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Editorial summary

In this review, Bergis-Ser and colleagues discuss how chromatin dynamics and nucleic acids metabolism impinge on genome integrity, both as sources of spontaneous lesions, and as key contributors of the DNA Damage response in plants.

Abstract

Maintenance of genome integrity is an essential process in all organisms. Mechanisms avoiding the formation of DNA lesions or mutations are well described in animals because of their relevance to human health and cancer. In plants, they are of growing interest because DNA damage accumulation is increasingly recognized as one of the consequences of stress. Although the cellular response to DNA damage is mostly studied in response to genotoxic treatments, the main source of DNA lesions is the cellular activity itself. This can occur through the production of reactive oxygen species as well as DNA processing mechanisms such as DNA replication or transcription, and chromatin dynamics. In addition, how lesions are formed and are repaired is greatly influenced by chromatin features and dynamics, and by DNA and RNA metabolism. Notably, actively transcribed regions or replicating DNA, because they are less condensed and are sites of DNA processing, are more exposed to DNA damage. However, at the same time, a wealth of cellular mechanisms cooperates to favor DNA repair at these genomic

loci. These intricate relationships that shape the distribution of mutations along the genome have been extensively studied in animals, but are much less put forward in plant studies. In this review, we summarize how chromatin dynamic influences lesion formation and DNA repair in plants, providing a comprehensive view of current knowledge, and highlighting open questions with regard to what is known in other organisms.

Introduction

DNA integrity is constantly threatened by the formation of lesions that result from cellular activities such as the production of reactive oxygen species by energy-producing organelles, or from DNA metabolism itself. Indeed, it is estimated that each human cell is subject to approximately 70 000 lesions per day[1]. Given that the human genome is about 3Gb long, and assuming that lesion number depends solely on genome size, this would correspond to 3 000 lesions per cell and per day in Arabidopsis, but almost 400 000 in wheat and roughly 60 000 in maize. Importantly, Arabidopsis is an outlier in terms of genome size compared to other plant species[2], which implies that spontaneous lesion accumulation in most plants is likely very frequent.

Because DNA lesions are extremely common, cells have evolved sophisticated mechanisms to repair each type of lesion, that are largely conserved in all eukaryotes including plants[3]. Punctual lesions that do not distort the DNA double-helix such as single-strand breaks (SSBs), or oxidized bases such as 8-oxo-guanine (8-oxo-G) are repaired through the base Excision Repair (BER) process. Bulky DNA adducts such as cyclobutane pyrimidine dimers (CPDs) or intra-strand crosslinks induced by UV light are repaired by nucleotide excision repair (NER) or by direct photoreactivation, a pathway in which enzymes called photolyases use light energy for the direct reversion of the lesion. Mismatches resulting from replication errors are dealt with by the mismatch repair pathway (MMR). Finally, repair of inter-strand cross-link, which can also be induced by UV light has been studied more recently in plants, and relies on the Fanconi Anemia pathway, like in other eukaryotes[4,5].

If left unrepaired, these lesions can further lead to double-strand breaks (DSBs) that are more harmful than the previously mentioned lesions, as they can lead to the loss of genetic information; or replication stress, because replicative DNA polymerases have very tight catalytic sites that cannot accommodate damaged bases[6]. These two types of DNA stress trigger the DNA Damage Response (DDR), that results in cell cycle arrest until DNA is repaired[7–9]. Briefly, DSBs and single-stranded DNA (ssDNA) that accumulates during replication stress activate the ATM and ATR kinases respectively. This leads to extensive chromatin modifications (detailed in this review) that favour DNA repair; and to the transcriptional regulation cell cycle and DNA repair genes, thus allowing to stop cell cycle progression until DNA damage is repaired[10–12].

Repair mechanisms activated by the DDR slightly differ according to the type of lesion. DSBs can be repaired via homologous recombination (HR), in which an undamaged DNA sequence sharing homology with the damaged region, often the sister chromatid, is used as a template for repair. During HR, the extremities of the DNA break are resected, and the RAD51 recombinase forms a nucleofilament

with ssDNA to allow homology search and strand invasion. Pairing of this DNA strand with the undamaged template next allows a DNA polymerase to repair the break[3,13]. Alternatively, DSBs can be repaired by the potentially error-prone Non-Homologous End Joining (NHEJ). Two independent pathways called canonical NHEJ and alternative NHEJ (alt-NHEJ) exist in plant cells. In cNHEJ, DNA ends are recognized by Ku proteins and religated, without DNA end resection[13]. By contrast, alt-NHEJ is initiated by end-resection, and relies on micro-homology, and on the activity of DNA polymerase theta[3,14]. In the case of replication stress, translesion-synthesis can bypass replication-blocking lesions by recruiting specialized DNA polymerases[6,15]. Alternatively, stalled forks can be rescued by activation of nearby replication origins, or repaired through HR and alt-NHEJ[15].

Although DDR signalling and DNA repair mechanisms have been extensively studied, most studies are performed using exogenously applied genotoxic stress. Recently, chromatin dynamics and nucleic acids metabolism (i.e. DNA replication and transcription) are emerging as important elements impinging on DNA lesion formation in plants. Reciprocally, the role of chromatin dynamics and nucleic acids metabolism in the maintenance of genome integrity is increasingly recognized. In this review, we will explore how chromatin features and DNA processing affect genome integrity and the repair machinery, which has profound consequences on the distribution of mutations on the genome. Fig. 1 summarizes the main mechanisms that will be discussed here, and Table 1 lists the main actors described in the review.

Genomic features and DNA processing as sources of DNA damage

Methylated DNA sequences are more prone to DNA damage and mutation accumulation

In Arabidopsis, GC->AT mutations are by far the most frequent, and the mutation rate of methylated cytosines is higher than that of unmethylated cytosines[16]. These mutations likely result both from the spontaneous deamination of methylated cytosines, that is known to be more frequent than that of unmethylated cytosines[17], and from the formation of pyrimidine dimers, because most mutations occurring at unmethylated cytosines are in di-pyrimidine contexts[16]. In addition, methylated cytosines are also more prone to forming CPDs[18]. Thus, UV-induced lesions that are enriched in the highly methylated constitutive heterochromatin[19] could thus also contribute to the mutability of methylated cytosines.

Another direct connection between DNA methylation and DNA damage comes from the molecular mechanisms underlying the DNA demethylation process itself, which share most of the machinery involved in BER (Fig. 2). BER is initiated by DNA glycosylases that remove the damaged base, leaving an abasic site. Several DNA glycosylases exist in plant genomes that show distinct specificities towards

different DNA lesions[20], among which Demeter-like (DML) DNA glycosylases are specifically involved in DNA demethylation[21,22]. Thus, methylated cytosines can behave as hotspots for the formation of DNA lesions, particularly if their methylation status is highly dynamic. Indeed, *zdp/ape2* double mutants that lack both pathways involved in the processing of abasic sites (Fig. 2) display severe developmental defects, due to the constitutive activation of the DDR, that can be rescued by inactivation of the ROS1 DNA glycosylase, indicating that active DNA demethylation is a source of DNA damage in plant cells[23]. Interestingly, in Arabidopsis and rice, mutation rates are higher on the 1-3 nucleotides situated on each side of methylated cytosines, which could be due to repair errors during the LP-BER process (Fig. 2[24]). However, the mutation rates of cytosines whose methylation status is dynamically regulated by ROS1 is lower than that of stably methylated cytosines[24]. Thus, although DNA demethylation creates DNA lesions, the efficiency and accuracy of DNA repair are likely higher at actively demethylated sites, suggesting that accessibility to the DNA repair machinery, rather than frequent methylation/demethylation cycles, is the main factor determining the mutation risk of methylated cytosines (Fig. 3a). Given that most crop genomes have much higher DNA methylation levels than Arabidopsis or rice due to their high TE (Transposable Elements) content[2], it will be interesting to find whether the methylation-related bias in the distribution of mutations along the genome are exacerbated in other plants.

Specificities of telomeric regions

Because eukaryotic chromosomes are linear, their extremities resemble DSBs, and thus need to be protected to avoid triggering DDR activation, and being inappropriately repaired, which could lead to chromosome fusions. This is achieved through the presence of telomeric repeats, that allow the recruitment of dedicated proteins to protect them[25]. Due to their G-rich sequence, telomeres accumulate about 100 times more 8-oxoG, the most common DNA lesion induced by reactive oxygen species, than the rest of the genome in control conditions in Arabidopsis[26]. This feature could require specific targeting of the DNA repair machinery to telomeres[27], but this issue has not been explored in plants as yet.

Topological stress and its resolution by topoisomerases

All DNA metabolic processes can induce topological stress in the form of negative or positive supercoiling. Because excessive supercoiling can induce breakage, these constraints are resolved by a dedicated class of enzymatic complexes called Topoisomerases, that can cut DNA, release the tension and religate the DNA molecule[28]. They are involved in all aspects of DNA metabolism, including DNA replication, transcription, DNA repair, DNA disentanglement, for example during endoreduplication, chromosome condensation, heterochromatin metabolism…[29]. In Arabidopsis, topoisomerases have

been shown to play essential roles in the maintenance of genome integrity. Pharmacological inhibition of TOPI enzymes leads to DNA breaks accumulation due to transcription/replication conflicts[30]. Topoisomerase II (TOPII) is also essential to DNA replication: *topII* mutants display DSB accumulation in meristematic cells, likely due to replication fork collapse[29]. Likewise, endoreduplication is severely compromised in *topVI* mutants[31–33], suggesting that TOPVI could play an important role in chromosome disentanglement. Interestingly, this essential activity of topoisomerases is in itself a threat for genome integrity, because they can form DNA-protein crosslink (DPC), if DNA protein intermediates of the reaction get stabilized [34]. Inactivation of DPCs repair leads to severe developmental defects, associated with a dramatic reduction of cell proliferation and spontaneous cell death[35], likely due to replication-associated DNA damage.

