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1 Chromatin dynamics and RNA metabolism are double-edged 2 swords for the maintenance of plant genome integrity

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14 Editorial summary

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

18 Abstract

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

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

35

36 Introduction

37 DNA integrity is constantly threatened by the formation of lesions that result from cellular activities
38 such as the production of reactive oxygen species by energy-producing organelles, or from DNA
39 metabolism itself. Indeed, it is estimated that each human cell is subject to approximately 70 000
40 lesions per day¹. Given that the human genome is about 3Gb long, and assuming that lesion number
41 depends solely on genome size, this would correspond to 3 000 lesions per cell and per day in
42 Arabidopsis, but almost 400 000 in wheat and roughly 60 000 in maize. Importantly, Arabidopsis is an
43 outlier in terms of genome size compared to other plant species², which implies that spontaneous
44 lesion accumulation in most plants is likely very frequent.

45 Because DNA lesions are extremely common, cells have evolved sophisticated mechanisms to repair
46 each type of lesion, that are largely conserved in all eukaryotes including plants³. Punctual lesions that
47 do not distort the DNA double-helix such as single-strand breaks (SSBs), or oxidized bases such as 8-
48 oxo-guanine (8-oxo-G) are repaired through the base Excision Repair (BER) process. Bulky DNA adducts
49 such as cyclobutane pyrimidine dimers (CPDs) or intra-strand crosslinks induced by UV light are
50 repaired by nucleotide excision repair (NER) or by direct photoreactivation, a pathway in which
51 enzymes called photolyases use light energy for the direct reversion of the lesion. Mismatches resulting
52 from replication errors are dealt with by the mismatch repair pathway (MMR). Finally, repair of inter-
53 strand cross-link, which can also be induced by UV light has been studied more recently in plants, and
54 relies on the Fanconi Anemia pathway, like in other eukaryotes^{4,5}.

55 If left unrepaired, these lesions can further lead to double-strand breaks (DSBs) that are more harmful
56 than the previously mentioned lesions, as they can lead to the loss of genetic information; or
57 replication stress, because replicative DNA polymerases have very tight catalytic sites that cannot
58 accommodate damaged bases⁶. These two types of DNA stress trigger the DNA Damage Response
59 (DDR), that results in cell cycle arrest until DNA is repaired⁷⁻⁹. Briefly, DSBs and single-stranded DNA
60 (ssDNA) that accumulates during replication stress activate the ATM and ATR kinases respectively. This
61 leads to extensive chromatin modifications (detailed in this review) that favour DNA repair; and to the
62 transcriptional regulation cell cycle and DNA repair genes, thus allowing to stop cell cycle progression
63 until DNA damage is repaired¹⁰⁻¹².

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

68 with ssDNA to allow homology search and strand invasion. Pairing of this DNA strand with the
69 undamaged template next allows a DNA polymerase to repair the break^{3,13}. Alternatively, DSBs can be
70 repaired by the potentially error-prone Non-Homologous End Joining (NHEJ). Two independent
71 pathways called canonical NHEJ and alternative NHEJ (alt-NHEJ) exist in plant cells. In cNHEJ, DNA ends
72 are recognized by Ku proteins and religated, without DNA end resection¹³. By contrast, alt-NHEJ is
73 initiated by end-resection, and relies on micro-homology, and on the activity of DNA polymerase
74 theta^{3,14}. In the case of replication stress, translesion-synthesis can bypass replication-blocking lesions
75 by recruiting specialized DNA polymerases^{6,15}. Alternatively, stalled forks can be rescued by activation
76 of nearby replication origins, or repaired through HR and alt-NHEJ¹⁵.

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

86 **Genomic features and DNA processing as sources of DNA damage**

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

88 In Arabidopsis, GC->AT mutations are by far the most frequent, and the mutation rate of methylated
89 cytosines is higher than that of unmethylated cytosines¹⁶. These mutations likely result both from the
90 spontaneous deamination of methylated cytosines, that is known to be more frequent than that of
91 unmethylated cytosines¹⁷, and from the formation of pyrimidine dimers, because most mutations
92 occurring at unmethylated cytosines are in di-pyrimidine contexts¹⁶. In addition, methylated cytosines
93 are also more prone to forming CPDs¹⁸. Thus, UV-induced lesions that are enriched in the highly
94 methylated constitutive heterochromatin¹⁹ could thus also contribute to the mutability of methylated
95 cytosines.

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

100 different DNA lesions²⁰, among which Demeter-like (DML) DNA glycosylases are specifically involved in
101 DNA demethylation^{21,22}. Thus, methylated cytosines can behave as hotspots for the formation of DNA
102 lesions, particularly if their methylation status is highly dynamic. Indeed, *zdp/ape2* double mutants
103 that lack both pathways involved in the processing of abasic sites (Fig. 2) display severe developmental
104 defects, due to the constitutive activation of the DDR, that can be rescued by inactivation of the ROS1
105 DNA glycosylase, indicating that active DNA demethylation is a source of DNA damage in plant cells²³.
106 Interestingly, in Arabidopsis and rice, mutation rates are higher on the 1-3 nucleotides situated on each
107 side of methylated cytosines, which could be due to repair errors during the LP-BER process (Fig. 2²⁴).
108 However, the mutation rates of cytosines whose methylation status is dynamically regulated by ROS1
109 is lower than that of stably methylated cytosines²⁴. Thus, although DNA demethylation creates DNA
110 lesions, the efficiency and accuracy of DNA repair are likely higher at actively demethylated sites,
111 suggesting that accessibility to the DNA repair machinery, rather than frequent
112 methylation/demethylation cycles, is the main factor determining the mutation risk of methylated
113 cytosines (Fig. 3a). Given that most crop genomes have much higher DNA methylation levels than
114 Arabidopsis or rice due to their high TE (Transposable Elements) content², it will be interesting to find
115 whether the methylation-related bias in the distribution of mutations along the genome are
116 exacerbated in other plants.

117 ***Specificities of telomeric regions***

118 Because eukaryotic chromosomes are linear, their extremities resemble DSBs, and thus need to be
119 protected to avoid triggering DDR activation, and being inappropriately repaired, which could lead to
120 chromosome fusions. This is achieved through the presence of telomeric repeats, that allow the
121 recruitment of dedicated proteins to protect them²⁵. Due to their G-rich sequence, telomeres
122 accumulate about 100 times more 8-oxoG, the most common DNA lesion induced by reactive oxygen
123 species, than the rest of the genome in control conditions in Arabidopsis²⁶. This feature could require
124 specific targeting of the DNA repair machinery to telomeres²⁷, but this issue has not been explored in
125 plants as yet.

126 ***Topological stress and its resolution by topoisomerases***

127 All DNA metabolic processes can induce topological stress in the form of negative or positive
128 supercoiling. Because excessive supercoiling can induce breakage, these constraints are resolved by a
129 dedicated class of enzymatic complexes called Topoisomerases, that can cut DNA, release the tension
130 and religate the DNA molecule²⁸. They are involved in all aspects of DNA metabolism, including DNA
131 replication, transcription, DNA repair, DNA disentanglement, for example during endoreduplication,
132 chromosome condensation, heterochromatin metabolism...²⁹. In Arabidopsis, topoisomerases have

133 been shown to play essential roles in the maintenance of genome integrity. Pharmacological inhibition
134 of TOPI enzymes leads to DNA breaks accumulation due to transcription/replication conflicts³⁰.
135 Topoisomerase II (TOPII) is also essential to DNA replication: *topII* mutants display DSB accumulation
136 in meristematic cells, likely due to replication fork collapse²⁹. Likewise, endoreduplication is severely
137 compromised in *topVI* mutants^{31–33}, suggesting that TOPVI could play an important role in chromosome
138 disentanglement. Interestingly, this essential activity of topoisomerases is in itself a threat for genome
139 integrity, because they can form DNA-protein crosslink (DPC), if DNA protein intermediates of the
140 reaction get stabilized³⁴. Inactivation of DPCs repair leads to severe developmental defects, associated
141 with a dramatic reduction of cell proliferation and spontaneous cell death³⁵, likely due to replication-
142 associated DNA damage.

143 ***DNA replication as a source of DNA damage***

144 DNA replication is a major source of DNA stress, not only because it generates topological stress in the
145 form of DNA supercoiling, but also because it leads to the accumulation of ssDNA that is more prone
146 to breakage than dsDNA. SsDNA is protected from damage by the RPA heterotrimer, that is structurally
147 very similar to the CST complex involved in telomere protection^{36,37}. RPAs allow the rapid detection of
148 fork stalling³⁶ by promoting the recruitment and activation of ATR⁷ that leads to fork stabilization and
149 repair. Because the ATR kinase is at the centre of the replication stress response, its inactivation is
150 lethal in mammals³⁸, although it is not in *Drosophila* or yeast. Initially, the ATR gene was thought to be
151 unessential in plants, because *Arabidopsis* mutants lacking ATR do not display any developmental
152 defects in the absence of exogenously applied genotoxic stress³⁹. However, maize and barley *atr*
153 mutants display severe growth defects, due to the spontaneous accumulation of DSBs in proliferating
154 cells^{40,41}. One possible explanation for the discrepancies between results obtained in *Arabidopsis* and
155 in barley or maize could stem from the respective size of their genomes and thus from the abundance
156 of DNA damage spontaneously accumulating during S-phase.

