

1 Coupling DNA damage and repair: an essential safeguard during
2 programmed DNA double strand breaks?

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18 DNA recombination and Repair.

19 **Abstract**

20 DNA double strand breaks (DSBs) are the most toxic DNA lesions given their oncogenic
21 potential. Nevertheless, programmed DSBs (prDSBs) contribute to several biological
22 processes. Formation of prDSBs is the price to pay to achieve these essential biological
23 functions. Generated by domesticated PiggyBac transposases, prDSBs have been integrated in
24 the life cycle of ciliates. Created by Spo11 during meiotic recombination, they constitute a
25 driving force of evolution and ensure balanced chromosome content for successful
26 reproduction. Produced by the RAG1/2 recombinase, they are required for the development of
27 the adaptive immune system in many species. The co-evolution of processes that couple
28 introduction of prDSBs to their accurate repair may constitute an effective safeguard against
29 genomic instability.

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31

32 **Introduction**

33 Living organisms are constantly exposed to genotoxic assaults, which can be of
34 endogenous origin such as cellular respiration or exogenous sources such as radiations or
35 chemical exposures. Several highly conserved DNA repair mechanisms have been selected
36 during evolution to cope with these various damages and maintain genomic integrity. Among
37 DNA lesions, DNA double strand breaks (DSBs) are considered the most toxic and at least
38 two DNA repair pathways (homologous recombination (HR) and non-homologous end
39 joining, or NHEJ) have evolved to cope with DSBs. In addition to repairing pathologic DSBs,
40 these DNA repair pathways are also important for the repair of physiological DSBs or
41 programmed DSBs (prDSBs) created during programmed genome rearrangements (PGR) in
42 ciliates, meiotic recombination for sexual reproduction, and V(D)J recombination. Defects in
43 these processes result in death of progeny (PGR), sterility or aneuploidy (meiotic
44 recombination), and severe immune deficiency (V(D)J recombination). Therefore, the
45 introduction of prDSBs is “the price to pay” for some physiological processes. One can argue
46 that efficient ways to control prDSBs have co-evolved to avoid the deleterious consequences
47 of their mis-repair. Here, we discuss the view that the timely and physical coupling of DNA
48 damage and repair may represent an efficient safeguard during prDSBs.

49

50 **Coupling DNA damage and NHEJ-mediated repair of prDSBs?**

51 NHEJ is one of the two main DSB repair mechanisms. It operates in all phases of the
52 cell cycle, in contrast to HR, which is excluded from G0/G1. Its catalytic process can be
53 schematically divided into three steps: (i) the heterodimer Ku70/80 identifies and is recruited
54 to the break, prior to the recruitment of the DNA-dependent protein kinase catalytic subunit
55 DNA-PKcs, forming the DNAPK holoenzyme; (ii) If needed, DNA ends are processed
56 (“cleaned”) by DNA polymerases, nucleases, and kinases. The processing step is important
57 during V(D)J recombination for the opening of RAG1/2-generated DNA hairpins by the
58 nuclease Artemis; (iii) the DSB is resealed by DNA ligaseIV assisted by the Xlf, MRI and
59 PAXX accessory factors (see [1] for a recent review on the actors of NHEJ).

60 ***Programmed Genome Rearrangement in ciliates: The prototypical example of*** 61 ***DNA coupling between DNA damage and repair.***

62 In ciliates, which constitute a monophyletic group of unicellular eukaryotes, the
63 somatic and germline functions of chromosomes are separated into two distinct types of

64 nuclei coexisting in the same cytoplasm [2-4]: (i) the diploid micronucleus (MIC),
65 transcriptionally silent during vegetative growth, undergoes meiosis and transmits the parental
66 germline genome to the zygotic nucleus of the following generation; (ii) the polyploid
67 somatic macronucleus (MAC), responsible for gene expression, directs the cell phenotype but
68 is destroyed at each sexual cycle (Figure 1A). Ciliates make their new MAC from a copy of
69 the zygotic nucleus, through a process involving several rounds of whole-genome endo-
70 duplication and massive **programmed genome rearrangements (PGR)** triggered by the
71 introduction of **tens of thousands of prDSBs** at multiple loci in the genome of the developing
72 new MAC.