DNA replication as a source of DNA damage

DNA replication is a major source of DNA stress, not only because it generates topological stress in the form of DNA supercoiling, but also because it leads to the accumulation of ssDNA that is more prone to breakage than dsDNA. SsDNA is protected from damage by the RPA heterotrimer, that is structurally very similar to the CST complex involved in telomere protection[36,37]. RPAs allow the rapid detection of fork stalling[36] by promoting the recruitment and activation of ATR[7] that leads to fork stabilization and repair. Because the ATR kinase is at the centre of the replication stress response, its inactivation is lethal in mammals[38], although it is not in Drosophila or yeast. Initially, the ATR gene was thought to be unessential in plants, because Arabidopsis mutants lacking ATR do not display any developmental defects in the absence of exogenously applied genotoxic stress[39]. However, maize and barley *atr* mutants display severe growth defects, due to the spontaneous accumulation of DSBs in proliferating cells[40,41]. One possible explanation for the discrepancies between results obtained in Arabidopsis and in barley or maize could stem from the respective size of their genomes and thus from the abundance of DNA damage spontaneously accumulating during S-phase.

The question of genomic and/or chromatin features associated with replication stress and spontaneous DSB formation has been extensively explored in many models, but comparatively less is known in plants. Mammalian genomes contain so-called common fragile sites (CFS) that are defined as loci prone to DNA breaks and chromosomal rearrangements in response to mild replication stress[42], whose expression is enhanced by ATR deficiency[43]. Similar fragile sites could exist in plants, and DNA breakage at these sites could explain the growth defects of maize or barley *atr* mutants. Several DNA structures have been associated with replication-associated break formation in many models such as bacteria, yeast or mammals[42,44]. These include R-loops (detailed in “RNA metabolism induces DNA damage” part), as well as secondary structures that can form on ssDNA after unwinding by replicative

helicases such as G-quadruplexes (G4), guanine-rich sequences folding into four stranded structures forming stacked tetrads, and hairpins that form in GC and AT-rich sequences respectively. G4 formation after DNA unwinding blocks DNA polymerase progression, and thus leads to polymerase/helicase uncoupling and ssDNA exposure. In addition G4s interfere with RPA loading, thereby compromising DDR signalling[45,46]. These replication-blocking structures are likely particularly scarce in Arabidopsis: G4 are widespread structures in all plant species except Arabidopsis[47], and the high proportion of TE and repetitive elements found in maize or barley genomes could increase the frequency of AT-rich sequences and thus favour the existence of CFS.

DNA sequences coding for ribosomal RNA (rDNA) loci are well documented to be prone to DNA damage, notably because they cause replication issues[48]: inhibition of DNA replication induces 45S rDNA fragility in maize, barley and rice[49]. rDNA genes are present in hundreds to thousands of copies in plant genomes, and are organized in large clusters[50]. One proposed explanation for their fragility is that rDNA loci need to be transcribed at very high levels throughout the cell cycle, including S-phase[48], causing transcription/replication conflicts (see below). In yeast and human cells, this problem is circumvented by the existence of the Replication Fork Barrier (RFB), a sequence located in the vicinity of the rDNA locus that can arrest the replication fork[51,52]. A RFB was identified in *Pisum sativum*, but until now, such sequences have not been studied in other plant models such as Arabidopsis[53]. Further evidence for the fact that rDNA behave as CFS in plant genomes comes from the gradual loss of rDNA copies in *fas* mutants, that are deficient for a sub-unit of the CAF1 complex[54], a heterotrimeric histone chaperone complex involved in the incorporation of H3-H4 dimers during DNA replication[55]. Loss of rDNA in *fas* mutants is mitotic, and correlates with spontaneous accumulation of DSBs that largely colocalize with the 45S rDNA locus[56]. The formation of these foci is ATR-dependent, but not restricted to S-phase cells[56], and may thus be associated with transcription and not only with replication[49]. Nevertheless, protein complexes involved in the resolution of replication-blocking structures and aberrant recombination intermediates are involved in the maintenance of rDNA copy-number[4,5], confirming that plant rDNA loci are prone to replication stress.

Like rDNA loci, telomeres are well known difficult-to-replicate regions of the genome[48]. This is in part due to their high GC content, that favours the formation of G-quadruplexes. In addition, they pose the so-called end-replication problem, because replication has to terminate at the end of the chromosome without meeting a replication fork travelling in the opposite direction[25]. This results in erosion of telomeres at each replication cycle, that is compensated by the action of telomerase, a ribonucleoprotein enzyme that contains a catalytic telomerase reverse transcriptase (TERT) and uses a long noncoding telomerase RNA component as a template to elongate telomeres[25].

Finally, fragile sites are also found in genomic regions that are comparatively poor in replication origins, because rescue of stalled forks by replication units coming from nearby origins becomes less efficient[42]. Interestingly, the size range of replication segments in late replicating regions is broader in maize (about 10-160kb[57]) than in Arabidopsis (about 10 to 100kb [58]), and the median size of early replicating segments is also larger. Thus, the maize genome may contain more origin-poor regions, in which DNA breaks are more likely to occur during DNA replication.

Overall, DNA-replication stalling at difficult-to-replicate sequences likely triggers the formation of DNA breaks in plant genomes even in the absence of genotoxic stress, as summarized on Fig. 3b. This phenomenon could be of importance for crops physiology, as plants with larger genomes seem to have a higher rate of spontaneous break formation than Arabidopsis. In addition, even in Arabidopsis where replication-associated DNA damage appears to be rare, its frequency has been reported to increase in response to various types of environmental stresses such as high-light or salt[59].

Is chromatin looping a potential source of DNA damage?

Very recently, chromatin topology itself was found to be a potential source of DNA lesions in the form of DSBs: in human cells, Topoisomerase 2B generates DSBs at promoter/enhancer loop anchors, independently of transcription[60]. The formation of higher-order chromatin structures such as loops generates topological stress that is resolved by Topoisomerases, and this localized DNA cleavage activity generates fragile sites that are transcription and replication-independent (Fig. 3c). Consistently, chromosome conformation was found to efficiently allow the prediction of sites of DSB formation[61]. Given that exposure to stress such as heat stress has been shown to result in profound changes in 3D chromatin organization and promoter/enhancer loops in plants[62], it will be interesting to determine whether this reprogramming of 3D chromatin organization results in the accumulation of DNA breaks at new anchor sites.

RNA metabolism induces DNA damage

Persistent RNA-DNA hybrids called R-loops can lead to DNA damage

R-loops are structures composed of a DNA-RNA hybrid and a displaced single-stranded DNA (ssDNA) that are key players of gene expression regulation[63], although they can be responsible for genome instability[64,65]. In plants, R-loop mediated genomic instability has been observed mainly in the chloroplast genome[66], but nuclear R-loops are also likely to impact genomic integrity.

The FLOWERING LOCUS C (*FLC*), a repressor of flowering, was the first characterized example of gene expression regulation by an anti-sense non-coding RNA thanks to R-loops formation[67]. Since

then, R-loops have been extensively studied[68–70], and mapped at the genome-wide level in several plant species[71]. They tend to accumulate on G-rich and A-rich regions primarily present in the promoters of several genes and on TEs. The very structure of R-loops makes them more susceptible to DNA damage because ssDNA is more susceptible than dsDNA to damage[72]. Yet, in yeast, only a subset of R-loops contributes to genomic instability[73], likely because specific proteins protect R-loop-forming loci from DNA damage. In Arabidopsis, two DNA/RNA binding proteins called AtALBA1 and AtALBA2 (acetylation lowers binding affinity 1 and 2) can bind the DNA-RNA hybrid and the displaced ssDNA of R-loops *in vitro*, respectively, and it likely play such a protective role on R-loops[72] (Fig. 4a).

Finally, R-loops could contribute to the genomic instability of *A. thaliana ddm1* (decrease in DNA methylation 1) mutants, that are deficient for a chromatin remodelling complex. R-loops accumulation in pericentromeric regions increases in the absence of the DDM1 protein: DDM1 would allow disrupting these loops thanks to its helicase activity and through the replacement of H2A.Z histone variants, mainly localised at chromosome arms, by H2A.W, variants that are usually associated with heterochromatin[74]. Thus, DDM1 is proposed to allow heterochromatin formation through the co-transcriptional clearance of R-loops, notably because this mechanism allows the restoration of dsDNA, which is the appropriate substrate for DNA methyl-transferases[74]. Efficient silencing of these heterochromatic regions is essential to the maintenance of genome integrity because it avoids TE reactivation and remobilisation, but it is also likely that avoiding excess R-loop accumulation in pericentromeric regions could directly prevent DNA damage accumulation in these regions (Fig. 4b).

Every above-mentioned change in R-loops homeostasis could lead to genome instability. However, these modifications were observed under artificial laboratory conditions, using mutants in which R-loop homeostasis was disturbed. Interestingly, in wheat (*Triticum dicoccoides*) some edaphic factors, such as soil type and water availability, were recently shown to impinge on the accumulation of RNA PolII, DSBs formation, and R-loops accumulation[75], suggesting R-loop homeostasis is a relevant factor for genome integrity under natural conditions.

Transcription/replication conflicts or transcription collisions are a source of genomic instability

Another source of genomic instability caused by RNA metabolism is the collision between two transcription units or between the transcription and replication machineries. This phenomenon is well documented in many organisms, such as humans[76–78], and the role of Topoisomerases in the resolution of topological problems associated with these conflicts is clearly established[79]. However, much less information can be found in plants.

Our understanding of mechanisms allowing plant cells to deal with transcription-associated conflicts greatly benefited from the detailed analysis of the intricate regulation of the *FLC* locus[80]. *FLC* is transcribed both in the sense orientation to produce the *FLC* mRNA and in the antisense orientation to produce a transcript named *COOLAIR*. Transcription of *FLC* and *COOLAIR* are mutually exclusive, and *COOLAIR* contributes to *FLC* repression during vernalization[81]. *COOLAIR* expression is fine-tuned by a R-loop formed in its promoter. Resolution of the R-loop allows *COOLAIR* transcription and recruitment of the chromatin modifier complex FLD/LD/SDG26 that controls H3K4me1 levels at this locus, leading to *FLC* repression[82,83]. At the genome-wide level, FLD was proposed to slow-down transcription at convergent transcription sites[84]. Importantly, FLD genetically interacts with Topoisomerase 1α, suggesting that FLD could allow the reduction of topological stresses triggered by collisions between transcription units[84] (Fig. 4c).