157 The question of genomic and/or chromatin features associated with replication stress and
158 spontaneous DSB formation has been extensively explored in many models, but comparatively less is
159 known in plants. Mammalian genomes contain so-called common fragile sites (CFS) that are defined
160 as loci prone to DNA breaks and chromosomal rearrangements in response to mild replication stress⁴²,
161 whose expression is enhanced by ATR deficiency⁴³. Similar fragile sites could exist in plants, and DNA
162 breakage at these sites could explain the growth defects of maize or barley *atr* mutants. Several DNA
163 structures have been associated with replication-associated break formation in many models such as
164 bacteria, yeast or mammals^{42,44}. These include R-loops (detailed in “RNA metabolism induces DNA
165 damage” part), as well as secondary structures that can form on ssDNA after unwinding by replicative

166 helicases such as G-quadruplexes (G4), guanine-rich sequences folding into four stranded structures
167 forming stacked tetrads, and hairpins that form in GC and AT-rich sequences respectively. G4
168 formation after DNA unwinding blocks DNA polymerase progression, and thus leads to
169 polymerase/helicase uncoupling and ssDNA exposure. In addition G4s interfere with RPA loading,
170 thereby compromising DDR signalling^{45,46}. These replication-blocking structures are likely particularly
171 scarce in Arabidopsis: G4 are widespread structures in all plant species except Arabidopsis⁴⁷, and the
172 high proportion of TE and repetitive elements found in maize or barley genomes could increase the
173 frequency of AT-rich sequences and thus favour the existence of CFS.

174 DNA sequences coding for ribosomal RNA (rDNA) loci are well documented to be prone to DNA damage,
175 notably because they cause replication issues⁴⁸: inhibition of DNA replication induces 45S rDNA fragility
176 in maize, barley and rice⁴⁹. rDNA genes are present in hundreds to thousands of copies in plant
177 genomes, and are organized in large clusters⁵⁰. One proposed explanation for their fragility is that rDNA
178 loci need to be transcribed at very high levels throughout the cell cycle, including S-phase⁴⁸, causing
179 transcription/replication conflicts (see below). In yeast and human cells, this problem is circumvented
180 by the existence of the Replication Fork Barrier (RFB), a sequence located in the vicinity of the rDNA
181 locus that can arrest the replication fork^{51,52}. A RFB was identified in *Pisum sativum*, but until now, such
182 sequences have not been studied in other plant models such as Arabidopsis⁵³. Further evidence for the
183 fact that rDNA behave as CFS in plant genomes comes from the gradual loss of rDNA copies in *fas*
184 mutants, that are deficient for a sub-unit of the CAF1 complex⁵⁴, a heterotrimeric histone chaperone
185 complex involved in the incorporation of H3-H4 dimers during DNA replication⁵⁵. Loss of rDNA in *fas*
186 mutants is mitotic, and correlates with spontaneous accumulation of DSBs that largely colocalize with
187 the 45S rDNA locus⁵⁶. The formation of these foci is ATR-dependent, but not restricted to S-phase
188 cells⁵⁶, and may thus be associated with transcription and not only with replication⁴⁹. Nevertheless,
189 protein complexes involved in the resolution of replication-blocking structures and aberrant
190 recombination intermediates are involved in the maintenance of rDNA copy-number^{4,5}, confirming
191 that plant rDNA loci are prone to replication stress.

192 Like rDNA loci, telomeres are well known difficult-to-replicate regions of the genome⁴⁸. This is in part
193 due to their high GC content, that favours the formation of G-quadruplexes. In addition, they pose the
194 so-called end-replication problem, because replication has to terminate at the end of the chromosome
195 without meeting a replication fork travelling in the opposite direction²⁵. This results in erosion of
196 telomeres at each replication cycle, that is compensated by the action of telomerase, a
197 ribonucleoprotein enzyme that contains a catalytic telomerase reverse transcriptase (TERT) and uses
198 a long noncoding telomerase RNA component as a template to elongate telomeres²⁵.

199 Finally, fragile sites are also found in genomic regions that are comparatively poor in replication origins,
200 because rescue of stalled forks by replication units coming from nearby origins becomes less efficient⁴².
201 Interestingly, the size range of replication segments in late replicating regions is broader in maize
202 (about 10-160kb⁵⁷) than in Arabidopsis (about 10 to 100kb⁵⁸), and the median size of early replicating
203 segments is also larger. Thus, the maize genome may contain more origin-poor regions, in which DNA
204 breaks are more likely to occur during DNA replication.

205 Overall, DNA-replication stalling at difficult-to-replicate sequences likely triggers the formation of DNA
206 breaks in plant genomes even in the absence of genotoxic stress, as summarized on Fig. 3b. This
207 phenomenon could be of importance for crops physiology, as plants with larger genomes seem to have
208 a higher rate of spontaneous break formation than Arabidopsis. In addition, even in Arabidopsis where
209 replication-associated DNA damage appears to be rare, its frequency has been reported to increase in
210 response to various types of environmental stresses such as high-light or salt⁵⁹.

211 ***Is chromatin looping a potential source of DNA damage?***

212 Very recently, chromatin topology itself was found to be a potential source of DNA lesions in the form
213 of DSBs: in human cells, Topoisomerase 2B generates DSBs at promoter/enhancer loop anchors,
214 independently of transcription⁶⁰. The formation of higher-order chromatin structures such as loops
215 generates topological stress that is resolved by Topoisomerases, and this localized DNA cleavage
216 activity generates fragile sites that are transcription and replication-independent (Fig. 3c). Consistently,
217 chromosome conformation was found to efficiently allow the prediction of sites of DSB formation⁶¹.
218 Given that exposure to stress such as heat stress has been shown to result in profound changes in 3D
219 chromatin organization and promoter/enhancer loops in plants⁶², it will be interesting to determine
220 whether this reprogramming of 3D chromatin organization results in the accumulation of DNA breaks
221 at new anchor sites.

222 **RNA metabolism induces DNA damage**

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

224 R-loops are structures composed of a DNA-RNA hybrid and a displaced single-stranded DNA
225 (ssDNA) that are key players of gene expression regulation⁶³, although they can be responsible for
226 genome instability^{64,65}. In plants, R-loop mediated genomic instability has been observed mainly in the
227 chloroplast genome⁶⁶, but nuclear R-loops are also likely to impact genomic integrity.

228 The FLOWERING LOCUS C (*FLC*), a repressor of flowering, was the first characterized example
229 of gene expression regulation by an anti-sense non-coding RNA thanks to R-loops formation⁶⁷. Since

230 then, R-loops have been extensively studied⁶⁸⁻⁷⁰, and mapped at the genome-wide level in several
231 plant species⁷¹. They tend to accumulate on G-rich and A-rich regions primarily present in the
232 promoters of several genes and on TEs. The very structure of R-loops makes them more susceptible to
233 DNA damage because ssDNA is more susceptible than dsDNA to damage⁷². Yet, in yeast, only a subset
234 of R-loops contributes to genomic instability⁷³, likely because specific proteins protect R-loop-forming
235 loci from DNA damage. In Arabidopsis, two DNA/RNA binding proteins called AtALBA1 and AtALBA2
236 (acetylation lowers binding affinity 1 and 2) can bind the DNA-RNA hybrid and the displaced ssDNA of
237 R-loops *in vitro*, respectively, and it likely play such a protective role on R-loops⁷² (Fig. 4a).

238 Finally, R-loops could contribute to the genomic instability of *A. thaliana ddm1* (decrease in DNA
239 methylation 1) mutants, that are deficient for a chromatin remodelling complex. R-loops accumulation
240 in pericentromeric regions increases in the absence of the DDM1 protein: DDM1 would allow
241 disrupting these loops thanks to its helicase activity and through the replacement of H2A.Z histone
242 variants, mainly localised at chromosome arms, by H2A.W, variants that are usually associated with
243 heterochromatin⁷⁴. Thus, DDM1 is proposed to allow heterochromatin formation through the co-
244 transcriptional clearance of R-loops, notably because this mechanism allows the restoration of dsDNA,
245 which is the appropriate substrate for DNA methyl-transferases⁷⁴. Efficient silencing of these
246 heterochromatic regions is essential to the maintenance of genome integrity because it avoids TE
247 reactivation and remobilisation, but it is also likely that avoiding excess R-loop accumulation in
248 pericentromeric regions could directly prevent DNA damage accumulation in these regions (Fig. 4b).

249 Every above-mentioned change in R-loops homeostasis could lead to genome instability. However,
250 these modifications were observed under artificial laboratory conditions, using mutants in which R-
251 loop homeostasis was disturbed. Interestingly, in wheat (*Triticum dicoccoides*) some edaphic factors,
252 such as soil type and water availability, were recently shown to impinge on the accumulation of RNA
253 PolIII, DSBs formation, and R-loops accumulation⁷⁵, suggesting R-loop homeostasis is a relevant factor
254 for genome integrity under natural conditions.

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

256 Another source of genomic instability caused by RNA metabolism is the collision between two
257 transcription units or between the transcription and replication machineries. This phenomenon is well
258 documented in many organisms, such as humans⁷⁶⁻⁷⁸, and the role of Topoisomerases in the resolution
259 of topological problems associated with these conflicts is clearly established⁷⁹. However, much less
260 information can be found in plants.

261 Our understanding of mechanisms allowing plant cells to deal with transcription-associated
262 conflicts greatly benefited from the detailed analysis of the intricate regulation of the *FLC* locus⁸⁰. *FLC*
263 is transcribed both in the sense orientation to produce the *FLC* mRNA and in the antisense orientation
264 to produce a transcript named *COOLAIR*. Transcription of *FLC* and *COOLAIR* are mutually exclusive, and
265 *COOLAIR* contributes to *FLC* repression during vernalization⁸¹. *COOLAIR* expression is fine-tuned by a
266 R-loop formed in its promoter. Resolution of the R-loop allows *COOLAIR* transcription and recruitment
267 of the chromatin modifier complex FLD/LD/SDG26 that controls H3K4me1 levels at this locus, leading
268 to *FLC* repression^{82,83}. At the genome-wide level, FLD was proposed to slow-down transcription at
269 convergent transcription sites⁸⁴. Importantly, FLD genetically interacts with Topoisomerase 1 α ,
270 suggesting that FLD could allow the reduction of topological stresses triggered by collisions between
271 transcription units⁸⁴ (Fig. 4c).