73 During PGR, *Paramecium tetraurelia* eliminates 25 to 30% of germline DNA from its
74 somatic genome [5, 6], including repeated sequences (transposable elements (TEs),
75 minisatellites) and ~45,000 TE-related short and noncoding **Internal Eliminated Sequences**
76 (**IESs**), usually found as single-copy elements and scattered all along the ~100-Mbp germline
77 genome. While repeated DNA is eliminated in a heterogeneous manner, IES excision in
78 *Paramecium* is precise at the nucleotide level. Because IESs interrupt almost half of genes in
79 the germline, their efficient and precise excision ensures that the somatic genome is correctly
80 assembled, a prerequisite for accurate gene expression and progeny survival.

81 *Paramecium* IESs are flanked by conserved TA dinucleotides. IES excision is initiated
82 by 4-base staggered double-strand DNA cleavages centered on each flanking TA [7]. The
83 endonuclease responsible for prDSB introduction at IES ends is a domesticated transposase
84 called **PiggyMac (Pgm)**, whose conserved DDD catalytic triad, characteristic of transposases
85 from the PiggyBac family, is essential for its function [8, 9]. Pgm is expressed during MAC
86 development and localizes specifically in the developing new MAC by the time DNA
87 elimination takes place. Five groups of Pgm-like domesticated PiggyBac transposases
88 (PgmL1 to PgmL5) assist Pgm in cleaving DNA [10]. Each PgmL can interact individually
89 with Pgm and is essential for IES excision genome-wide. None of them harbors a fully
90 conserved catalytic site, suggesting that they play an architectural role during assembly of the
91 IES excision complex, with PgmL1 and PgmL3 fine-tuning the precise positioning of DNA
92 cleavage at IES boundaries. Once introduced, chromosomal prDSBs are repaired by the
93 Ligase IV- and Xrcc4-dependent **classical NHEJ pathway (C-NHEJ)** [11]. NHEJ-mediated
94 DSB repair of excision sites must be efficient and precise in order to preserve the coding
95 capacity of the rearranged somatic genome, especially for intragenic IESs. This precision is
96 likely driven through the pairing of conserved TAs at each 5' overhang, removal of the

97 terminal 5' nucleotide, and addition of one nucleotide at recessed 3' ends [7], ensuring that
98 open reading frames are faithfully reconstituted upon IES excision.

99 Functional studies of Ku70/Ku80, the earliest-acting NHEJ factors that bind broken DNA
100 ends and protect them against extensive resection, suggest that different solutions to the
101 problem have evolved among ciliate species.

102 *P. tetraurelia* harbors two almost identical *KU70* genes and three more divergent
103 *KU80* paralogs, a single of which (*KU80c*) is specifically expressed during MAC
104 development [12]. In a similar way to Pgm, the development-specific Ku70/Ku80c
105 heterodimer localizes in the new MAC during PGR and expression of *KU70* or *KU80c* is
106 essential for the recovery of a functional somatic genome. Ku80c interacts with Pgm when
107 both proteins are co-expressed in a heterologous system, a property shared by PiggyBac
108 transposases and Ku proteins from other organisms [13]. Strikingly, the depletion of Ku80c
109 abolishes DNA cleavage at IES ends, resulting in retention of all 45,000 IESs genome-wide
110 [12]. In *Paramecium*, therefore, Ku interaction with Pgm during MAC development is
111 thought to license Pgm-dependent DNA cleavage through a mechanism that remains to be
112 established. Such **tight coupling between DSB introduction and repair** would ensure that
113 DSBs are introduced only if Ku proteins are present to channel broken ends to the NHEJ
114 pathway (Figure 1B).