Like transcription collision events, transcription/replication conflicts threaten genome integrity[85]. The *FLC* locus may also prove to be a case-study that will become relevant to our global understanding of transcription-replication conflicts in plants. Indeed, it can interfere with replication depending on its orientation respective to fork progression. At the genome-wide level, the resolution of R-loops by FCA and FY is required to avoid replication fork progression impediment[86], suggesting that transcription/replication conflicts are a major source of DNA damage in plant genomes, and that the machinery identified for its role in the regulation of convergent transcription events could also participate in the limitation of transcription/replication conflicts (Fig. 4c). Consistently, inhibition of topoisomerase 1 leads to R-loop accumulation and genome instability, and the cellular response to these defects requires ATM activity[30]. A hypomorphic mutation of the catalytic sub-unit of DNA polymerase ε rescues the hypersensitivity of the *atm* mutant to TOP1 inhibition, indicating that slowing-down replication alleviates DNA-damage accumulation triggered by TOP1 inactivation, likely by reducing transcription/replication conflicts[30].

Finally, it is reasonable to expect replication-transcription collisions on genes' transcription start sites (TSS), as origins of replication (ORI) often colocalise with these regions in *Arabidopsis thaliana*[87].

Chromatin organization and dynamics are essential to the maintenance of genome integrity

Chromatin features and dynamics are well-recognized key elements in the control of genome stability, notably through their role in the control of TE mobilization. Here we will focus on mechanisms that are specific to the maintenance of genome integrity i.e., on the direct effects of chromatin features and dynamics at the site of DNA damage.

Can chromatin condensation protect DNA from damaging agents?

Extreme chromatin condensation is commonly found in organs or cell types that are strongly exposed to environmental insults such as gametes, pollen grains or seeds[88,89]. In animals, chromatin compaction in male gametes mediated by protamins incorporation is thought to protect DNA from genotoxic stress[90]. Protamins are not conserved in plants, but the histone variant H2B.8 has recently been postulated to play a similar role in generative nuclei of pollen grains[91]. Similarly, nuclei of dry-seed embryos are extremely small and contain highly condensed chromatin, and seed imbibition triggers gradual chromatin decondensation and increase in nuclear size[92], suggesting that chromatin compaction is an important parameter to protect DNA from accumulation of damage.

Whether or not heterochromatin is generally better protected from DNA damage and accumulation of mutations in all organs and cell types is a complex question. Indeed, in human cells, compacted chromatin was found to be 5 to 50 times less prone than decondensed chromatin to accumulate DSBs after ionizing irradiation[93,94]. In addition, naked DNA is more susceptible than nucleosome-associated DNA to all kinds of DNA lesions and even more strikingly, how DNA interacts with nucleosomes shapes the distribution of damage, resulting in a marked bias for the accumulation of lesions on the DNA strand facing away from the histone surface[95].

The few studies performed in plants draw a mixed picture of the relationships between chromatin compaction and DNA damage accumulation. In Lolium, putative fragile sites identified as regions faintly stained with DAPI in mitosis due to lower DNA compaction are not more susceptible to γ-ray-induced DSBs than other regions of the genome[96]. Conversely, genotoxic stress seems to increase chromatin compaction in *Medicago* through the activation of the SUVH2 histone methyl-transferase[97], which supports the notion that chromatin condensation might act as a protective mechanism against the occurrence of further damage. Likewise, auxin starvation increases chromatin accessibility, which results in higher sensitivity to DNA damaging agents in tobacco cell cultures[98]. In the same line, loss of rDNA in *fas* mutants seems to result from enhanced chromatin accessibility. These mutants display a global replacement of the H3.1 histone variant, that is normally incorporated during DNA replication, by the H3.3 variant that is normally incorporated independently of DNA-replication at highly transcribed genes[99,100]. This excess incorporation of H3.3 depends on the NAP1;1, 2 and 3 histone chaperones, known to be involved in histone trafficking, nucleosome assembly and disassembly[101]: inactivation of these three chaperones rescues the genomic instability of *fas1* mutant by restoring H3.3/H3.1 ratios and decreasing the accessibility of chromatin, demonstrating that chromatin compaction plays a key role to avoid illegitimate recombination events that arise when nonhomologous recombination occurs[102].

Paradoxically, although expressed regions of the genome are more accessible and could thus be expected to accumulate more lesions, coding sequences of genes actually accumulate fewer mutations than the rest of the genome in Arabidopsis, and this phenomenon cannot be solely attributed to counter-selection of deleterious mutations through evolution[103,104]. This apparent contradiction likely results from the fact that in spite of the protective role of chromatin condensation, it simultaneously precludes access to the DNA repair machinery.

Chromatin modifications favour the recruitment of the genome surveillance machinery at transcriptionally active loci and limit recombination events during DNA replication

The general assumption that mutations occur randomly has recently been challenged by the demonstration that the mutation rate is not uniform along the genome in *Arabidopsis* and rice[16,103–105], and that EMS-induced mutations are unevenly distributed and accumulate preferentially in lowly expressed regions and heterochromatin in Arabidopsis[106], likely because the poor accessibility of silent loci precludes their efficient repair, as observed in Mammals[107]. Because EMS-induced mutations in rice and wheat were mapped through exome sequences, their relative frequency in eu- and heterochromatin cannot be compared, but the probability of EMS-induced mutations seems to be affected by the methylation status of cytosines in both species [108,109].

In addition, the repair machinery can be actively targeted to expressed genes. The activity of the MMR pathway depends on the chromatin context[110], and is targeted to protein-coding genes through the ability of the MSH6 protein to recognizes the H3K4me1 mark, that is specifically enriched at transcribed gene body[111]. This suggests that cellular mechanisms cooperate to target the machinery involved in the repair of punctual DNA lesions preferentially to coding sequences. This hypothesis is further supported by the existence of the transcription-coupled NER pathway (see below).

DSB repair also seems to be favoured in euchromatic regions. Indeed, PDS5C, a protein involved in HR, binds H3K4me1, and is enriched in coding sequences and essential genes[111]. Similarly, the SMC5/6 (structural maintenance complex) complex is recruited to DSBs thanks to the transcriptional coactivator ADA2b (Alteration deficiency in Activation Complex b)[112], that is enriched at transcriptionally active genes. This targeting of the HR machinery to transcriptionally active regions may also involve a specific class of sncRNA called diRNAs (Damaged-Induced RNAs[113]) whose role will be discussed later in this review (Fig. 5a).

During DNA replication, the repair of broken replication forks is also tightly controlled by histone modifications (Fig. 5b). In *atxr5/6* mutants, deficiency in H3K27me1 deposition leads to re-replication of heterochromatin[114], through the activation of the DDR[115]: TONSOKU (TSK), a key player in the

initiation of HR-mediated repair at stalled replication forks, binds exclusively to unmethylated histone H3.1, and facilitates the recruitment of Polθ, the DNA polymerase responsible for alt-NHEJ[116]. Newly incorporated H3.1 histones very rapidly become mono-methylated on lysin 27 by ATRX5 and 6 that are thought to travel with the replication fork[114], which restricts TSK binding and thus Polθ recruitment, thereby avoiding the duplication of heterochromatic sequences[116].

Thus, a wealth of mechanisms cooperates to favour DNA repair on open, accessible chromatin, particularly in transcriptionally active regions and newly replicated regions. However, dedicated mechanisms also allow driving the repair machinery to heterochromatin. Indeed, the DDB2 protein is both a key player of the GGR-NER (Global Genome Repair-Nucleotide Excision Repair) pathway[117] and of DNA methylation[118,119]. The simultaneous involvement of DDB2 in DNA repair and DNA methylation might play two roles: it may facilitate the recruitment of the DNA repair machinery to regions of the genome that are less accessible thanks to the ability of DDB2 to interact with methylated regions, and allow the restauration of appropriate chromatin states once DNA repair is complete.

Overall, these studies demonstrate that chromatin organization and function are important factors influencing the formation of DNA damage and the activity of the genome surveillance machinery. In addition to that, chromatin dynamics plays a direct role in the response to DNA damage and in the DNA repair process.

Histone post-translational modifications occur at the site of damage and are essential for DNA repair

In all eukaryotes including plants, the best characterized chromatin modification related to the DDR is the accumulation of γH2AX (H2AX variant phosphorylated on S139) around DSBs, thanks to the activity of ATM and ATR kinases[120,121]. The H2AX histone variant is present on all the genome, and enriched on gene body, but depleted at centromeres[122], allowing rapid detection of DSBs without requiring the *de novo* incorporation of histone variants. γH2AX allows signalling of DNA damage and recruitment of repair factors at break sites through the involvement of γH2AX readers. Because most players involved in this process are present in plant genomes and required for DSB repair [123–125], this sequence of events is likely conserved. Very recently, two putative readers of γH2AX: XIP (H2AX Interacting Protein)[126] and BCP4 (BRCT Protein 4)[127] have been identified, and co-localize with γH2AX, but their precise function remains to be elucidated. In human cells and yeast, γH2AX distribution seems to be governed by pre-existing chromatin conformation: the spreading of this histone mark near the DNA break seems to be controlled by cohesin-mediated loop-extrusion, and to cover the entire TAD (topologically associating domain) in which the break formed[128]. Plant genomes are organized in TAD-like structures that resemble TADs, in the sense that loci inside these regions tend to interact more with each other than

with the rest of the genome[129]. However, TAD-like structures are not functionally equivalent to TADs as they are extremely gene-poor, and genes are found mainly on the borders of these structures[129]. It is also interesting to note that these TAD-like structures are scarcely found in Arabidopsis compared to plant species with bigger genomes such as rice[130]. Therefore, the mechanisms governing the distribution of γH2AX in plants may differ significantly from what has been discovered in other eukaryotes, and may be different from one plant species to another.