272 Like transcription collision events, transcription/replication conflicts threaten genome integrity⁸⁵. The
273 *FLC* locus may also prove to be a case-study that will become relevant to our global understanding of
274 transcription-replication conflicts in plants. Indeed, it can interfere with replication depending on its
275 orientation respective to fork progression. At the genome-wide level, the resolution of R-loops by FCA
276 and FY is required to avoid replication fork progression impediment⁸⁶, suggesting that
277 transcription/replication conflicts are a major source of DNA damage in plant genomes, and that the
278 machinery identified for its role in the regulation of convergent transcription events could also
279 participate in the limitation of transcription/replication conflicts (Fig. 4c). Consistently, inhibition of
280 topoisomerase 1 leads to R-loop accumulation and genome instability, and the cellular response to
281 these defects requires ATM activity³⁰. A hypomorphic mutation of the catalytic sub-unit of DNA
282 polymerase ϵ rescues the hypersensitivity of the *atm* mutant to TOP1 inhibition, indicating that
283 slowing-down replication alleviates DNA-damage accumulation triggered by TOP1 inactivation, likely
284 by reducing transcription/replication conflicts³⁰.

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

287 **Chromatin organization and dynamics are essential to the maintenance of** 288 **genome integrity**

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

293 ***Can chromatin condensation protect DNA from damaging agents?***

294 Extreme chromatin condensation is commonly found in organs or cell types that are strongly exposed
295 to environmental insults such as gametes, pollen grains or seeds^{88,89}. In animals, chromatin compaction
296 in male gametes mediated by protamins incorporation is thought to protect DNA from genotoxic
297 stress⁹⁰. Protamins are not conserved in plants, but the histone variant H2B.8 has recently been
298 postulated to play a similar role in generative nuclei of pollen grains⁹¹. Similarly, nuclei of dry-seed
299 embryos are extremely small and contain highly condensed chromatin, and seed imbibition triggers
300 gradual chromatin decondensation and increase in nuclear size⁹², suggesting that chromatin
301 compaction is an important parameter to protect DNA from accumulation of damage.

302 Whether or not heterochromatin is generally better protected from DNA damage and accumulation of
303 mutations in all organs and cell types is a complex question. Indeed, in human cells, compacted
304 chromatin was found to be 5 to 50 times less prone than decondensed chromatin to accumulate DSBs
305 after ionizing irradiation^{93,94}. In addition, naked DNA is more susceptible than nucleosome-associated
306 DNA to all kinds of DNA lesions and even more strikingly, how DNA interacts with nucleosomes shapes
307 the distribution of damage, resulting in a marked bias for the accumulation of lesions on the DNA
308 strand facing away from the histone surface⁹⁵.

309 The few studies performed in plants draw a mixed picture of the relationships between chromatin
310 compaction and DNA damage accumulation. In *Lolium*, putative fragile sites identified as regions faintly
311 stained with DAPI in mitosis due to lower DNA compaction are not more susceptible to γ -ray-induced
312 DSBs than other regions of the genome⁹⁶. Conversely, genotoxic stress seems to increase chromatin
313 compaction in *Medicago* through the activation of the SUVH2 histone methyl-transferase⁹⁷, which
314 supports the notion that chromatin condensation might act as a protective mechanism against the
315 occurrence of further damage. Likewise, auxin starvation increases chromatin accessibility, which
316 results in higher sensitivity to DNA damaging agents in tobacco cell cultures⁹⁸. In the same line, loss of
317 rDNA in *fas* mutants seems to result from enhanced chromatin accessibility. These mutants display a
318 global replacement of the H3.1 histone variant, that is normally incorporated during DNA replication,
319 by the H3.3 variant that is normally incorporated independently of DNA-replication at highly
320 transcribed genes^{99,100}. This excess incorporation of H3.3 depends on the NAP1;1, 2 and 3 histone
321 chaperones, known to be involved in histone trafficking, nucleosome assembly and disassembly¹⁰¹:
322 inactivation of these three chaperones rescues the genomic instability of *fas1* mutant by restoring
323 H3.3/H3.1 ratios and decreasing the accessibility of chromatin, demonstrating that chromatin
324 compaction plays a key role to avoid illegitimate recombination events that arise when
325 nonhomologous recombination occurs¹⁰².

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

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

334 The general assumption that mutations occur randomly has recently been challenged by the
335 demonstration that the mutation rate is not uniform along the genome in *Arabidopsis* and rice^{16,103–105},
336 and that EMS-induced mutations are unevenly distributed and accumulate preferentially in lowly
337 expressed regions and heterochromatin in *Arabidopsis*¹⁰⁶, likely because the poor accessibility of silent
338 loci precludes their efficient repair, as observed in Mammals¹⁰⁷. Because EMS-induced mutations in
339 rice and wheat were mapped through exome sequences, their relative frequency in eu- and
340 heterochromatin cannot be compared, but the probability of EMS-induced mutations seems to be
341 affected by the methylation status of cytosines in both species^{108,109}.

342 In addition, the repair machinery can be actively targeted to expressed genes. The activity of the MMR
343 pathway depends on the chromatin context¹¹⁰, and is targeted to protein-coding genes through the
344 ability of the MSH6 protein to recognize the H3K4me1 mark, that is specifically enriched at transcribed
345 gene body¹¹¹. This suggests that cellular mechanisms cooperate to target the machinery involved in
346 the repair of punctual DNA lesions preferentially to coding sequences. This hypothesis is further
347 supported by the existence of the transcription-coupled NER pathway (see below).

348 DSB repair also seems to be favoured in euchromatic regions. Indeed, PDS5C, a protein involved in HR,
349 binds H3K4me1, and is enriched in coding sequences and essential genes¹¹¹. Similarly, the SMC5/6
350 (structural maintenance complex) complex is recruited to DSBs thanks to the transcriptional
351 coactivator ADA2b (Alteration deficiency in Activation Complex b)¹¹², that is enriched at
352 transcriptionally active genes. This targeting of the HR machinery to transcriptionally active regions
353 may also involve a specific class of sncRNA called diRNAs (Damaged-Induced RNAs¹¹³) whose role will
354 be discussed later in this review (Fig. 5a).

355 During DNA replication, the repair of broken replication forks is also tightly controlled by histone
356 modifications (Fig. 5b). In *atxr5/6* mutants, deficiency in H3K27me1 deposition leads to re-replication
357 of heterochromatin¹¹⁴, through the activation of the DDR¹¹⁵: TONSOKU (TSK), a key player in the

358 initiation of HR-mediated repair at stalled replication forks, binds exclusively to unmethylated histone
359 H3.1, and facilitates the recruitment of Pol θ , the DNA polymerase responsible for alt-NHEJ¹¹⁶. Newly
360 incorporated H3.1 histones very rapidly become mono-methylated on lysin 27 by ATRX5 and 6 that are
361 thought to travel with the replication fork¹¹⁴, which restricts TSK binding and thus Pol θ recruitment,
362 thereby avoiding the duplication of heterochromatic sequences¹¹⁶.

363 Thus, a wealth of mechanisms cooperates to favour DNA repair on open, accessible chromatin,
364 particularly in transcriptionally active regions and newly replicated regions. However, dedicated
365 mechanisms also allow driving the repair machinery to heterochromatin. Indeed, the DDB2 protein is
366 both a key player of the GGR-NER (Global Genome Repair-Nucleotide Excision Repair) pathway¹¹⁷ and
367 of DNA methylation^{118,119}. The simultaneous involvement of DDB2 in DNA repair and DNA methylation
368 might play two roles: it may facilitate the recruitment of the DNA repair machinery to regions of the
369 genome that are less accessible thanks to the ability of DDB2 to interact with methylated regions, and
370 allow the restauration of appropriate chromatin states once DNA repair is complete.

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

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

376 In all eukaryotes including plants, the best characterized chromatin modification related to the DDR is
377 the accumulation of γ H2AX (H2AX variant phosphorylated on S139) around DSBs, thanks to the activity
378 of ATM and ATR kinases^{120,121}. The H2AX histone variant is present on all the genome, and enriched on
379 gene body, but depleted at centromeres¹²², allowing rapid detection of DSBs without requiring the *de*
380 *novo* incorporation of histone variants. γ H2AX allows signalling of DNA damage and recruitment of
381 repair factors at break sites through the involvement of γ H2AX readers. Because most players involved
382 in this process are present in plant genomes and required for DSB repair^{123–125}, this sequence of events
383 is likely conserved. Very recently, two putative readers of γ H2AX: XIP (H2AX Interacting Protein)¹²⁶ and
384 BCP4 (BRCT Protein 4)¹²⁷ have been identified, and co-localize with γ H2AX, but their precise function
385 remains to be elucidated. In human cells and yeast, γ H2AX distribution seems to be governed by pre-
386 existing chromatin conformation: the spreading of this histone mark near the DNA break seems to be
387 controlled by cohesin-mediated loop-extrusion, and to cover the entire TAD (topologically associating
388 domain) in which the break formed¹²⁸. Plant genomes are organized in TAD-like structures that
389 resemble TADs, in the sense that loci inside these regions tend to interact more with each other than

390 with the rest of the genome¹²⁹. However, TAD-like structures are not functionally equivalent to TADs
391 as they are extremely gene-poor, and genes are found mainly on the borders of these structures¹²⁹. It
392 is also interesting to note that these TAD-like structures are scarcely found in Arabidopsis compared
393 to plant species with bigger genomes such as rice¹³⁰. Therefore, the mechanisms governing the
394 distribution of γ H2AX in plants may differ significantly from what has been discovered in other
395 eukaryotes, and may be different from one plant species to another.