115 In *Tetrahymena thermophila*, most of the ~12,000 IESs identified in the germline
116 genome lie in non-coding regions [14]. They are also excised by domesticated transposases
117 including a Pgm ortholog (Tpb2, see [15]) and a Pgm-like protein (Lia5, see [16]). Likewise,
118 the C-NHEJ pathway carries out the repair of intergenic IES excision sites but Tpb2 does not
119 require the presence of Ku80 to cleave IES ends [17], suggesting that excision of
120 *Tetrahymena* intergenic IESs has not imposed the same constraints on the system as
121 compared with excision of *Paramecium* IESs, the majority of which are intragenic. *T.*
122 *thermophila* also harbors 12 intragenic IESs that do not rely on Tpb2 for their elimination.
123 Remarkably, their excision is extremely precise and carried out by two distinct domesticated
124 PiggyBac transposases, Tpb1 and Tpb6 (Figure 1B), both of which are fused to a Ku80
125 domain at their N-terminal end [18, 19]. Even though the biological importance of the Ku80
126 domain still must be tested experimentally, it is tempting to speculate that Ku-transposase
127 fusions have been selected in *Tetrahymena* to secure the precise excision of intragenic IESs.

128 In ciliates, Ku80, whether as a separate factor (*P. tetraurelia*) or linked to the
129 transposase (*T. thermophila*), appears to play an essential role, independent of its *bona fide*
130 DNA repair factor function, upstream of the prDSB during PGR.

131

132 ***V(D)J recombination: coupling DNA damage and repair to avoid genomic***
133 ***instability?***

134 **V(D)J recombination** is the molecular process by which exons encoding the variable
135 domain of immunoglobulins and T cell receptors are assembled prior to their expression, thus
136 ensuring the generation of an almost infinite possibility of antigenic recognition specificities
137 by the adaptive immune system B and T lymphocytes [20]. It is essentially a mechanism
138 related to “cut and paste” transposition, in which previously scattered variable (V), diversity
139 (D), and joining (J) segments are physically associated on the DNA by a combinatorial
140 somatic rearrangement process. V(D)J recombination is initiated by a site specific prDSB
141 introduced by the lymphoid specific, domesticated transposase **RAG1 and RAG2** on
142 recombination signal sequences (RSS) that flank all the rearranging V, D, and J segments [21,
143 22], a catalytic mechanism that evolved from the ancient *Transib* transposon [23].

144 Although V(D)J recombination proceeds through the introduction of the most toxic
145 DNA lesion, it is the “price to pay” for the development of an efficient adaptive immunity,
146 and has been selected for this purpose since the jawless vertebrates [24]. Indeed, abortive
147 V(D)J recombination caused by either the inability to introduce the prDSB by RAG1/2 or the
148 inefficiency in processing/repairing these breaks results in the early arrest of B and T cell
149 maturation and the ensuing Severe Combined Immune Deficiency (**SCID**) both in humans
150 and mice [25]. Mouse models also revealed the substantial oncogenic power of V(D)J
151 recombination with the early onset of aggressive pro-B cell lymphomas in mice harboring
152 NHEJ deficiency coupled with TP53 targeted inactivation [26]. Likewise, RAG1/2 may have
153 oncogenic mutator functions driving leukemias in humans, such as observed in the context of
154 leukemias harboring the ETV6-RUNX1 chromosomal translocation [27].

155 V(D)J recombination occurs during the G0/G1 phase of the cell cycle, resulting in
156 prDSBs that are repaired through **NHEJ** [28]. The NHEJ factor Cernunnos was identified
157 through the analysis of immune compromised patients with clinical presentation resembling
158 that of patients with Lig4 syndromes but lacking DNA Ligase IV mutations [29]. Cernunnos
159 shares sequence homology and interacts with XRCC4 (X4), two features at the basis of its
160 concomitant identification as XRCC4-like factor (Xlf) [30]. XRCC4 and Cernunnos/Xlf
161 interact through their globular head domains, forming long filaments readily visible by
162 electronic microscopy and live imaging [31-35]. Given this structure, it was proposed that the

163 X4/Xlf filament could form a synapse that would facilitate DNA end tethering for their
164 subsequent ligation by NHEJ.