Besides γH2AX accumulation, many other histone modifications are deposited or removed at the sites of DNA damage in yeast and animals[131]. In plants, a few histone modifications have been shown to play a role in DSB repair. First, H3K4me2 removal at damaged sites by the LDL1 protein would be important to release the chromatin remodeller RAD54 sites during HR, thereby allowing normal progression of the repair process[132]. By contrast, SUVH2, that is required for the deposition of H3K9me2, partially co-localizes with γH2AX foci in *Medicago*, and appears to recruit RAD51[97]. Lastly, the Polymerase-Associated Factor Complex (PAF1C) was recently identified as a new component of DSB repair[133]. PAF1C recruits the E2 Ubiquitin-conjugating enzymes UBC1/2 and E3 ligases HUB1/2 that mediate H2B mono-ubiquitination at DSBs, thereby promoting DNA repair through HR[133]. Given that several chromatin modifiers have been identified as ATM or ATR targets[134], other histone modifications are likely to play a role in DSB repairs in plants.

Chromatin remodelling and mobility are essential to efficient DNA repair

As discussed above, association of DNA with nucleosomes into chromatin can prevent access of the DNA repair machinery to these lesions. Therefore, the activity of remodellers is crucial to give access to the repair machinery in[95]. ATP-dependent Chromatin Remodellers (ACRs) are multi-subunit protein complexes that utilize the energy freed by ATP-hydrolysis to slide, evict, exchange or dismantle nucleosomes. They are classified according to the family of their catalytic sub-units between four groups: SWI/SNF2 (Switch Sucrose Non-Fermentable), SWR1 (SWI/SNF-related 1), CHD (Chromodomain Helicase DNA) and ISWI1 (Imitation Switch 1). Their contribution to excision repair pathways or photoreactivation has been little investigated in plants[135]. Campi and colleagues did report increased CPD accumulation and UV sensitivity in maize and Arabidopsis plants deficient for a number of chromatin-remodellers, but whether this reflects a direct role of the corresponding protein to facilitate access of the repair machinery to DNA has not been formally proven[136].

Chromatin remodelling is also essential to all steps of DSB repair, namely DNA end resection (a step that is required for all DSB repair pathways except cNHEJ), and the HR-specific processes (homology search, strand invasion and holiday junction resolution[137]). Members of all families have been shown to contribute to DSB repair in animals and yeast and these roles are likely to be conserved in

plants[135,138]. Strikingly, in many instances, direct evidence for the role of ACRs subunits in plant DSB repair remains scarce. For example, systematic analysis of SWI/SNF2 mutant and RNAi lines revealed that most of them were hyper-sensitive to γ-irradiation[139], but since then, the direct involvement of most of these factors in the DNA repair process was not further investigated. Because detailed description of all ACRs putatively involved in DSB repair is available in[135,138], we will focus on the few ACRs that have been unequivocally involved in DNA repair (listed on Fig. 5c). First, the SWI3B chromatin remodeler was recently shown to promote dissociation of SMC5 from chromatin, to facilitate its recruitment to DSBs[140] where it contributes to DNA repair, possibly by promoting cohesion between the damaged DNA molecule and the undamaged repair template[141]. Second, CHR721, a novel member of the SMARCAL1 sub-family known to reanneal stalled replication forks in response to DNA damage in human[142], was identified in rice[143]. CHR721 interacts with the DNA repair protein RPA1A[143] and with the DNA polymerase processivity factor PCNA[144], suggesting that it is directly involved in DSB repair and/or replication stress response and fork stabilization, like its human homolog[145]. Finally, the best characterized member in the SWI/SNF2 family for its role in DSB repair is RAD54, that unlike other ACRs, is a core component of the enzymatic machinery responsible for HR and DSB repair[146]: it participates to the stabilization of nucleoprotein filaments, facilitates D-loop formation and heteroduplex extension[147]. In plants, it has been shown to form DNA repair foci that are mobile, and tend to localize at the nuclear periphery[148,149]. Also, it likely favours homologous recombination by mediating homologous loci pairing following γ-irradiation[150]. Interestingly, DSB induction results in a global increase in chromatin mobility, particularly in the S and G2 phases of the cell cycle, suggesting that homology search could be favoured both by a global increase in chromatin mobility, and by RAD54-mediated movement of the damaged site[151].

Members of the INO80/SWR1 family have also been implicated in DSB repair: *ino80* mutants are deficient for somatic HR[152], and mutants lacking the *arp4* and *arp5* sub-units of the same complex are hypersensitive to DNA damage[153]. Importantly, the accumulation of γH2AX foci and the expression of DNA repair genes are unaffected in *ino80* mutants, providing evidence for its direct role in the DNA repair process downstream of γH2AX foci formation[154]. Finally, the SWR1 complex is required for HR but not for cNHEJ[155]. This observation clearly supports a direct role of SWR1 in the repair process, because cNHEJ does not require DNA end resection and extensive chromatin remodelling like HR or alt-NHEJ. In addition, PIE1, the catalytic sub-unit of the SWR1 complex, is phosphorylated by ATM and ATR in response to γ-irradiation[134].

Finally, several ACRs have been shown to be targeted by post-translational modifications associated with DNA repair. This is the case for the SWI/SNF2-related protein BRAHMA, that can be SUMOylated

by MMS21[156], a sub-unit of the SMC5/6 complex[156,157]. Likewise, phospho-proteomics identified several chromatin remodellers as targets of ATM and ATR, supporting their role in the DDR[134], but a global view of the respective roles of chromatin remodellers during the plant DDR is still missing.

Role of RNA metabolism in the maintenance of genome integrity

Transcriptional changes in protein coding genes are obvious central mechanisms involved in the DDR, but they have been extensively reviewed elsewhere[7–9] and will not be detailed here. In this paragraph, we will briefly review the direct involvement of RNAs and RNA metabolism in DNA repair.

Transcription-coupled DNA repair avoids accumulation of mutation in transcriptionally active regions

In addition to the recruitment of various DNA repair components at region enriched in active chromatin marks that we described earlier, transcription itself is coupled to DNA repair. Indeed, repair of UV-induced DNA damage is more active at transcribed genes, with a strong preference for the transcribed strand[158]. When RNA PolII encounters a transcription-blocking lesion, RNA elongation stops, which leads to the recruitment of the nucleotide excision repair machinery[159]. The components of Transcription Coupled (TC)-DNA repair are conserved between eukaryotes, and Arabidopsis mutants lacking these proteins are hypersensitive to UV-irradiation. The current model of TC-NER in plants is thus the following (Fig. 6a). Stalled RNA PolII is recognized by the SWI/SNF chromatin remodeller CHR8 (CSB in humans[139]). CHR8 then recruits the Cockaine Syndrome A-DDB1-CRL4 complex which ubiquitinates CHR8[160,161], and the UVSSA and UBP12 (UVSS7 in humans) proteins that stabilize it[162]. Finally, core NER proteins such as TFIIH and the XPG and XPF endonucleases are recruited to allow DNA excision on each side of the lesion. The RPA protein coats the ssDNA formed in front of the newly generated gap, until it is filled by the joint activity of a DNA polymerase and a ligase[3], and transcription re-initiation is likely mediated by the RDO2 (TFIIS in humans) elongation factor[162]. Interestingly, TC-repair is strongly modulated by the circadian clock in Arabidopsis[158], highlighting the crucial importance of this DNA repair pathway in organisms for whom sunlight is an indispensable source of energy.

The diverse roles of non-coding RNAs

The existence of mechanisms targeting NER towards heterochromatin appears all the more important considering UV-induced damage is particularly abundant in heterochromatin[19]. This is achieved through the so-called GG-NER (Global Genome NER) process (Fig. 6a). In this pathway, the NER machinery is targeted to heterochromatic regions thanks to siRNA produced by the RdDM (RNA-Directed DNA Methylation) machinery: UV-induced DNA damage triggers the production of 21-nt RNAs

called uviRNA that are loaded by the AGO1 protein, which in turn guides DDB2 recruitment to damaged regions, thereby promoting NER at damaged sites[163].

RNA metabolism occurring directly at the damaged site may also play a role in DSB repair. Indeed, DSB induction inside a reporter transgene was found to induce the production of small non-coding RNAs called diRNAs (Damage-Induced RNAs) by RNA PolIV at the vicinity of the break. These diRNAs were proposed to favour DSB repair, although the underlying mechanism remains unclear in plants[164]. This model was later complexified by the finding that diRNAs do not seem to be produced at DSBs triggered outside of a transgene either by CRISPR or TALEN, suggesting that they originate exclusively from highly expressed loci and resemble small RNAs produced by post-transcriptional gene silencing[165]. Furthermore, efficient DNA repair was observed at loci where diRNA production could not be detected[165], which contradicts the initial hypothesis that these snRNA would be important for DNA repair. Thus, diRNA may result from the production of aberrant transcript at damaged sites, in which case their role could be to silence damaged loci and avoid the accumulation of aberrant transcript until DNA is repaired. However, in mammals, diRNA were found to allow the recruitment of chromatin modifiers and remodellers, thereby facilitating the access of the DNA repair machinery and promoting HR[166]. Because HR was not directly measured in plants[165], one cannot rule out that diRNA accumulation could function to favour HR on highly expressed loci (Fig. 6b), whereas diRNA-free loci could be repaired by alt-NHEJ. Further studies will be required to clarify this point.

Finally, a number of long non coding RNAs (lncRNAs) have been shown to be induced by DNA damage, and to be involved in Arabidopsis' tolerance to genotoxins[167,168]. They could play a role in the post-transcriptional regulation of the DDR, but may also contribute to DNA repair or DDR signalling through various mechanisms, possibly by interacting with protein complexes[169]. Molecular dissection of their precise function should help clarify this point.

Concluding remarks

Although many questions remain open, the current knowledge regarding the relationships between chromatin dynamics and maintenance of genome integrity provides and interesting frame-work to understand how DNA lesions form and are repaired or lead to mutations. It is now clearly established that several mechanisms cooperate to safeguard the genetic information more efficiently in coding regions. These mechanisms allow compensating the greater exposure of expressed regions to damage, and safeguarding essential genetic information. Conversely, more frequent lesion accumulation and less efficient repair in non-coding sequences may provide plasticity to plant genomes and allow the emergence of genetic diversity, notably in terms of gene regulation.

