimprich, A genome-wide siRNA screen reveals diverse cellular processes and pathways that mediate genome stability, *Mol. Cell* 35 (2009) 228–239.
- [52] A. Madireddy, S.T. Kosiyatrakul, R.A. Boisvert, E. Herrera-Moyano, M.L. Garcia-Rubio, J. Gerhardt, E.A. Vuono, N. Owen, Z. Yan, S. Olson, A. Aguilera, N.G. Howlett, C.L. Schildkraut, FANCD2 facilitates replication through common fragile sites, *Mol. Cell* 64 (2016) 388–404.
- [53] A. Aguilera, T. Garcia-Muse, R loops: from transcription byproducts to threats to genome stability, *Mol. Cell* 46 (2012) 115–124.
- [54] T. Garcia-Muse, A. Aguilera, Transcription-replication conflicts: how they occur and how they are resolved, *Nat. Rev. Mol. Cell Biol.* 17 (2016) 553–563.
- [55] D. Cortez, Preventing replication fork collapse to maintain genome integrity, *DNA Repair (Amst)* 32 (2015) 149–157.
- [56] A.K. Tehranchi, M.D. Blankschien, Y. Zhang, J.A. Halliday, A. Srivatsan, J. Peng, C. Herman, J.D. Wang, The transcription factor DksA prevents conflicts between DNA replication and transcription machinery, *Cell* 141 (2010) 595–605.
- [57] D. Dutta, K. Shatalin, V. Epshtein, M.E. Gottesman, E. Nudler, Linking RNA polymerase backtracking to genome instability in *E. coli*, *Cell* 146 (2011) 533–543.
- [58] H. Merrih, Y. Zhang, A.D. Grossman, J.D. Wang, Replication-transcription conflicts in bacteria, *Nat. Rev. Microbiol.* 10 (2012) 449–458.

- [59] A. Helmrich, M. Ballarino, E. Nudler, L. Tora, Transcription-replication encounters, consequences and genomic instability, *Nat. Struct. Mol. Biol.* 20 (2013) 412–418.
- [60] A. Cristini, J.H. Park, G. Capranico, G. Legube, G. Favre, O. Sordet, DNA-PK triggers histone ubiquitination and signaling in response to DNA double-strand breaks produced during the repair of transcription-blocking topoisomerase I lesions, *Nucleic Acids Res.* 44 (2016) 1161–1178.
- [61] M.C. Haffner, M.J. Aryee, A. Toubaji, D.M. Esopi, R. Albadine, B. Gurel, W.B. Isaacs, G.S. Bova, W. Liu, J. Xu, A.K. Meeker, G. Netto, A.M. De Marzo, W.G. Nelson, S. Yegnasubramanian, Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements, *Nat. Genet.* 42 (2010) 668–675.
- [62] B.G. Ju, V.V. Lunyak, V. Perissi, I. Garcia-Bassets, D.W. Rose, C.K. Glass, M.G. Rosenfeld, A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription, *Science* 312 (2006) 1798–1802.
- [63] C. Lin, L. Yang, B. Tanasa, K. Hutt, B.G. Ju, K. Ohgi, J. Zhang, D.W. Rose, X.D. Fu, C.K. Glass, M.G. Rosenfeld, Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer, *Cell* 139 (2009) 1069–1083.
- [64] L.M. Williamson, S.P. Lees-Miller, Estrogen receptor alpha-mediated transcription induces cell cycle-dependent DNA double-strand breaks, *Carcinogenesis* 32 (2011) 279–285.
- [65] R. Madabhushi, F. Gao, A.R. Pfening, L. Pan, S. Yamakawa, J. Seo, R. Rueda, T.X. Phan, H. Yamakawa, P.C. Pao, R.T. Stott, E. Gjoneska, A. Nott, S. Cho, M. Kellis, L.H. Tsai, Activity-induced DNA breaks govern the expression of neuronal early-response genes, *Cell* 161 (2015) 1592–1605.
- [66] E. Suberbielle, P.E. Sanchez, A.V. Kravitz, X. Wang, K. Ho, K. Eilertson, N. Devidze, A.C. Kreitzer, L. Mucke, Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-beta, *Nat. Neurosci.* 16 (2013) 613–621.
- [67] R.E. Jensen, I. Herskowitz, Directionality and regulation of cassette substitution in yeast, *Cold Spring Harb. Symp. Quant. Biol.* 49 (1984) 97–104.
- [68] C.I. White, J.E. Haber, Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*, *EMBO J.* 9 (1990) 663–673.
- [69] P. Rouet, F. Smih, M. Jasin, Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease, *Mol. Cell. Biol.* 14 (1994) 8096–8106.