396 Besides γ H2AX accumulation, many other histone modifications are deposited or removed at the sites
397 of DNA damage in yeast and animals¹³¹. In plants, a few histone modifications have been shown to play
398 a role in DSB repair. First, H3K4me2 removal at damaged sites by the LDL1 protein would be important
399 to release the chromatin remodeller RAD54 sites during HR, thereby allowing normal progression of
400 the repair process¹³². By contrast, SUVH2, that is required for the deposition of H3K9me2, partially co-
401 localizes with γ H2AX foci in *Medicago*, and appears to recruit RAD51⁹⁷. Lastly, the Polymerase-
402 Associated Factor Complex (PAF1C) was recently identified as a new component of DSB repair¹³³.
403 PAF1C recruits the E2 Ubiquitin-conjugating enzymes UBC1/2 and E3 ligases HUB1/2 that mediate H2B
404 mono-ubiquitination at DSBs, thereby promoting DNA repair through HR¹³³. Given that several
405 chromatin modifiers have been identified as ATM or ATR targets¹³⁴, other histone modifications are
406 likely to play a role in DSB repairs in plants.

407 ***Chromatin remodelling and mobility are essential to efficient DNA repair***

408 As discussed above, association of DNA with nucleosomes into chromatin can prevent access of the
409 DNA repair machinery to these lesions. Therefore, the activity of remodellers is crucial to give access
410 to the repair machinery in⁹⁵. ATP-dependent Chromatin Remodellers (ACRs) are multi-subunit protein
411 complexes that utilize the energy freed by ATP-hydrolysis to slide, evict, exchange or dismantle
412 nucleosomes. They are classified according to the family of their catalytic sub-units between four
413 groups: SWI/SNF2 (Switch Sucrose Non-Fermentable), SWR1 (SWI/SNF-related 1), CHD
414 (Chromodomain Helicase DNA) and ISWI1 (Imitation Switch 1). Their contribution to excision repair
415 pathways or photoreactivation has been little investigated in plants¹³⁵. Campi and colleagues did
416 report increased CPD accumulation and UV sensitivity in maize and Arabidopsis plants deficient for a
417 number of chromatin-remodellers, but whether this reflects a direct role of the corresponding protein
418 to facilitate access of the repair machinery to DNA has not been formally proven¹³⁶.

419 Chromatin remodelling is also essential to all steps of DSB repair, namely DNA end resection (a step
420 that is required for all DSB repair pathways except cNHEJ), and the HR-specific processes (homology
421 search, strand invasion and holiday junction resolution¹³⁷). Members of all families have been shown
422 to contribute to DSB repair in animals and yeast and these roles are likely to be conserved in

423 plants^{135,138}. Strikingly, in many instances, direct evidence for the role of ACRs subunits in plant DSB
424 repair remains scarce. For example, systematic analysis of SWI/SNF2 mutant and RNAi lines revealed
425 that most of them were hyper-sensitive to γ -irradiation¹³⁹, but since then, the direct involvement of
426 most of these factors in the DNA repair process was not further investigated. Because detailed
427 description of all ACRs putatively involved in DSB repair is available in^{135,138}, we will focus on the few
428 ACRs that have been unequivocally involved in DNA repair (listed on Fig. 5c). First, the SWI3B chromatin
429 remodeler was recently shown to promote dissociation of SMC5 from chromatin, to facilitate its
430 recruitment to DSBs¹⁴⁰ where it contributes to DNA repair, possibly by promoting cohesion between
431 the damaged DNA molecule and the undamaged repair template¹⁴¹. Second, CHR721, a novel member
432 of the SMARCAL1 sub-family known to reanneal stalled replication forks in response to DNA damage
433 in human¹⁴², was identified in rice¹⁴³. CHR721 interacts with the DNA repair protein RPA1A¹⁴³ and with
434 the DNA polymerase processivity factor PCNA¹⁴⁴, suggesting that it is directly involved in DSB repair
435 and/or replication stress response and fork stabilization, like its human homolog¹⁴⁵. Finally, the best
436 characterized member in the SWI/SNF2 family for its role in DSB repair is RAD54, that unlike other
437 ACRs, is a core component of the enzymatic machinery responsible for HR and DSB repair¹⁴⁶: it
438 participates to the stabilization of nucleoprotein filaments, facilitates D-loop formation and
439 heteroduplex extension¹⁴⁷. In plants, it has been shown to form DNA repair foci that are mobile, and
440 tend to localize at the nuclear periphery^{148,149}. Also, it likely favours homologous recombination by
441 mediating homologous loci pairing following γ -irradiation¹⁵⁰. Interestingly, DSB induction results in a
442 global increase in chromatin mobility, particularly in the S and G2 phases of the cell cycle, suggesting
443 that homology search could be favoured both by a global increase in chromatin mobility, and by
444 RAD54-mediated movement of the damaged site¹⁵¹.

445 Members of the INO80/SWR1 family have also been implicated in DSB repair: *ino80* mutants are
446 deficient for somatic HR¹⁵², and mutants lacking the *arp4* and *arp5* sub-units of the same complex are
447 hypersensitive to DNA damage¹⁵³. Importantly, the accumulation of γ H2AX foci and the expression of
448 DNA repair genes are unaffected in *ino80* mutants, providing evidence for its direct role in the DNA
449 repair process downstream of γ H2AX foci formation¹⁵⁴. Finally, the SWR1 complex is required for HR
450 but not for cNHEJ¹⁵⁵. This observation clearly supports a direct role of SWR1 in the repair process,
451 because cNHEJ does not require DNA end resection and extensive chromatin remodelling like HR or
452 alt-NHEJ. In addition, PIE1, the catalytic sub-unit of the SWR1 complex, is phosphorylated by ATM and
453 ATR in response to γ -irradiation¹³⁴.

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

456 by MMS21¹⁵⁶, a sub-unit of the SMC5/6 complex^{156,157}. Likewise, phospho-proteomics identified
457 several chromatin remodellers as targets of ATM and ATR, supporting their role in the DDR¹³⁴, but a
458 global view of the respective roles of chromatin remodellers during the plant DDR is still missing.

459 **Role of RNA metabolism in the maintenance of genome integrity**

460 Transcriptional changes in protein coding genes are obvious central mechanisms involved in the DDR,
461 but they have been extensively reviewed elsewhere⁷⁻⁹ and will not be detailed here. In this paragraph,
462 we will briefly review the direct involvement of RNAs and RNA metabolism in DNA repair.

463 **Transcription-coupled DNA repair avoids accumulation of mutation in transcriptionally active** 464 **regions**

465 In addition to the recruitment of various DNA repair components at region enriched in active
466 chromatin marks that we described earlier, transcription itself is coupled to DNA repair. Indeed, repair
467 of UV-induced DNA damage is more active at transcribed genes, with a strong preference for the
468 transcribed strand¹⁵⁸. When RNA PolII encounters a transcription-blocking lesion, RNA elongation stops,
469 which leads to the recruitment of the nucleotide excision repair machinery¹⁵⁹. The components of
470 Transcription Coupled (TC)-DNA repair are conserved between eukaryotes, and Arabidopsis mutants
471 lacking these proteins are hypersensitive to UV-irradiation. The current model of TC-NER in plants is
472 thus the following (Fig. 6a). Stalled RNA PolII is recognized by the SWI/SNF chromatin remodeller CHR8
473 (CSB in humans¹³⁹). CHR8 then recruits the Cockaine Syndrome A-DDB1-CRL4 complex which
474 ubiquitinates CHR8^{160,161}, and the UVSSA and UBP12 (UVSS7 in humans) proteins that stabilize it¹⁶².
475 Finally, core NER proteins such as TFIIH and the XPG and XPF endonucleases are recruited to allow DNA
476 excision on each side of the lesion. The RPA protein coats the ssDNA formed in front of the newly
477 generated gap, until it is filled by the joint activity of a DNA polymerase and a ligase³, and transcription
478 re-initiation is likely mediated by the RDO2 (TFIIS in humans) elongation factor¹⁶². Interestingly, TC-
479 repair is strongly modulated by the circadian clock in Arabidopsis¹⁵⁸, highlighting the crucial importance
480 of this DNA repair pathway in organisms for whom sunlight is an indispensable source of energy.

481 **The diverse roles of non-coding RNAs**

482 The existence of mechanisms targeting NER towards heterochromatin appears all the more important
483 considering UV-induced damage is particularly abundant in heterochromatin¹⁹. This is achieved
484 through the so-called GG-NER (Global Genome NER) process (Fig. 6a). In this pathway, the NER
485 machinery is targeted to heterochromatic regions thanks to siRNA produced by the RdDM (RNA-
486 Directed DNA Methylation) machinery: UV-induced DNA damage triggers the production of 21-nt RNAs

487 called uviRNA that are loaded by the AGO1 protein, which in turn guides DDB2 recruitment to damaged
488 regions, thereby promoting NER at damaged sites¹⁶³.