On a more mechanistic point of view, future work should help better understand how chromatin dynamics contributes to DNA repair globally, and depending on the chromatin context. Furthermore, several lines of evidence point to a direct relationship between the maintenance of genome and epigenome integrity. Indeed, many mutants deficient for DNA replication proteins have been identified in genetic screens designed to find components of the constitutive heterochromatin maintenance (see[170] and references therein). A challenge for the years to come will be to dissect this coupling between the maintenance of the genetic and the epigenetic information, and notably to understand how chromatin states are restored after DNA replication or repair.

Finally, another challenge will be to understand how different cell types deal with lesion formation and repair, and how specific chromatin features or responses to DNA damage could help safeguard genome integrity more efficiently in stem cells than in other cell types, a key point in organisms such as plants in which most of the development occurs post-embryonically.

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Competing interests

Authors declare no competing interests.

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Contributions

C.R. and C.B-S. conceptualized the manuscript framework. C.R., C.B-S., and M.R. wrote the original draft of the manuscript. C.B-S, C.R., and M.B. produced, reviewed and edited the figures. M.B., D.L., and C.B. reviewed the manuscript. C.B-S. and C.R. edited the manuscript.

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943 **Table 1: Summary of the main proteins involved in DNA repair or chromatin modification**
944 **mentioned in the Figures**

Figure number	Protein/gene name	Full name	Roles	Key Publications
Fig 2.	ARP, APE1L, APE	Apurinic endonuclease-redox protein Apurinic/apyrimidinic endonuclease APE-like	DNA repair proteins: Endonucleases that process the 5' and 3'end of the AP site	171
Fig 2.	DML	Demeter-like	Glycosylases involved in DNA demethylation	21
Fig 2.	XRCC1	X-Ray Repair Cross Complementing 1	Coordinates the stages of BER thanks to its scaffolding role	172
Fig 2.	ZDP	zinc finger DNA 3'-phosphoesterase	DNA dephosphorylase: Dephosphorylates the 3'end of the AP site	171
Fig 3.A	AtALBA1/2	Acetylation Lowers Binding Affinity	Protect R-loop structures to avoid DNA breaks	72
Fig 3.B	DDM1	Decrease in DNA Methylation 1	Chromatin remodeler: Avoids unscheduled R-loops that trigger DNA damage	74
Fig 3.C	*COOLAIR*	COOLAIR	FLC antisense transcript down-regulating FLC after R-loops resolution on its promoter	82,83
Fig 3.C	FCA/FY	Flowering time Control protein A/ Flowering Y	RNA binding (FCA) and RNA processing (FY) proteins that allow R-loops resolution and replication fork progression	86
Fig 3.C	FLC	Flowering Locus C	Flowering repressor which transcription and replication are controlled by R-loops regulation	80,83
Fig 3.C	FLD/LD/SDG26 complex	Flowering Locus D/ Luminidependens/ Set Domain Group 26	Protein complex comprising the Histone Demathylase FLD, the Histone methyl-transferase SDG26, and LD, a putative transcription factor: Decreases H3K4me1 level that can lead to chromatin silencing and remodeling	82,84
Fig 4.A	ADA2b	Alteration Deficiency in Activation	Transcriptional co-activator: Recruits SMC5/6 at transcriptionally active genes	112
Fig 4.A	MSH6	MutS Homolog 6	MMR protein: Targets the DNA repair machinery to protein-coding genes	111
Fig 4.A	PDS5C	Precocious dissociation of sisters 5	Associate protein of cohesins, involved in homologous recombination and binds to the active chromatin mark H3K4me1	111
Fig 4.A	SMC5/6	Structural maintenance of chromosomes proteins 5 and 6	DNA repair, perhaps through cohesion between damaged DNA and undamaged DNA template	141

Fig 3.B	ATR	Ataxia Telangiectasia-Mutated and Rad3-related	Kinase activating the DDR pathway in response to replication stress	[7,9]
Fig 4.B	ATRX5/6	Arabidopsis Trithorax Related 5 and 6	Histone methyltransferases that deposit the H3K27me1 mark: Avoid unscheduled re-replication by monomethylating H3K27 thus avoiding Polθ binding	[116]
Fig 4.B	Polθ	DNA polymerase Theta	TLS polymerase: rescue of stalled forks rescue thanks to alt-NHEJ repair pathway	[116]
Fig 4.B	TSK	TONSOKU	Facilitates Polθ recruitment at newly synthesized chromatin wrapped with unmethylated histones H3.1	[116]
Fig 4.C	ATM	Ataxia Telangiectasia-Mutated	Kinase activating the DDR pathway when DSBs occur	[7,9]
Fig 4.C	BRAHMA	BRAHMA	Could contribute to DSBs repair thanks to its SUMOylation by a subunit of SMC5/6 complex	[156]
Fig 4.C	CHR721	Chromatin 721	Could be involved in DSBs repair and replication stress response	[143,144]
Fig 4.C	INO80/SWR1	INOSITOL 80/ Switch-Sucrose Non-Fermentable Related 1	Chromatin remodeler Involved in DNA repair through homologous recombination	[152,155]
Fig 4.C	RAD54	Radiation 54	Chromatin remodeler involved in the enzymatic machinery for HR and DSBs repair	[146,150]
Fig 4.C	SWI3B	SWITCH	Dissociates SMC5 from chromatin and helps with its recruitment to DSBs	[140]
Fig 5.A	AGO1	Argonaute 1	sRNA-binding protein: Guides DDB2 recruitment to damaged regions when loaded with UV-induced RNAs	[163]
Fig 5.A	CHR8	Chromatin Remodeller 8	Chromatin remodeller that recognizes stalled RNA PolII and recruits CSA/DDB1/CRL4 complex, UVSSA, and UBP12	[160–162]
Fig 5.A	UVSSA;UBP12	UV stimulated scaffold protein A/ Ubiquitin Binding Protein 12	Stabilize CSA/DDB1/CRL4 complex	[162]
Fig 5.A	CSA/DDB1/CRL4 complex	Cockaine Syndrome A/DNA Damage Binding 1/Cullin 4 Ring Ligase 4	Protein complex that ubiquitinates CHR8 and helps with the recruitment of nucleotide excision repair proteins	[160]
Fig 5.A	DDB2	DNA Damage Binding 2	Forms a heterodimer with DDB1 that recognized DNA lesions: Controls DNA methylation pattern genome wide and is involved in DNA repair through the GGR-NER pathway	[117–119]

Fig 5.A	TFIIH	Transcription Factor IIH	Heterotetramer with helicase activity: Helps to partially unwind DNA followed by XPG and XPF recruitment	[3]
Fig 5.A	XPG; XPF	Xeroderma pigmentosum G and F	Endonucleases that excise DNA around lesions	[3]

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Figures

Figure 1: Pathways involved in chromatin dynamics or RNA metabolism involved in DNA damage formation or genome maintenance in plants

Left Panel: From top to bottom; specific genomic features such as the abundance of methylated cytosines can lead to compacted chromatin and spontaneous lesion formation through deamination, leading to the accumulation of mutations even when the BER pathway is mobilized (Fig. 2 and Fig. 3a). Telomeres are prone de oxidation due to their G-rich sequences, must be shielded from the DSB repair machinery to avoid inappropriate repair, and pose the end-replication problem. Topological stress can induce breaks and topoisomerases relieve this stress by cutting DNA, thus endangering DNA integrity. At difficult-to-replicate sites, secondary structures can form on ssDNA during replication, thus inducing replication stress followed by DNA damage (Fig. 3b). R-loops can form during transcription and lead to DNA damage if they are not protected or timely resolved (Fig. 4a and b). Transcription-replication conflicts can lead to topological stress and R-loops followed by DNA damage when not resolved (Fig. 4c).

Right panel: From top to bottom; chromatin condensation can help to prevent DNA damage under certain stress conditions. The deposition of specific histone marks that are hallmarks of expressed genes helps to recruit DNA repair agents to damaged sites at coding sequences (Fig. 5a and b), and facilitates the repair process. H2AX phosphorylation at DSB sites helps to recruit the repair machinery (Fig. 5c). ATP-dependent chromatin remodellers help to repair damaged DNA, notably through homologous recombination, but also by allowing access of the repair machinery to DNA (Fig. 5c). Transcription can directly lead to repair when encountering a lesion thanks to the transcription-coupled nucleotide excision repair pathway (Fig. 6a). Non-coding RNAs can be produced at damaged sites and helps to recruit the DNA repair machinery (Fig. 6b). Created with BioRender.com

Figure 2: Active DNA demethylation relies on the BER pathway

BER is initiated by DNA glycosylases that remove the damaged base, leaving an AP (apurinic/apyrimidic) site. Next, the 5’ and 3’ ends of the AP site are processed either by AP lyases and subsequent dephosphorylation of the 3’ end of the AP site by the ZDP (zinc-finger DNA 3’-phosphoesterase) phosphatase or by the successive action of AP endonucleases (ARP, APE1L and APE2 in Arabidopsis) and removal of the 5’deoxyribose-5-phosphate extremity by an enzyme with dRP lyase activity[171]. Gaps can be repaired either through short-patch BER, in which a single nucleotide is incorporated, and that requires only the activity of a DNA polymerase and a DNA ligase[171], or via the long-patch BER (LP-BER) in which case several nucleotides (around 3) are newly incorporated[173]. In addition to a DNA

polymerase and a DNA ligase, LP-BER also involves a FLAP-endonuclease to displace one strand and allow the incorporation of more than one nucleotide. Finally, the scaffold protein XRCC1 functions to coordinate the several stages of this process[172]. Created with BioRender.com

Figure 3: Chromatin features and DNA transactions as sources of DNA damage

a: Methylated cytosines are particularly prone to damage and mutation accumulation through two processes. First, they lead to the accumulation of AP sites, due to the activity of the BER pathway. In addition, in heterochromatic regions that are particularly enriched in methylated cytosines, recruitment of the repair machinery is less efficient, which increases the frequency of damage and accumulation of mutations.

b: Several genomic features or cellular processes can lead to fork stalling. Replication-blocking structures include R-loops, G4, hairpins, highly transcribed regions, and telomeres. In addition, fork rescue is less efficient in origin-poor regions. Regions enriched in these particular structures could correspond to common fragile sites. All these processes can cause fork stalling that can in turn lead to catastrophic damage accumulation, particularly in the absence of ATR, at least in plants with large genomes.

c: The formation of chromatin loops generates topological stress on the DNA double-helix that can be resolved by topoisomerases. In animals this process has been shown to lead to DNA damage formation independently of transcription of replication at loop-anchoring points. Whether this process also occurs in plants remains to be established. Created with BioRender.com

Figure 4: Several pathways cooperate to avoid DNA damage accumulation associated with RNA metabolism

a: R-loops are fragile structures, notably because ssDNA is more sensitive to DNA damaging agents, like MMS, but also to oxidation or cellular enzymatic activities. ALBA proteins are thought to protect ssDNA at R-loops by physically interacting with the ssDNA component of the R-loop.

b: In heterochromatic regions, DDM1 contributes to chromatin compaction and R-loop resolution by facilitating the replacement of the H2A.Z histone variant by the H2A.W histone variant. This pathway may avoid excess R-loop accumulation and thus DNA damage formation.

c: Top: Converging transcription units can generate topological stress on DNA. These colliding transcription events are prevented by the coordinated action of FLD/LD and SDG26 that slow down transcription by reducing H3K4me1 levels at these loci. In the absence of this machinery, transcription speed is not reduced which may lead to DNA damage.