- [70] J.S. Iacovoni, P. Caron, I. Lassadi, E. Nicolas, L. Massip, D. Trouche, G. Legube, High-resolution profiling of gamma-H2AX around DNA double strand breaks in the mammalian genome, *EMBO J.* 29 (2010) 1446–1457.
- [71] N.M. Shanbhag, I.U. Rafalska-Metcalf, C. Balane-Bolivar, S.M. Janicki, R.A. Greenberg, ATM-dependent chromatin changes silence transcription *in cis* to DNA double-strand breaks, *Cell* 141 (2010) 970–981.
- [72] E. Berkovich, R.J. Monnat Jr., M.B. Kastan, Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair, *Nat. Cell Biol.* 9 (2007) 683–690.
- [73] M. van Sluis, B. McStay, A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage, *Genes Dev.* 29 (2015) 1151–1163.
- [74] G. D'Alessandro, d'Adda di Fagagna, F., Transcription and DNA damage: holding hands or crossing swords? *J. Mol. Biol.* (2016) pii: S0022-2836(16)30471-5.
- [75] T. Pankotai, C. Bonhomme, D. Chen, E. Soutoglou, DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks, *Nat. Struct. Mol. Biol.* 19 (2012) 276–282.
- [76] L.V. Solovjeva, M.P. Svetlova, V.O. Chagin, N.V. Tomilin, Inhibition of transcription at radiation-induced nuclear foci of phosphorylated histone H2AX in mammalian cells, *Chromosom. Res.* 15 (2007) 787–797.
- [77] F. Gong, L.Y. Chiu, B. Cox, F. Aymard, T. Clouaire, J.W. Leung, M. Cammarata, M. Perez, P. Agarwal, J.S. Brodbelt, G. Legube, K.M. Miller, Screen identifies bromodomain protein ZMYND8 in chromatin recognition of transcription-associated DNA damage that promotes homologous recombination, *Genes Dev.* 29 (2015) 197–211.
- [78] S.E. Lee, A. Pelliccioli, J. Demeter, M.P. Vaze, A.P. Gasch, A. Malkova, P.O. Brown, D. Botstein, T. Stearns, M. Foiani, J.E. Haber, Arrest, adaptation, and recovery following a chromosome double-strand break in *Saccharomyces cerevisiae*, *Cold Spring Harb. Symp. Quant. Biol.* 65 (2000) 303–314.
- [79] J.A. Kim, M. Kruhlak, F. Dotiwala, A. Nussenzweig, J.E. Haber, Heterochromatin is refractory to gamma-H2AX modification in yeast and mammals, *J. Cell Biol.* 178 (2007) 209–218.
- [80] N. Manfrini, M. Clerici, M. Wery, C.V. Colombo, M. Describes, A. Morillon, F. d'Adda di Fagagna, M.P. Longhese, Resection is responsible for loss of transcription around a double-strand break in *Saccharomyces cerevisiae*, *elife* 4 (2015) e08942.
- [81] P. Caron, F. Aymard, J.S. Iacovoni, S. Briois, Y. Canitrot, B. Bugler, L. Massip, A. Losada, G. Legube, Cohesin protects genes against gammaH2AX induced by DNA double-strand breaks, *PLoS Genet.* 8 (2012) e1002460.
- [82] J. Kim, D. Sturgill, A.D. Tran, D.A. Sinclair, P. Oberdoerffer, Controlled DNA double-strand break induction in mice reveals post-damage transcriptome stability, *Nucleic Acids Res.* 44 (2016) e64.
- [83] A. Kakarougkas, A. Ismail, A.L. Chambers, E. Riballo, A.D. Herbert, J. Kunzel, M. Lobrich, P.A. Jeggo, J.A. Downs, Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin, *Mol. Cell* 55 (2014) 723–732.
- [84] A. Ui, Y. Nagaura, A. Yasui, Transcriptional elongation factor ENL phosphorylated by ATM recruits polycomb and switches off transcription for DSB repair, *Mol. Cell* 58 (2015) 468–482.
- [85] K.M. Miller, J.V. Tjeertes, J. Coates, G. Legube, S.E. Polo, S. Britton, S.P. Jackson, Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining, *Nat. Struct. Mol. Biol.* 17 (2010) 1144–1151.
- [86] F. Aymard, B. Bugler, C.K. Schmidt, E. Guillou, P. Caron, S. Briois, J.S. Iacovoni, V. Daburon, K.M. Miller, S.P. Jackson, G. Legube, Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks, *Nat. Struct. Mol. Biol.* 21 (2014) 366–374.
- [87] J. Tang, N.W. Cho, G. Cui, E.M. Manion, N.M. Shanbhag, M.V. Botuyan, G. Mer, R.A. Greenberg, Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination, *Nat. Struct. Mol. Biol.* 20 (2013) 317–325.