489 RNA metabolism occurring directly at the damaged site may also play a role in DSB repair. Indeed, DSB
490 induction inside a reporter transgene was found to induce the production of small non-coding RNAs
491 called diRNAs (Damage-Induced RNAs) by RNA PolIV at the vicinity of the break. These diRNAs were
492 proposed to favour DSB repair, although the underlying mechanism remains unclear in plants¹⁶⁴. This
493 model was later complexified by the finding that diRNAs do not seem to be produced at DSBs triggered
494 outside of a transgene either by CRISPR or TALEN, suggesting that they originate exclusively from highly
495 expressed loci and resemble small RNAs produced by post-transcriptional gene silencing¹⁶⁵.
496 Furthermore, efficient DNA repair was observed at loci where diRNA production could not be
497 detected¹⁶⁵, which contradicts the initial hypothesis that these snRNA would be important for DNA
498 repair. Thus, diRNA may result from the production of aberrant transcript at damaged sites, in which
499 case their role could be to silence damaged loci and avoid the accumulation of aberrant transcript until
500 DNA is repaired. However, in mammals, diRNA were found to allow the recruitment of chromatin
501 modifiers and remodellers, thereby facilitating the access of the DNA repair machinery and promoting
502 HR¹⁶⁶. Because HR was not directly measured in plants¹⁶⁵, one cannot rule out that diRNA accumulation
503 could function to favour HR on highly expressed loci (Fig. 6b), whereas diRNA-free loci could be
504 repaired by alt-NHEJ. Further studies will be required to clarify this point.

505 Finally, a number of long non coding RNAs (lncRNAs) have been shown to be induced by DNA damage,
506 and to be involved in Arabidopsis' tolerance to genotoxins^{167,168}. They could play a role in the post-
507 transcriptional regulation of the DDR, but may also contribute to DNA repair or DDR signalling through
508 various mechanisms, possibly by interacting with protein complexes¹⁶⁹. Molecular dissection of their
509 precise function should help clarify this point.

510 **Concluding remarks**

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

519 On a more mechanistic point of view, future work should help better understand how chromatin
520 dynamics contributes to DNA repair globally, and depending on the chromatin context. Furthermore,
521 several lines of evidence point to a direct relationship between the maintenance of genome and
522 epigenome integrity. Indeed, many mutants deficient for DNA replication proteins have been identified
523 in genetic screens designed to find components of the constitutive heterochromatin maintenance
524 (see¹⁷⁰ and references therein). A challenge for the years to come will be to dissect this coupling
525 between the maintenance of the genetic and the epigenetic information, and notably to understand
526 how chromatin states are restored after DNA replication or repair.

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

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533

534 **Competing interests**

535 Authors declare no competing interests.

536

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544 C.R. and C.B-S. conceptualized the manuscript framework. C.R., C.B-S., and M.R. wrote the original
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546 and C.B. reviewed the manuscript. C.B-S. and C.R. edited the manuscript.

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

Figure number	Protein/gene name	Full name	Roles	Key Publications
Fig 2.	ARP, APE1L, APE	Apurinic endonuclease-redox protein Apurinic/aprimidinic 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	<i>COOLAIR</i>	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 Demethylase 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 remodeler that recognizes stalled RNA PolIII 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

945

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947

948 **Figures**

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

951 Left Panel: From top to bottom; specific genomic features such as the abundance of methylated
952 cytosines can lead to compacted chromatin and spontaneous lesion formation through deamination,
953 leading to the accumulation of mutations even when the BER pathway is mobilized (Fig. 2 and Fig. 3a).
954 Telomeres are prone de oxidation due to their G-rich sequences, must be shielded from the DSB repair
955 machinery to avoid inappropriate repair, and pose the end-replication problem. Topological stress can
956 induce breaks and topoisomerases relieve this stress by cutting DNA, thus endangering DNA integrity.
957 At difficult-to-replicate sites, secondary structures can form on ssDNA during replication, thus inducing
958 replication stress followed by DNA damage (Fig. 3b). R-loops can form during transcription and lead to
959 DNA damage if they are not protected or timely resolved (Fig. 4a and b). Transcription-replication
960 conflicts can lead to topological stress and R-loops followed by DNA damage when not resolved (Fig.
961 4c).

962
963 Right panel: From top to bottom; chromatin condensation can help to prevent DNA damage under
964 certain stress conditions. The deposition of specific histone marks that are hallmarks of expressed
965 genes helps to recruit DNA repair agents to damaged sites at coding sequences (Fig. 5a and b), and
966 facilitates the repair process. H2AX phosphorylation at DSB sites helps to recruit the repair machinery
967 (Fig. 5c). ATP-dependent chromatin remodellers help to repair damaged DNA, notably through
968 homologous recombination, but also by allowing access of the repair machinery to DNA (Fig. 5c).
969 Transcription can directly lead to repair when encountering a lesion thanks to the transcription-
970 coupled nucleotide excision repair pathway (Fig. 6a). Non-coding RNAs can be produced at damaged
971 sites and helps to recruit the DNA repair machinery (Fig. 6b). Created with BioRender.com

972

973 **Figure 2: Active DNA demethylation relies on the BER pathway**

974 BER is initiated by DNA glycosylases that remove the damaged base, leaving an AP (apurinic/apyrimidic)
975 site. Next, the 5' and 3' ends of the AP site are processed either by AP lyases and subsequent
976 dephosphorylation of the 3' end of the AP site by the ZDP (zinc-finger DNA 3'-phosphoesterase)
977 phosphatase or by the successive action of AP endonucleases (ARP, APE1L and APE2 in Arabidopsis)
978 and removal of the 5' deoxyribose-5-phosphate extremity by an enzyme with dRP lyase activity¹⁷¹. Gaps
979 can be repaired either through short-patch BER, in which a single nucleotide is incorporated, and that
980 requires only the activity of a DNA polymerase and a DNA ligase¹⁷¹, or via the long-patch BER (LP-BER)
981 in which case several nucleotides (around 3) are newly incorporated¹⁷³. In addition to a DNA

982 polymerase and a DNA ligase, LP-BER also involves a FLAP-endonuclease to displace one strand and
983 allow the incorporation of more than one nucleotide. Finally, the scaffold protein XRCC1 functions to
984 coordinate the several stages of this process¹⁷². Created with BioRender.com

985

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

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

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

998 **c:** The formation of chromatin loops generates topological stress on the DNA double-helix that can be
999 resolved by topoisomerases. In animals this process has been shown to lead to DNA damage formation
1000 independently of transcription or replication at loop-anchoring points. Whether this process also
1001 occurs in plants remains to be established. Created with BioRender.com

1002

1003 **Figure 4: Several pathways cooperate to avoid DNA damage accumulation associated with RNA** 1004 **metabolism**

1005 **a:** R-loops are fragile structures, notably because ssDNA is more sensitive to DNA damaging agents,
1006 like MMS, but also to oxidation or cellular enzymatic activities. ALBA proteins are thought to protect
1007 ssDNA at R-loops by physically interacting with the ssDNA component of the R-loop.

1008 **b:** In heterochromatic regions, DDM1 contributes to chromatin compaction and R-loop resolution by
1009 facilitating the replacement of the H2A.Z histone variant by the H2A.W histone variant. This pathway
1010 may avoid excess R-loop accumulation and thus DNA damage formation.

1011 **c:** Top: Converging transcription units can generate topological stress on DNA. These colliding
1012 transcription events are prevented by the coordinated action of FLD/LD and SDG26 that slow down
1013 transcription by reducing H3K4me1 levels at these loci. In the absence of this machinery, transcription
1014 speed is not reduced which may lead to DNA damage.

1015 Bottom: The R-loop formed at the *FLC* locus interferes with replication progression when in the head-
1016 on orientation. This suggests that the machinery involved in R-loop resolution could be required for
1017 unhampered replication fork progression: FY and FCA resolve the R-loop and recruit FLD/SDG26/LD
1018 complexes, thereby allowing H3K4me1 demethylation and slowing-down fork progression to alleviate
1019 transcription/replication conflicts. In the absence of FCA and FY, replication progression is blocked by
1020 the persisting R-loop. Created with BioRender.com

1021

1022 **Figure 5: Chromatin modifications and dynamics play central roles in the maintenance of genome**
1023 **integrity**

1024 **a:** Several mechanisms cooperate to recruit DNA repair machineries at transcriptionally active regions.
1025 Active chromatin is characterized by high levels of H3K4me1 and histone acetylation. H3K4me1 is
1026 recognized by the MSH6 protein (involved in MMR) and the PDS5C protein (involved in DSB repair) via
1027 their Tudor domains. Finally, Ada2b that is usually associated to histone acetylation recruits SMC5/6
1028 complexes that are involved in DSB repair.

1029 **b:** Histone modifications avoid illegitimate recombination at replication forks. At newly replicated
1030 chromatin, histones are unmethylated which allows the recruitment of TSK and Pol theta at stalled
1031 forks to promote fork rescue. Further away from the fork, ATXR5 and ATXR6 allow H3K27me1
1032 deposition which precludes TSK recruitment and thus avoids illegitimate repair events leading to re-
1033 replication events.

1034 **c:** At DSBs, phosphorylation of histone H2AX creates a signalling platform that is crucial to recruit the
1035 repair machinery. Among proteins involved in the repair process, ACRs likely play a key role in allowing
1036 access of damaged DNA to the repair enzymes, as well as homology search in the case of HR. ACRs
1037 with a confirmed role in HR are INO80, SWR1, SWi3B, and RAD54 that play a key role in homology
1038 search. BRAHMA is also likely to play a role because it is post-translationally modified in response to
1039 DSBs, but its role has not been fully elucidated yet. Likewise, CHR721 may be involved, but it could also
1040 play a role in fork repair during replication stress. Created with BioRender.com

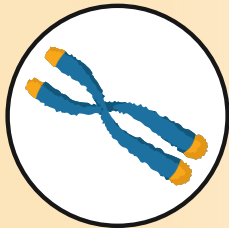
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1042 **Figure 6: RNA metabolism plays a key role in the maintenance of genome integrity**

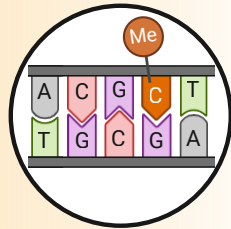
1043 **a:** Two distinct mechanisms are involved in the recruitment of the NER machinery on chromatin. At
1044 actively transcribed genes, RNA PolIII progression arrest due to bulky lesions such as CPD triggers the
1045 recruitment of a complex comprising CHR8, UVSSA, and UBP12, and due to the ubiquitination of CHR8
1046 by the CSA/DDB1/CRL4 complex. Next, this complex allows recruiting the NER machinery comprising
1047 TFIIH, XPG, and XPF. At heterochromatin, uviRNAs produced by the RdDM machinery allow the
1048 sequence-dependent recruitment of AGO1 and DDB2 that activates the NER machinery.