Bottom: The R-loop formed at the *FLC* locus interferes with replication progression when in the head-on orientation. This suggests that the machinery involved in R-loop resolution could be required for unhampered replication fork progression: FY and FCA resolve the R-loop and recruit FLD/SDG26/LD complexes, thereby allowing H3K4me1 demethylation and slowing-down fork progression to alleviate transcription/replication conflicts. In the absence of FCA and FY, replication progression is blocked by the persisting R-loop. Created with BioRender.com

Figure 5: Chromatin modifications and dynamics play central roles in the maintenance of genome integrity

a: Several mechanisms cooperate to recruit DNA repair machineries at transcriptionally active regions. Active chromatin is characterized by high levels of H3K4me1 and histone acetylation. H3K4me1 is recognized by the MSH6 protein (involved in MMR) and the PDS5C protein (involved in DSB repair) via their Tudor domains. Finally, Ada2b that is usually associated to histone acetylation recruits SMC5/6 complexes that are involved in DSB repair.

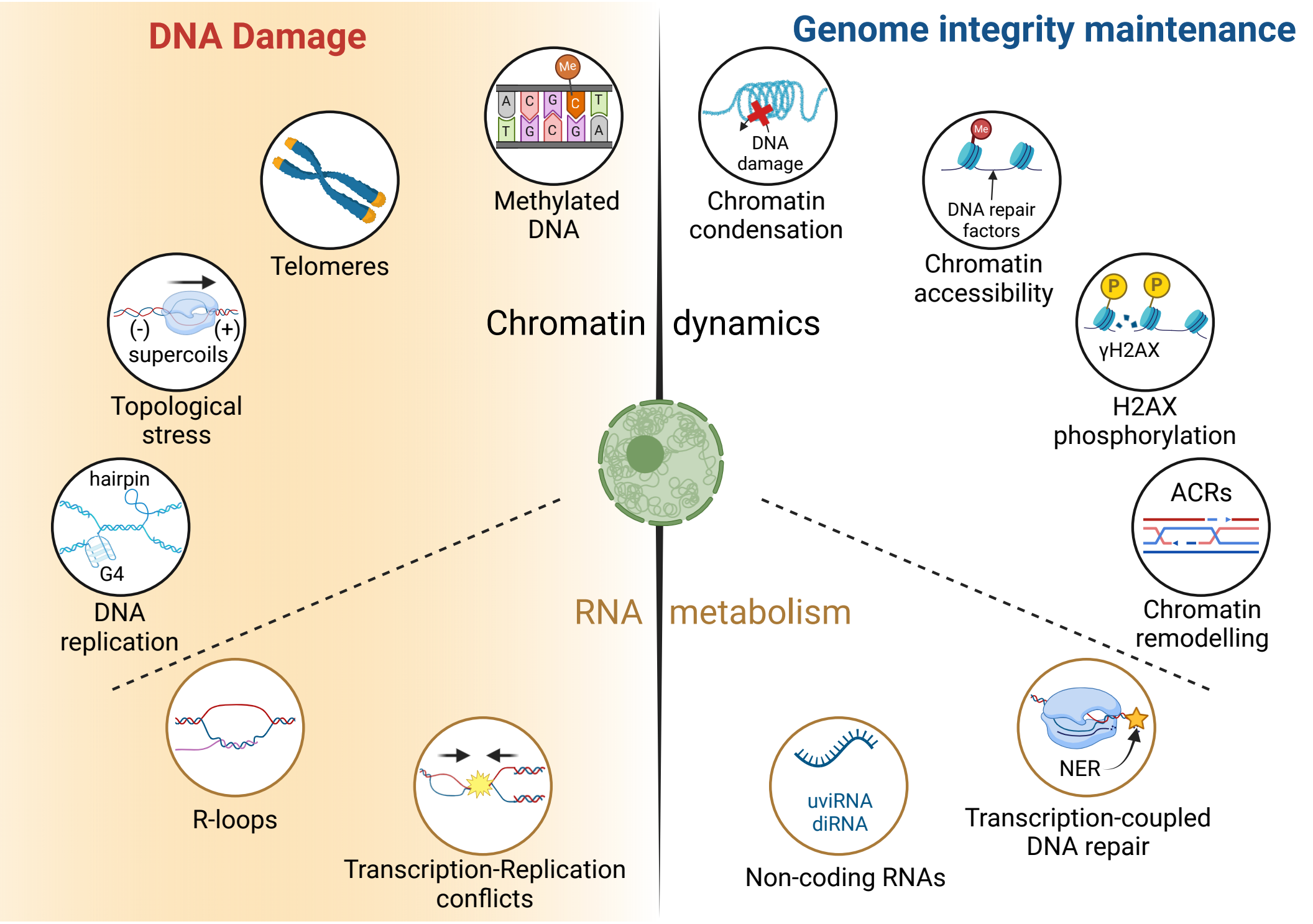
b: Histone modifications avoid illegitimate recombination at replication forks. At newly replicated chromatin, histones are unmethylated which allows the recruitment of TSK and Pol theta at stalled forks to promote fork rescue. Further away from the fork, ATXR5 and ATXR6 allow H3K27me1 deposition which precludes TSK recruitment and thus avoids illegitimate repair events leading to re-replication events.

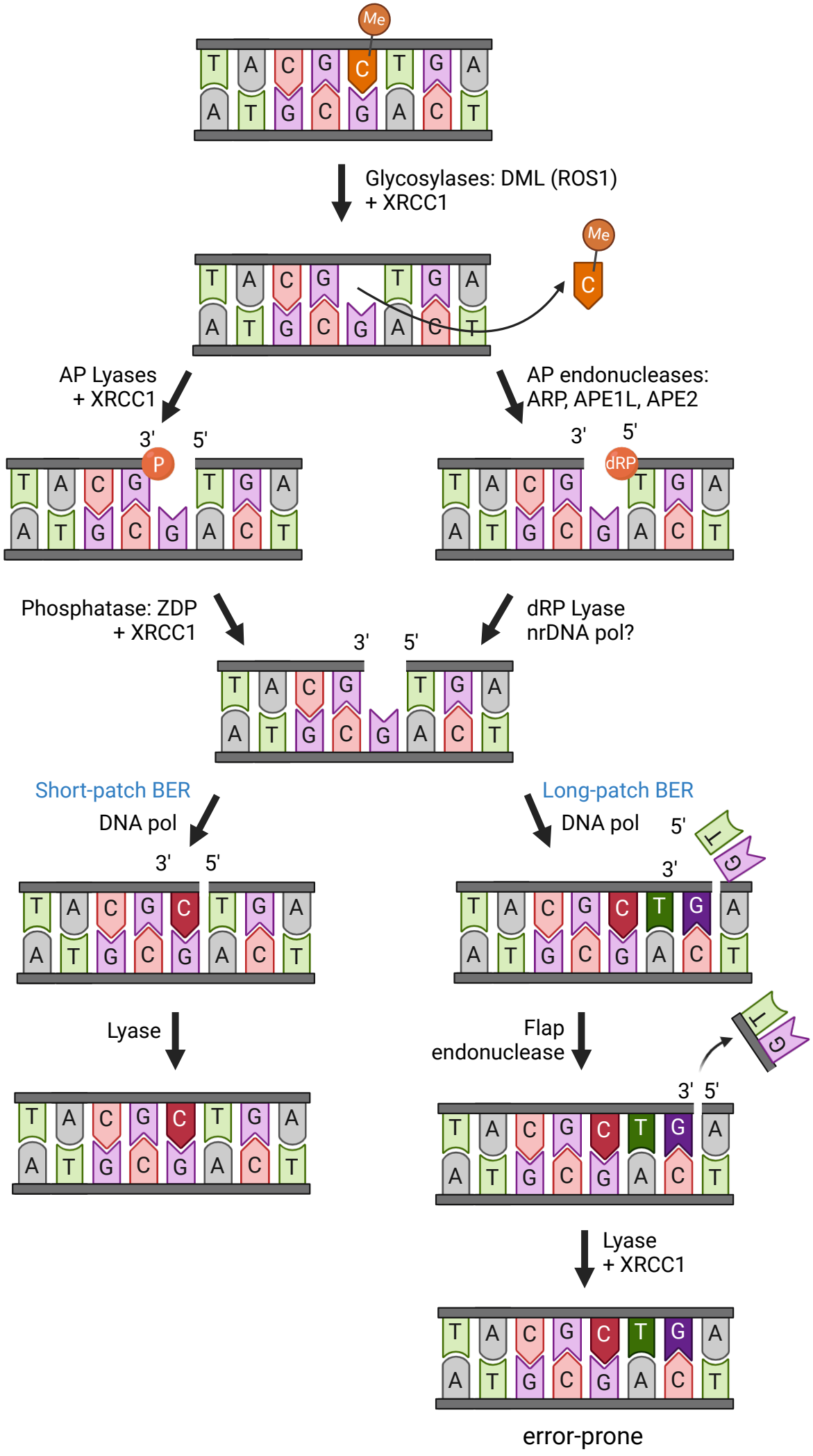
c: At DSBs, phosphorylation of histone H2AX creates a signalling platform that is crucial to recruit the repair machinery. Among proteins involved in the repair process, ACRs likely play a key role in allowing access of damaged DNA to the repair enzymes, as well as homology search in the case of HR. ACRs with a confirmed role in HR are INO80, SWR1, SWi3B, and RAD54 that play a key role in homology search. BRAHMA is also likely to play a role because it is post-translationally modified in response to DSBs, but its role has not been fully elucidated yet. Likewise, CHR721 may be involved, but it could also play a role in fork repair during replication stress. Created with BioRender.com