- [88] E. Mladenov, S. Magin, A. Soni, G. Iliakis, DNA double-strand-break repair in higher eukaryotes and its role in genomic instability and cancer: cell cycle and proliferation-dependent regulation, *Semin. Cancer Biol.* 37–38 (2016) 51–64.
- [89] C. Meers, H. Keskin, F. Storici, DNA repair by RNA: templated, or not templated, that is the question, *DNA Repair (Amst)* 44 (2016) 17–21.

- [90] B.J. Thomas, R. Rothstein, Elevated recombination rates in transcriptionally active DNA, *Cell* 56 (1989) 619–630.
- [91] J.A. Nickoloff, R.J. Reynolds, Transcription stimulates homologous recombination in mammalian cells, *Mol. Cell. Biol.* 10 (1990) 4837–4845.
- [92] P. Gottipati, T.N. Cassel, L. Savolainen, T. Helleday, Transcription-associated recombination is dependent on replication in mammalian cells, *Mol. Cell. Biol.* 28 (2008) 154–164.
- [93] S. Jinks-Robertson, A.S. Bhagwat, Transcription-associated mutagenesis, *Annu. Rev. Genet.* 48 (2014) 341–359.
- [94] A. Aguilera, The connection between transcription and genomic instability, *EMBO J.* 21 (2002) 195–201.
- [95] P. Chaurasia, R. Sen, T.K. Pandita, S.R. Bhaumik, Preferential repair of DNA double-strand break at the active gene *in vivo*, *J. Biol. Chem.* 287 (2012) 36,414–36,422.
- [96] C. Allen, C.A. Miller, J.A. Nickoloff, The mutagenic potential of a single DNA double-strand break in a mammalian chromosome is not influenced by transcription, *DNA Repair (Amst)* 2 (2003) 1147–1156.
- [97] A. Gunn, N. Bennardo, A. Cheng, J.M. Stark, Correct end use during end joining of multiple chromosomal double strand breaks is influenced by repair protein RAD50, DNA-dependent protein kinase DNA-PKcs, and transcription context, *J. Biol. Chem.* 286 (2011) 42,470–42,482.
- [98] L. Massip, P. Caron, J.S. Iacovoni, D. Trouche, G. Legube, Deciphering the chromatin landscape induced around DNA double strand breaks, *Cell Cycle* 9 (2010) 2963–2972.
- [99] L. Wei, S. Nakajima, S. Bohm, K.A. Bernstein, Z. Shen, M. Tsang, A.S. Levine, L. Lan, DNA damage during the G0/G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) E3495–E3504.
- [100] S.X. Pfister, S. Ahrabi, L.P. Zalmas, S. Sarkar, F. Aymard, C.Z. Bachrati, T. Helleday, G. Legube, N.B. La Thangue, A.C. Porter, T.C. Humphrey, SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability, *Cell Rep.* 7 (2014) 2006–2018.
- [101] S. Carvalho, A.C. Vitor, S.C. Sridhara, F.B. Martins, A.C. Raposo, J.M. Desterro, J. Ferreira, S.F. de Almeida, SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint, *elife* 3 (2014) e02482.
- [102] M. Daugaard, A. Baude, K. Fugger, L.K. Povlsen, H. Beck, C.S. Sorensen, N.H. Petersen, P.H. Sorensen, C. Lukas, J. Bartek, J. Lukas, M. Rohde, M. Jaattela, LEDGF (p75) promotes DNA-end resection and homologous recombination, *Nat. Struct. Mol. Biol.* 19 (2012) 803–810.
- [103] G.G. Sharma, S. So, A. Gupta, R. Kumar, C. Cayrou, N. Avvakumov, U. Bhadra, R.K. Pandita, M.H. Porteus, D.J. Chen, J. Cote, T.K. Pandita, MOF and histone H4 acetylation at lysine 16 are critical for DNA damage response and double-strand break repair, *Mol. Cell. Biol.* 30 (2010) 3582–3595.
- [104] K. Jacquet, A. Fradet-Turcotte, N. Avvakumov, J.P. Lambert, C. Roques, R.K. Pandita, E. Paquet, P. Herst, A.C. Gingras, T.K. Pandita, G. Legube, Y. Doyon, D. Durocher, J. Cote, The TIP60 complex regulates bivalent chromatin recognition by 53BP1 through direct H4K20me binding and H2AK15 acetylation, *Mol. Cell* 62 (2016) 409–421.