1049 **b:** diRNAs appear to play a role in DSB repair. They are produced at highly transcribed loci and could
1050 function to recruit chromatin remodellers to promoter HR-mediated DNA repair. Created with
1051 BioRender.com
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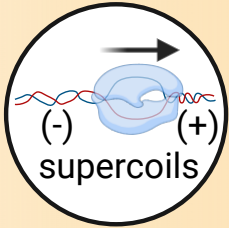
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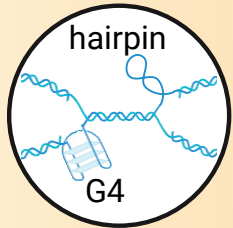
Telomeres



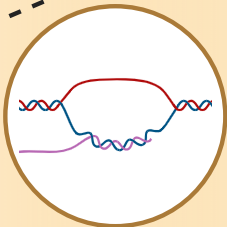
Methylated DNA



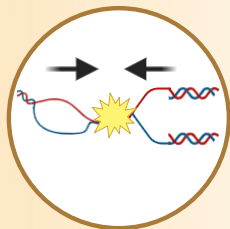
Topological stress



DNA replication

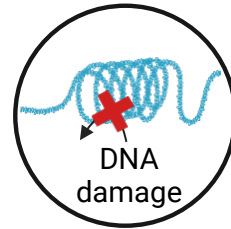