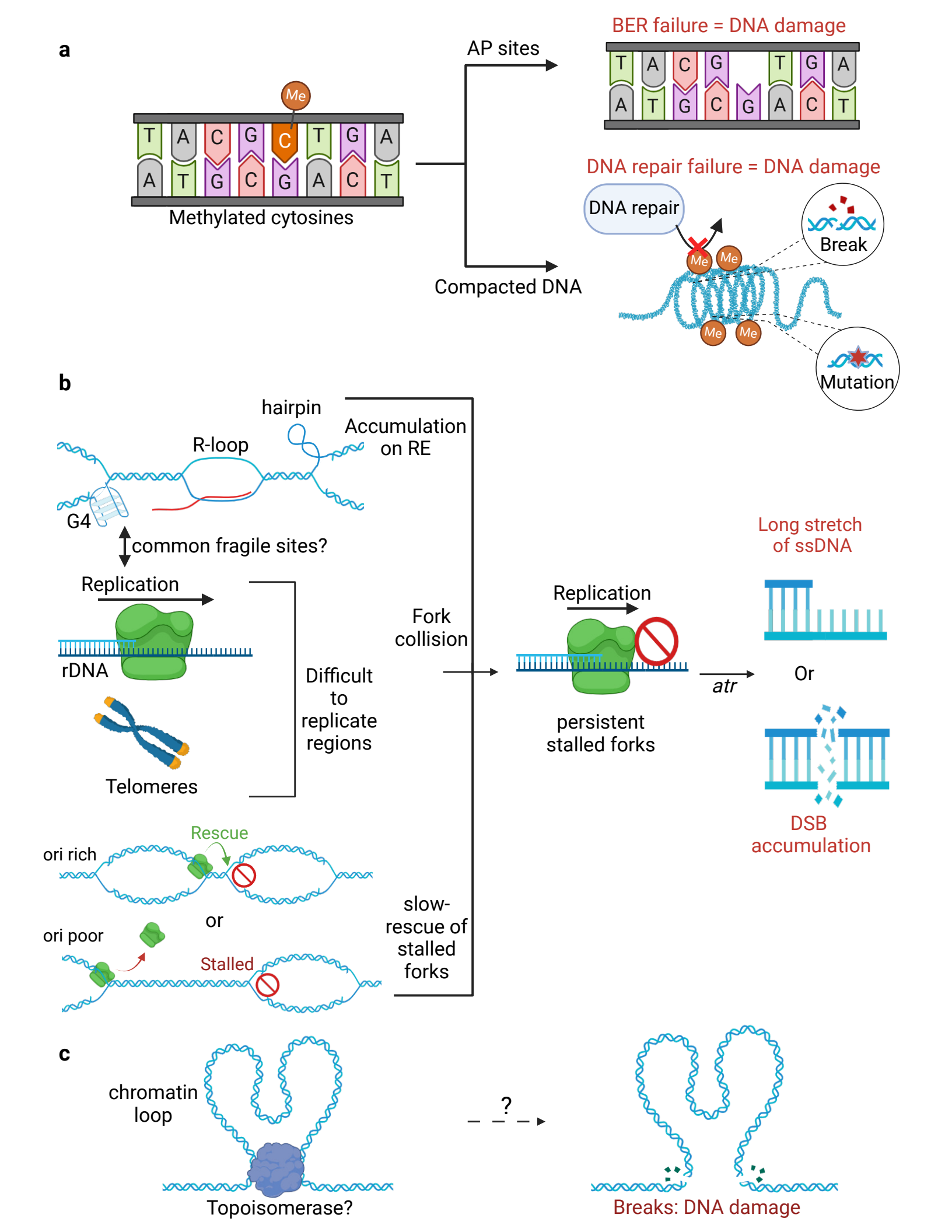
Figure 6: RNA metabolism plays a key role in the maintenance of genome integrity

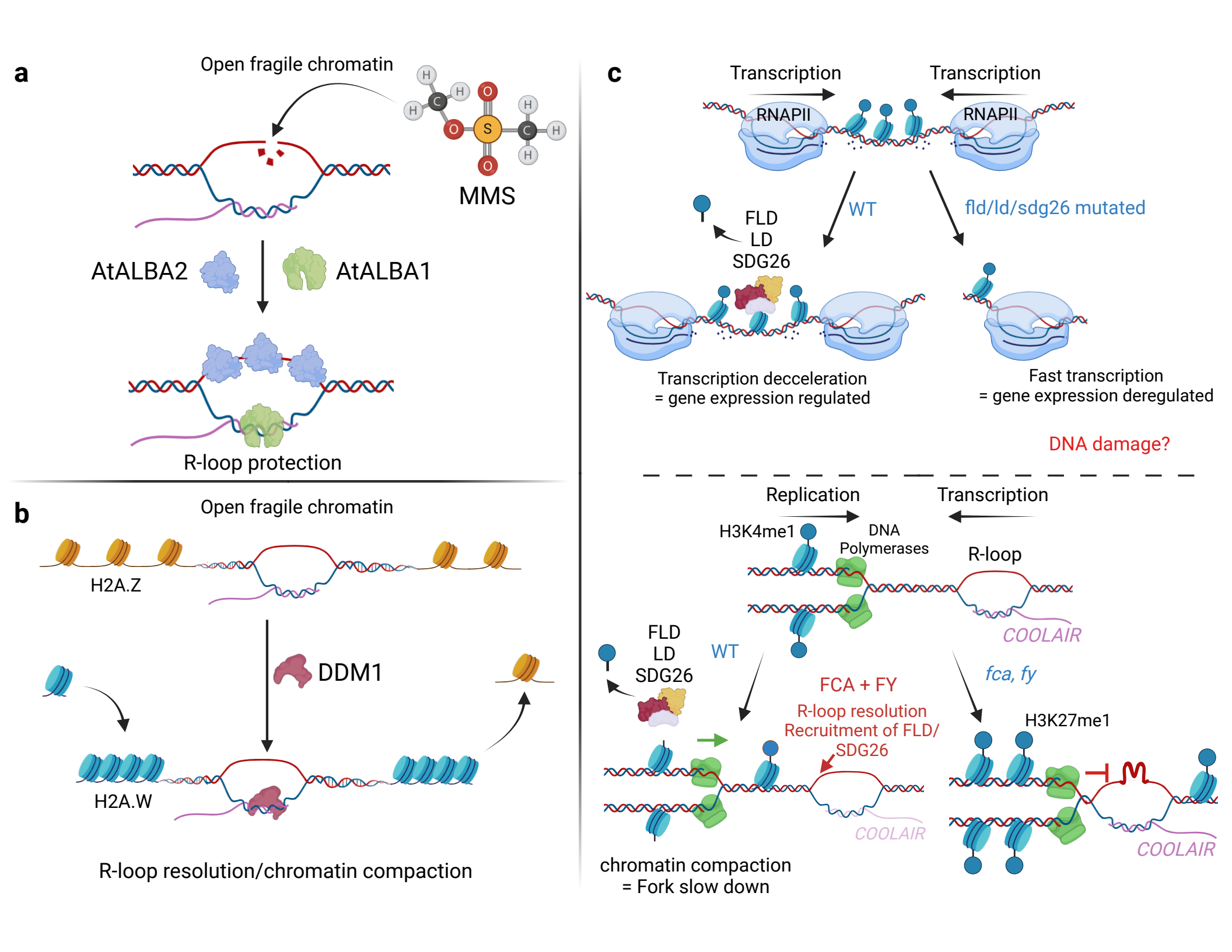
a: Two distinct mechanisms are involved in the recruitment of the NER machinery on chromatin. At actively transcribed genes, RNA PolII progression arrest due to bulky lesions such as CPD triggers the recruitment of a complex comprising CHR8, UVSSA, and UBP12, and due to the ubiquitination of CHR8 by the CSA/DDB1/CRL4 complex. Next, this complex allows recruiting the NER machinery comprising TFIIH, XPG, and XPF. At heterochromatin, uviRNAs produced by the RdDM machinery allow the sequence-dependent recruitment of AGO1 and DDB2 that activates the NER machinery.