- [105] A. Fradet-Turcotte, M.D. Canny, C. Escribano-Diaz, A. Orthwein, C.C. Leung, H. Huang, M.C. Landry, J. Kitevski-LeBlanc, S.M. Noordermeer, F. Sicheri, D. Durocher, 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark, *Nature* 499 (2013) 50–54.
- [106] T. Clouaire, G. Legube, DNA double strand break repair pathway choice: a chromatin based decision? *Nucleus* 6 (2015) 107–113.
- [107] A. Orthwein, S.M. Noordermeer, M.D. Wilson, S. Landry, R.I. Enchev, A. Sherker, M. Munro, J. Pinder, J. Salsman, G. Dellaire, B. Xia, M. Peter, D. Durocher, A mechanism for the suppression of homologous recombination in G1 cells, *Nature* 528 (2015) 422–426.
- [108] C.C. Ebmeier, D.J. Taatjes, Activator-mediator binding regulates mediator-cofactor interactions, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 11,283–11,288.
- [109] E. Maldonado, R. Shiekhhattar, M. Sheldon, H. Cho, R. Drapkin, P. Rickert, E. Lees, C.W. Anderson, S. Linn, D. Reinberg, A human RNA polymerase II complex associated with SRB and DNA-repair proteins, *Nature* 381 (1996) 86–89.
- [110] F. Aymard, M. Aguirrebengoa, E. Guillou, B.M. Javierre, B. Bugler, C. Arnould, V. Rocher, J.S. Iacovoni, A. Biernacka, M. Skrzypczak, K. Ginalski, M. Rowicka, P. Fraser, G. Legube, Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes, *Nat. Struct. Mol. Biol.* (2017) <http://dx.doi.org/10.1038/nsmb.3387> [Epub ahead of print].
- [111] C. Lukas, V. Savic, S. Bekker-Jensen, C. Doil, B. Neumann, R.S. Pedersen, M. Grofte, K.L. Chan, I.D. Hickson, J. Bartek, J. Lukas, 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress, *Nat. Cell Biol.* 13 (2011) 243–253.
- [112] F. Storici, K. Bebenek, T.A. Kunkel, D.A. Gordenin, M.A. Resnick, RNA-templated DNA repair, *Nature* 447 (2007) 338–341.
- [113] H. Keskin, Y. Shen, F. Huang, M. Patel, T. Yang, K. Ashley, A.V. Mazin, F. Storici, Transcript-RNA-templated DNA recombination and repair, *Nature* 515 (2014) 436–439.
- [114] A. Chakraborty, N. Tapryal, T. Venkova, N. Horikoshi, R.K. Pandita, A.H. Sarker, P.S. Sarkar, T.K. Pandita, T.K. Hazra, Classical non-homologous end-joining pathway utilizes nascent RNA for error-free double-strand break repair of transcribed genes, *Nat. Commun.* 7 (2016) 13,049.
- [115] F. d'Adda di Fagagna, A direct role for small non-coding RNAs in DNA damage response, *Trends Cell Biol.* 24 (2014) 171–178.
- [116] J.S. Khanduja, I.A. Calvo, R.I. Joh, I.T. Hill, M. Motamedi, Nuclear noncoding RNAs and genome stability, *Mol. Cell* 63 (2016) 7–20.
- [117] Y.G. Yang, Y. Qi, RNA-directed repair of DNA double-strand breaks, *DNA Repair (Amst)* 32 (2015) 82–85.
- [118] K.M. Michalik, R. Bottcher, K. Forstemann, A small RNA response at DNA ends in *Drosophila*, *Nucleic Acids Res.* 40 (2012) 9596–9603.
- [119] S. Francia, F. Michelini, A. Saxena, D. Tang, M. de Hoon, V. Anelli, M. Mione, P. Carninci, d'Adda di Fagagna, F., Site-specific DICER and DROSHA RNA products control the DNA-damage response, *Nature* 488 (2012) 231–235.
- [120] K. Skourti-Stathaki, K. Kamieniarz-Gdula, N.J. Proudfoot, R-loops induce repressive chromatin marks over mammalian gene terminators, *Nature* 516 (2014) 436–439.
- [121] C. Ohle, R. Tesorero, G. Schermann, N. Dobrev, I. Sinning, T. Fischer, Transient RNA-DNA hybrids are required for efficient double-strand break repair, *Cell* 167 (2016) e7.
- [122] P.C. Hanawalt, G. Spivak, Transcription-coupled DNA repair: two decades of progress and surprises, *Nat. Rev. Mol. Cell Biol.* 9 (2008) 958–970.
- [123] J.A. Marteijn, H. Lans, W. Vermeulen, J.H. Hoeijmakers, Understanding nucleotide excision repair and its roles in cancer and ageing, *Nat. Rev. Mol. Cell Biol.* 15 (2014) 465–481.