R-loops

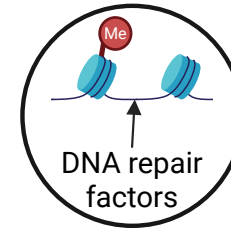


Transcription-Replication conflicts

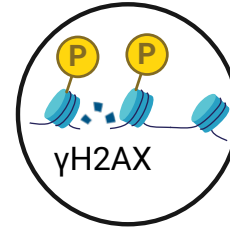
Genome integrity maintenance



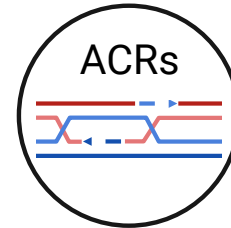
Chromatin condensation



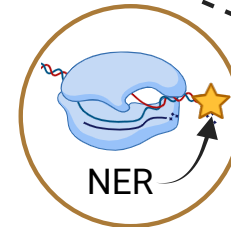
Chromatin accessibility



H2AX phosphorylation



Chromatin remodelling



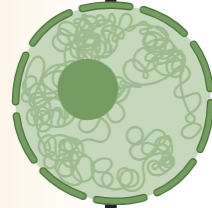
Transcription-coupled DNA repair

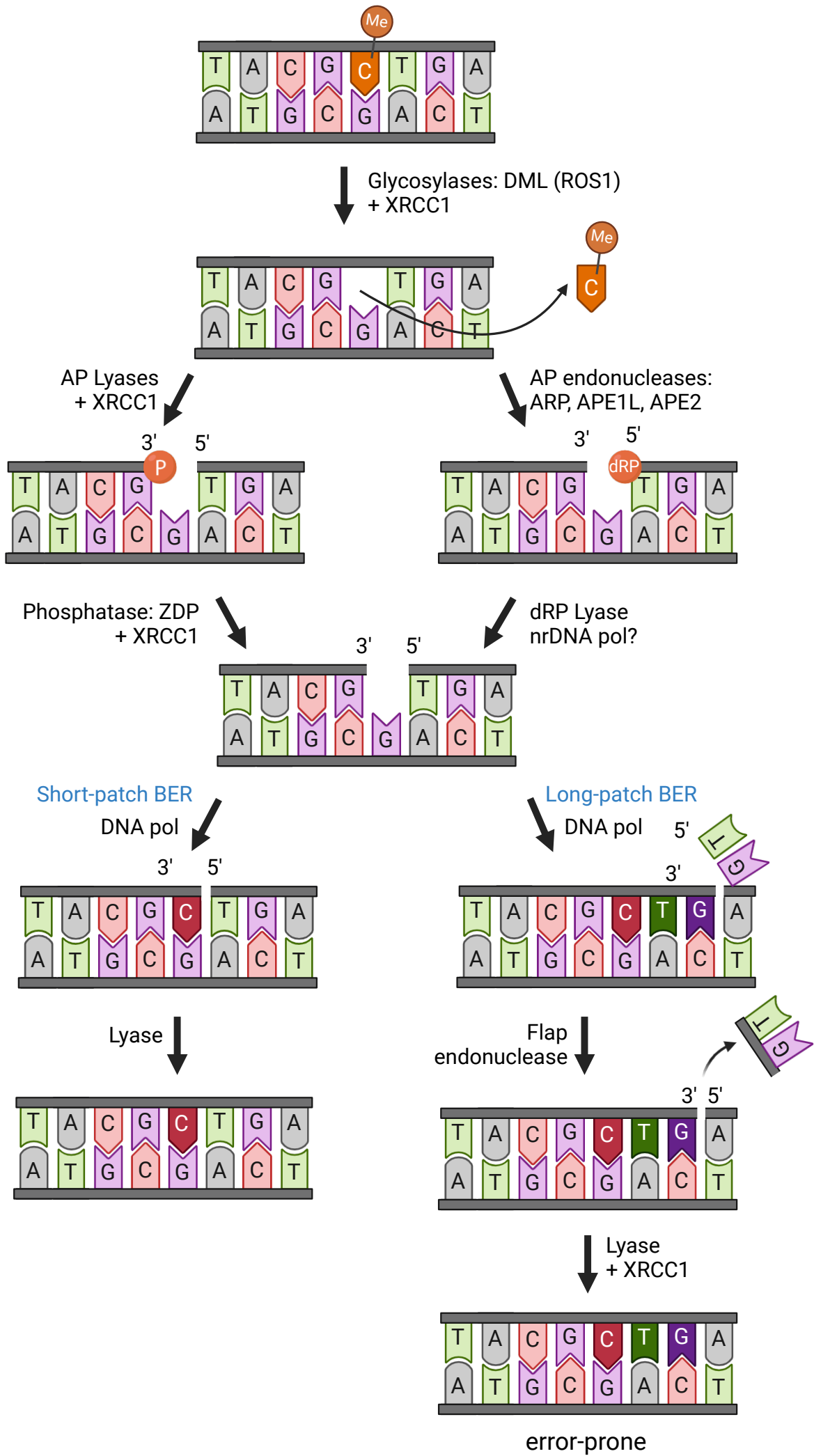


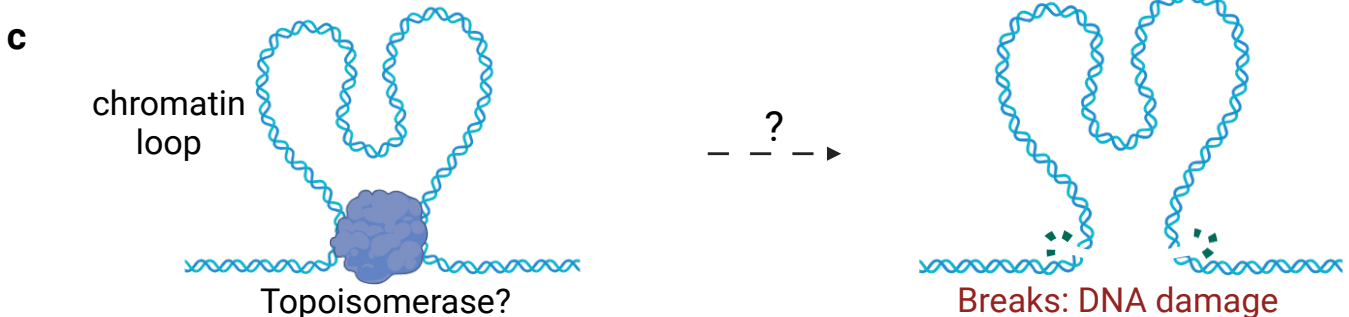
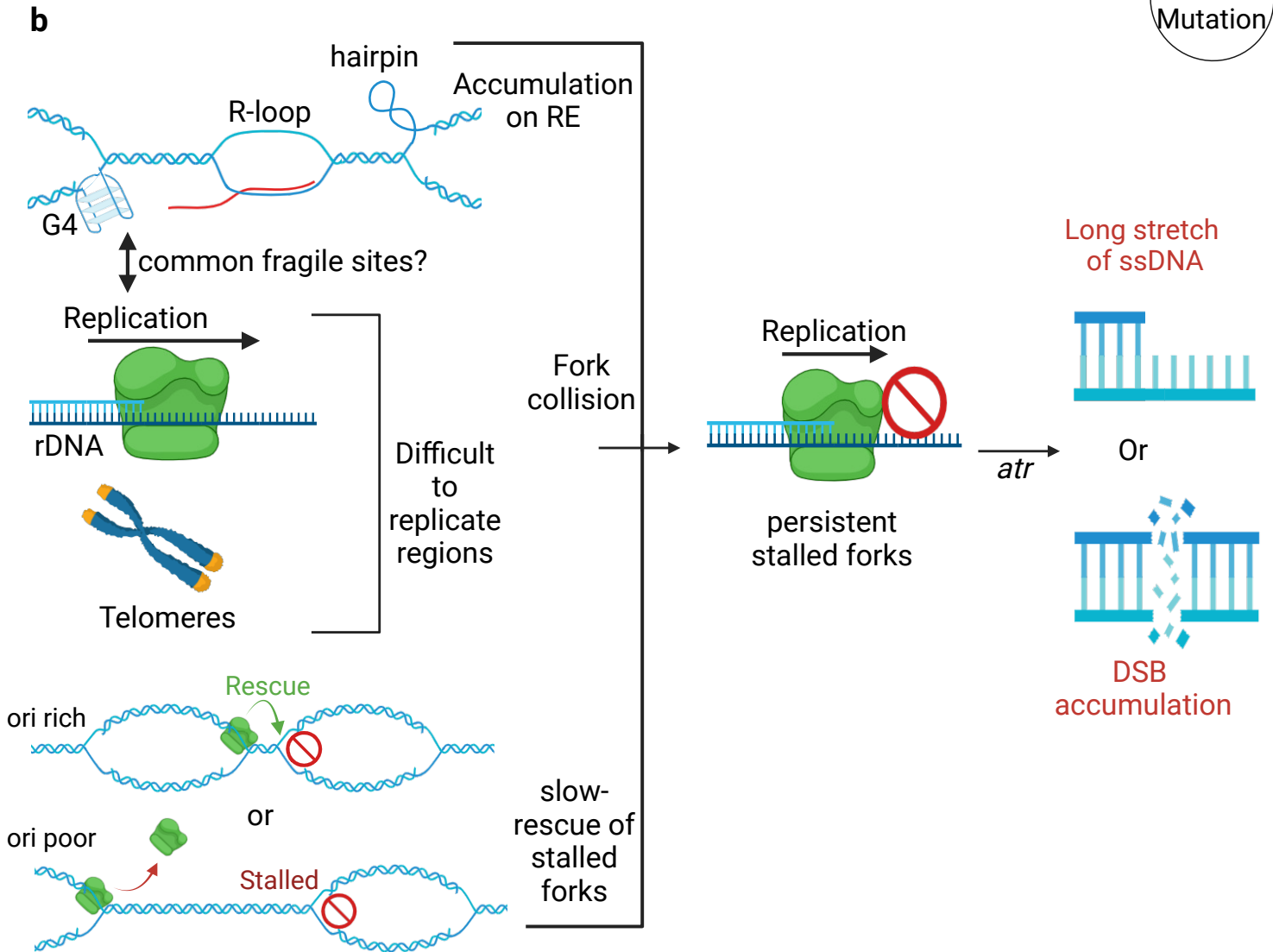
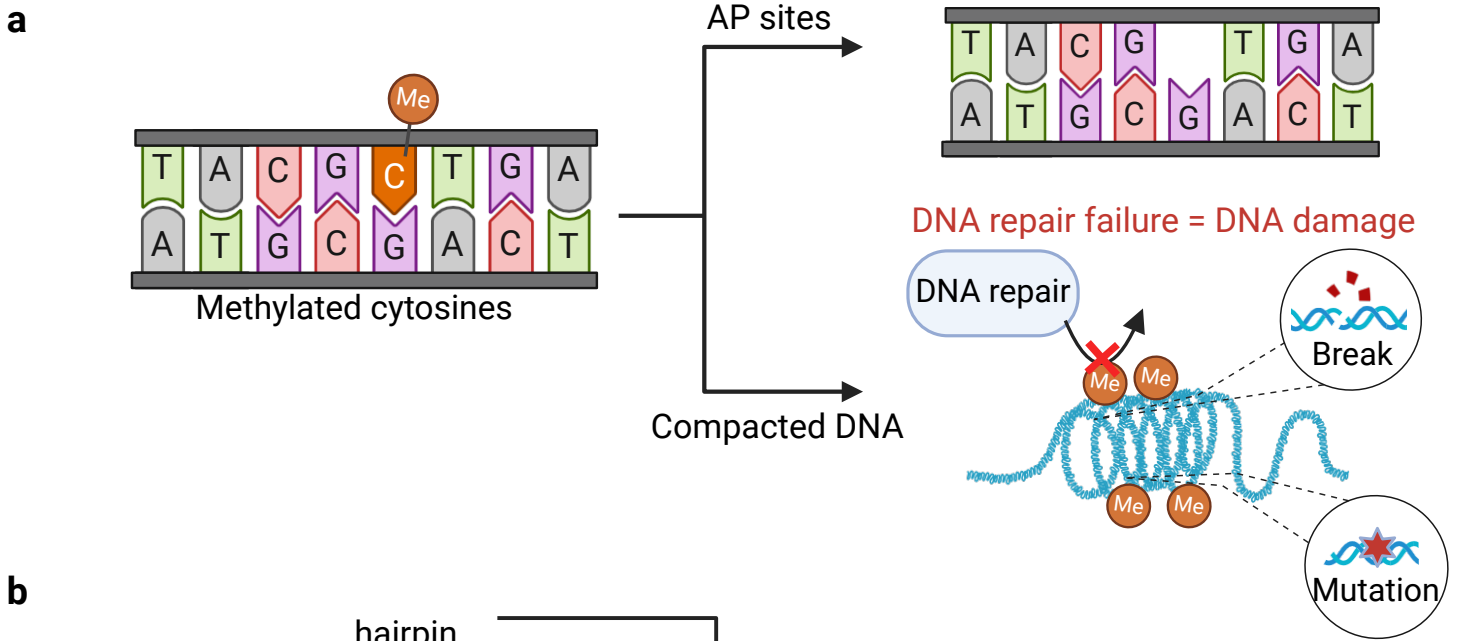
Non-coding RNAs