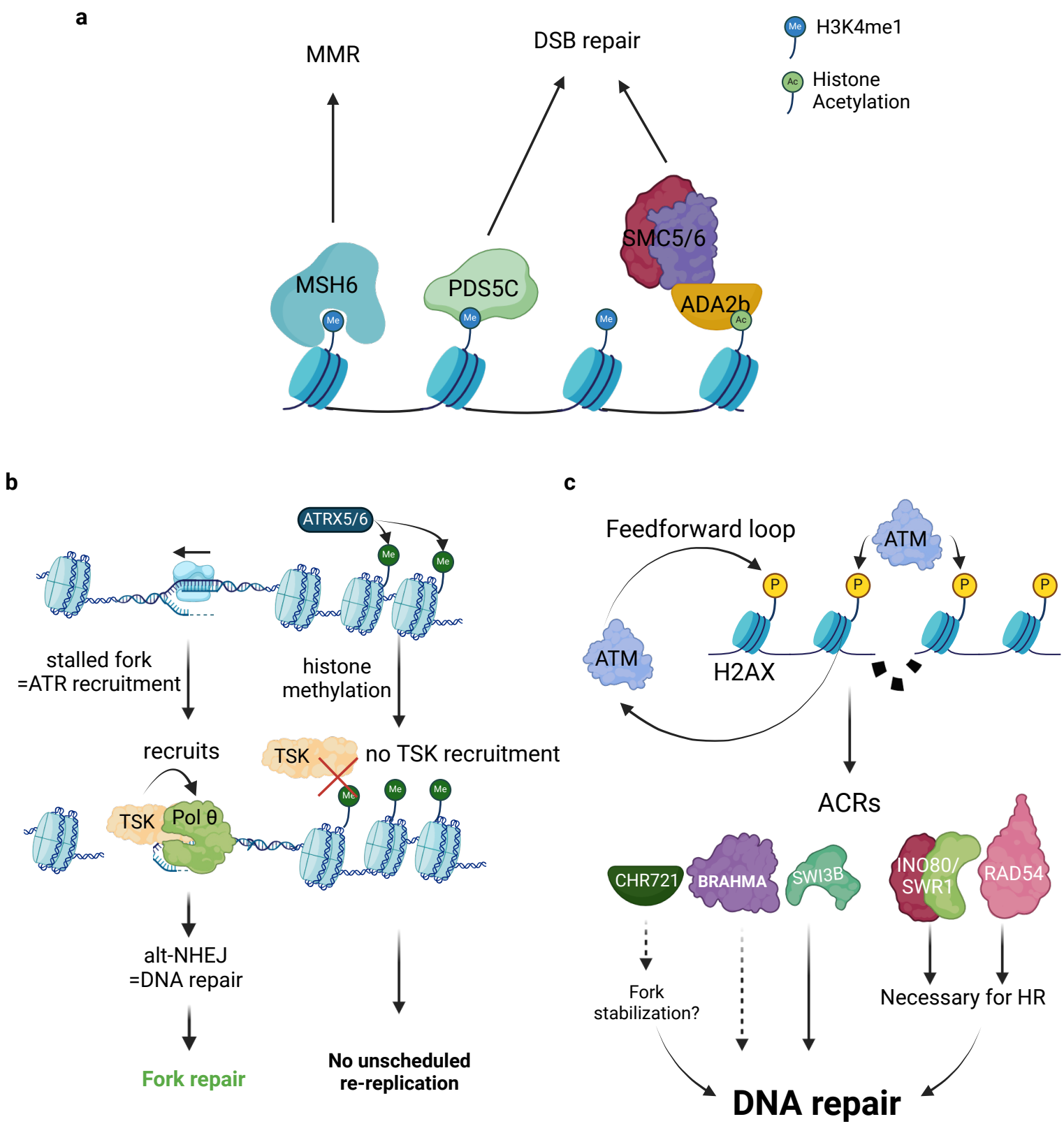
b: diRNAs appear to play a role in DSB repair. They are produced at highly transcribed loci and could function to recruit chromatin remodellers to promoter HR-mediated DNA repair. Created with BioRender.com

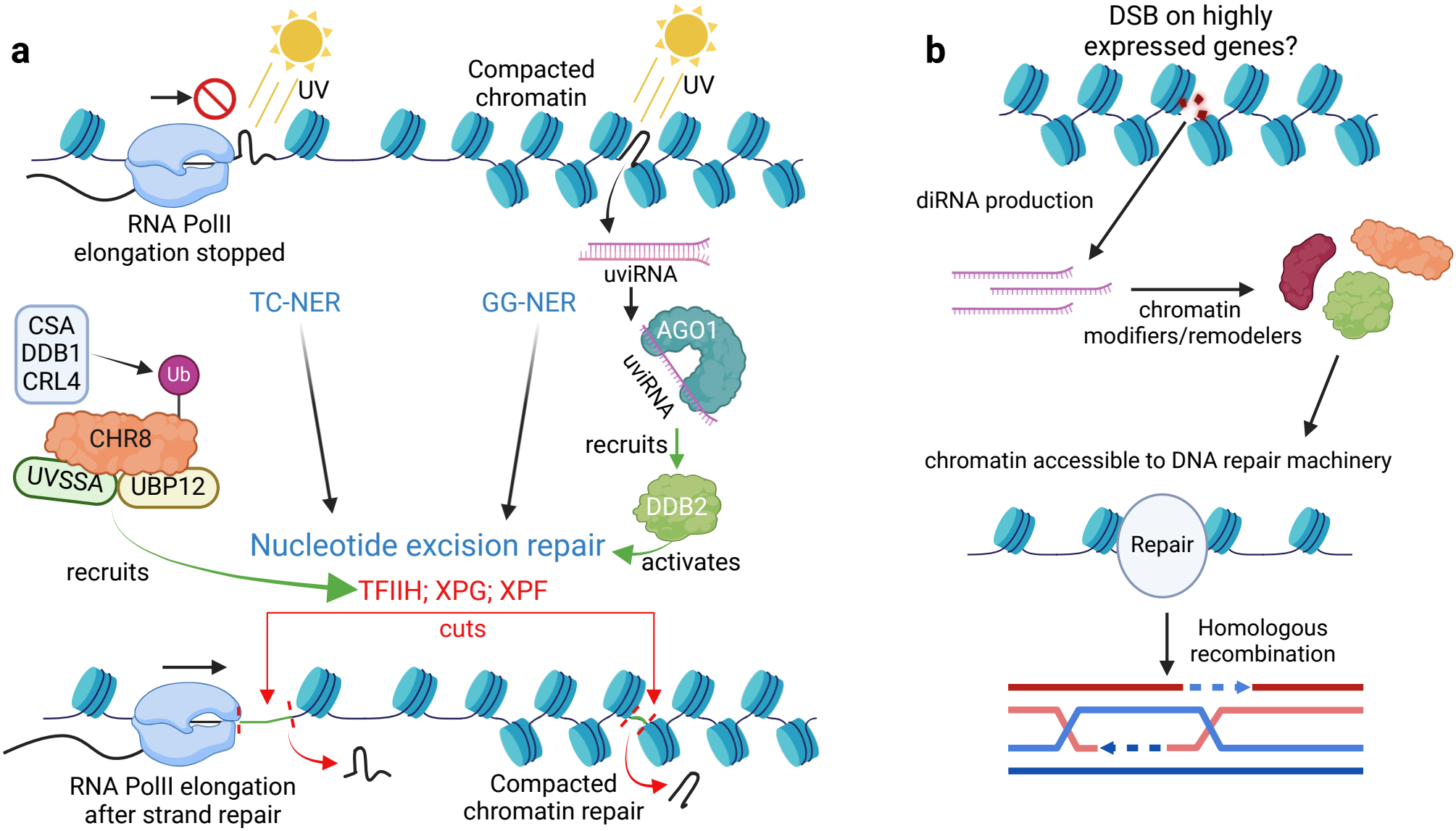

DNA Damage
Genome integrity maintenance
Telomeres
Me
A C G C T
T G C G A
Methylated DNA
DNA damage
Chromatin condensation
Me
DNA repair factors
Chromatin accessibility
(-) (+) supercoils
Topological stress
Chromatin dynamics
P P
γH2AX
H2AX phosphorylation
hairpin
G4
DNA replication
ACRs
Chromatin remodelling
RNA metabolism
R-loops
Transcription-Replication conflicts
uviRNA
diRNA
Non-coding RNAs
NER
Transcription-coupled DNA repair


Me
Glycosylases: DML (ROS1)
+ XRCC1
Me
AP Lyases
+ XRCC1
AP endonucleases:
ARP, APE1L, APE2
3'
5'
P
dRP
Phosphatase: ZDP
+ XRCC1
dRP Lyase
nrDNA pol?
Short-patch BER
DNA pol
Lyase
Long-patch BER
DNA pol
Flap
endonuclease
Lyase
+ XRCC1
error-prone


a
Me
T A C G C T G A
A T G C G A C T
Methylated cytosines
AP sites
BER failure = DNA damage
T A C G T G A
A T G C G A C T
DNA repair failure = DNA damage
DNA repair
Me
Me
Me
Me
Break
Mutation
Compacted DNA
b
hairpin
Accumulation on RE
R-loop
G4
common fragile sites?
Replication
rDNA
Telomeres
Difficult to replicate regions
Fork collision
Replication
persistent stalled forks
atr
Long stretch of ssDNA
Or
DSB accumulation
Rescue
ori rich
or
ori poor
Stalled
slow-rescue of stalled forks
c
chromatin loop
Topoisomerase?
?
Breaks: DNA damage


a
Open fragile chromatin
MMS
AtALBA2
AtALBA1
R-loop protection
b
Open fragile chromatin
H2A.Z
DDM1
H2A.W
R-loop resolution/chromatin compaction
c
Transcription
Transcription
RNAPII
RNAPII
WT
fld/ld/sdg26 mutated
FLD
LD
SDG26
Transcription decceleration
= gene expression regulated
Fast transcription
= gene expression deregulated
DNA damage?
Replication
Transcription
H3K4me1
DNA
Polymerases
R-loop
COOLAIR
FLD
LD
SDG26
WT
fca, fy
FCA + FY
R-loop resolution
Recruitment of FLD/
SDG26
H3K27me1
COOLAIR
COOLAIR
chromatin compaction
= Fork slow down


a
MMR
DSB repair
H3K4me1
Histone Acetylation
MSH6
PDS5C
SMC5/6
ADA2b
Me
Ac
b
ATRX5/6
stalled fork
=ATR recruitment
histone
methylation
recruits
TSK
Pol θ
no TSK recruitment
alt-NHEJ
=DNA repair
Fork repair
No unscheduled
re-replication
c
Feedforward loop
ATM
H2AX
P
ACRs
CHR721
BRAHMA
SWI3B
INO80/
SWR1
RAD54
Fork
stabilization?
Necessary for HR
DNA repair


a
UV
Compacted chromatin
UV
RNA PolII elongation stopped
uviRNA
TC-NER
GG-NER
AGO1
uviRNA
CSA
DDB1
CRL4
Ub
CHR8
UVSSA
UBP12
recruits
DDB2
Nucleotide excision repair
activates
recruits
TFIIH; XPG; XPF
cuts
RNA PolII elongation after strand repair
Compacted chromatin repair
b
DSB on highly expressed genes?
diRNA production
chromatin modifiers/remodelers
chromatin accessible to DNA repair machinery
Repair
Homologous recombination