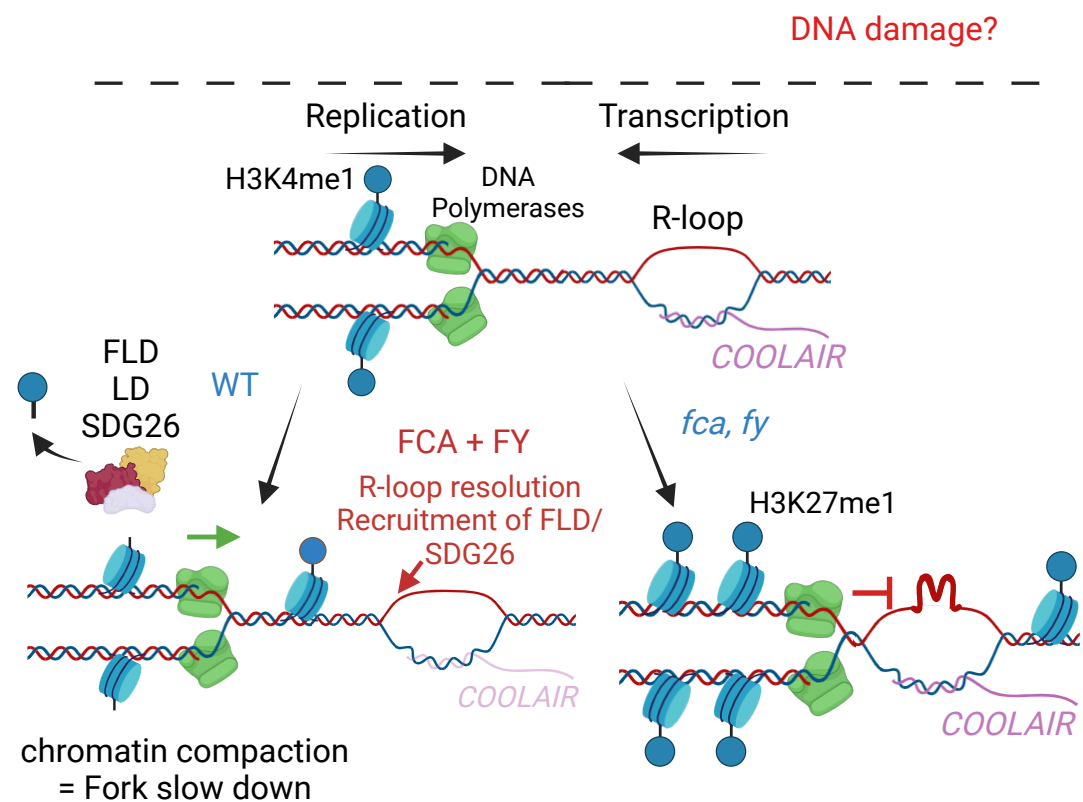
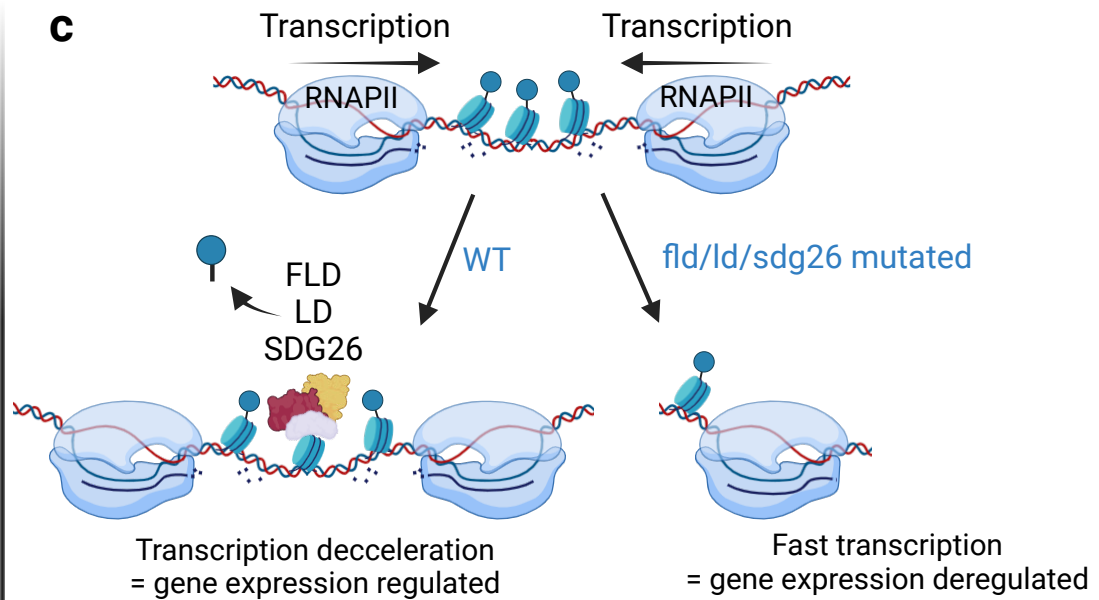
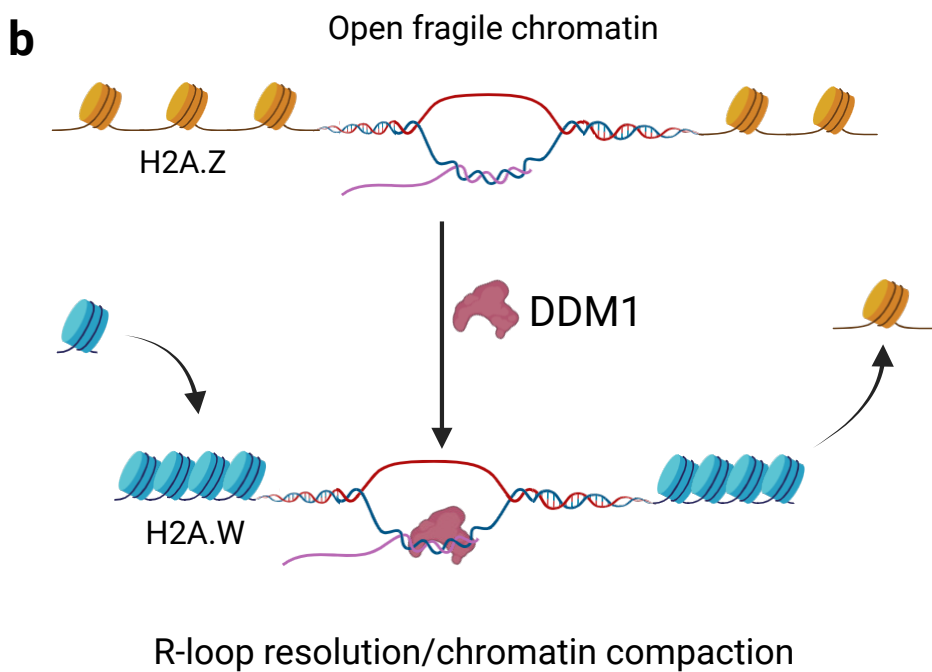
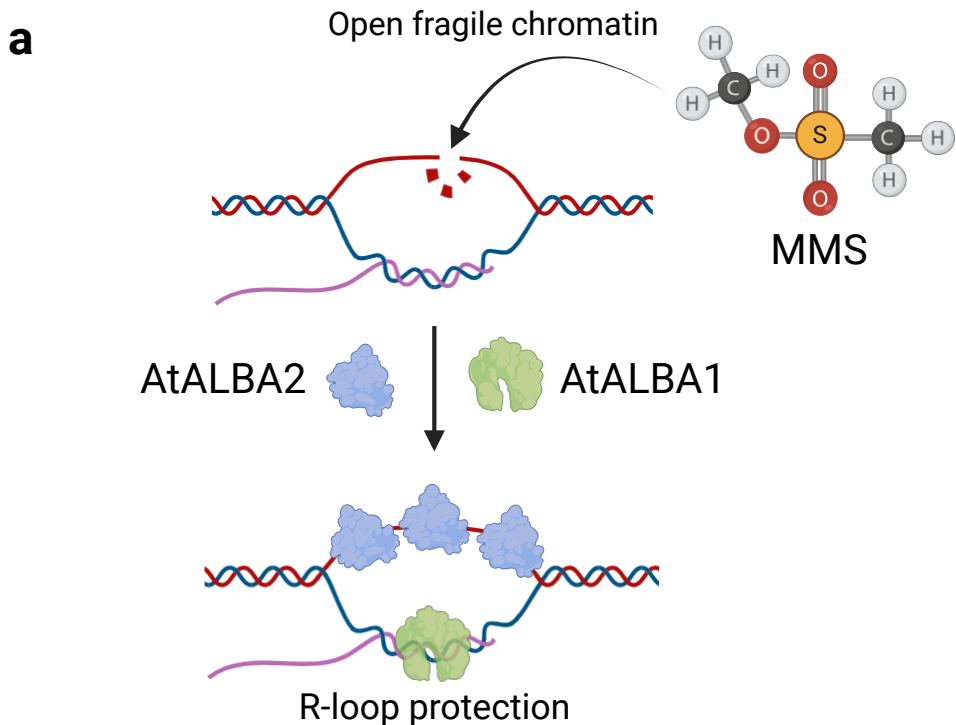
Chromatin dynamics

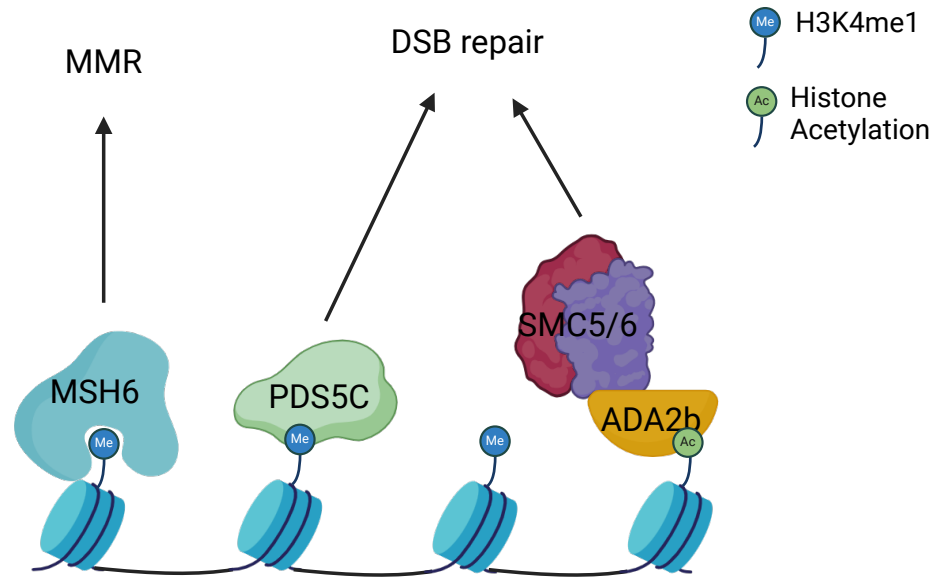
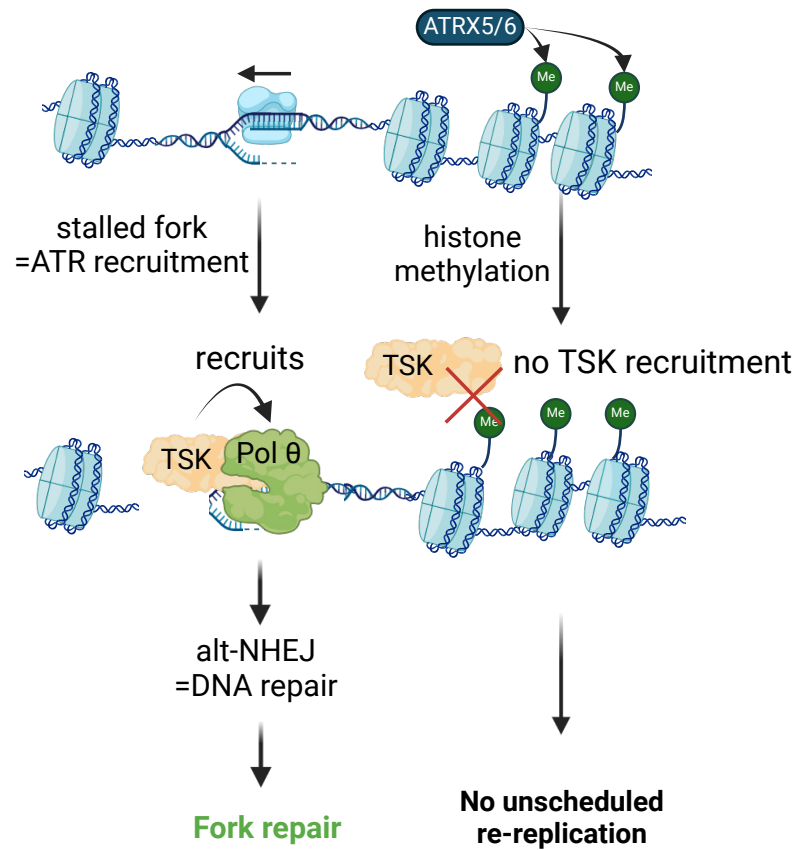
RNA metabolism









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