165 It was anticipated that because Xlf represents a *bona fide* NHEJ factor, its deficiency
166 would result in impaired V(D)J recombination. However, V(D)J recombination did not seem
167 to be significantly affected *in vivo* in the lymphoid lineages either in Xlf-deficient human
168 patients or mouse models [36-38]. First, B cell maturation in the bone marrow of Cernunnos
169 patients was not arrested at the pro-B cell stage as expected and found in case of RAG1/2,
170 Artemis, or DNA ligaseIV deficiencies. Second, Xlf KO mouse did not experience severe
171 immune deficiency, only a slight decrease in lymphocyte numbers. Third, Xlf deficiency did
172 not result in V(D)J recombination-driven chromosomal translocation or development of pro-B
173 cell lymphoma when introduced on a TP53^{-/-} background as observed with all the other models
174 of NHEJxTP53 combined inactivations. Together, it appears that Cernunnos/Xlf is
175 dispensable for V(D)J recombination although its absence clearly results in a major DNA
176 repair defect when it comes to random or accidental DNA lesions such as the ones inflicted by
177 ionizing radiations (IR).

178 One striking difference between IR- and V(D)J-driven DSBs is the presence of RAG1/2
179 in the latter. The RAG1/2 complex is known to remain on the DSB it has initiated as the post
180 cleavage complex (PCC) [21], providing a possible means to tether DNA ends, which would
181 be redundant to the expected function of the X4/Xlf filament (Figure 2A). Under this
182 hypothesis, the sole presence of the PCC would provide a DNA repair synapse
183 complementing the absence of the X4/Xlf filament during V(D)J recombination while such
184 synapse would be missing at genotoxic-driven DSBs. The stability of the PCC relies on the C
185 terminus region of RAG2, a region outside of the core and not essential for V(D)J
186 recombination, as shown *in vitro* and *in vivo* in the RAG2^{cc} mouse model specifically
187 engineered to restrict RAG2 to its core domain [39, 40]. V(D)J recombination is not grossly
188 affected in RAG2^{cc} or Xlf^{-/-} single mutant conditions, but is fully abrogated in RAG2^{cc}xXlf^{-/-}
189 mice, resulting in SCID animals devoid of mature B and T lymphocytes [41]. Based on these
190 observations a 2-synapse model (Fig. 2A) was proposed in which the PCC complex on one
191 hand and the X4/Xlf filament on the other hand help maintain genome integrity during V(D)J
192 recombination. A similar functional redundancy operating specifically during V(D)J
193 recombination was observed between Xlf and ATM or H2AX [42], and 53BP1 [43, 44] as
194 well as between Xlf and the recently described NHEJ factors PAXX [45-50] or MRI [51] thus
195 establishing a “synthetic dysfunction“ among these factors (Fig. 2B). RAG2^{cc} and ATM or
196 PAXX combined deficiencies do not result in impaired V(D)J recombination, suggesting that

197 these three factors are epistatic, opposite to that of Xlf (Figure 2B) [41, 49]. It will be
198 interesting to better understand the status of XRCC4 in this two-tier mechanism given its
199 physical interaction with Xlf. However, XRCC4 KO mice are embryonic lethal and
200 demonstrate impaired V(D)J recombination in fetuses because XRCC4 is required for DNA-
201 ligase IV stability [52, 53]. Hence, XRCC4 KO phenocopies DNA-Lig4 KO condition. In
202 contrast, the absence of immune deficiency in human primordial dwarfism with microcephaly
203 syndrome caused by hypomorphic (yet severe) XRCC4 mutations suggests that, like Xlf,
204 XRCC4 is not required for V(D)J recombination [54].

205 The demonstration of these functional interplays between RAG2, Xlf, and other DNA
206 repair factors during V(D)J recombination suggests the possible existence of a “coupling” of
207 DNA damage and repair during V(D)J recombination like the one described during PGR in
208 ciliates. The challenge is now to fully understand how functional links between the RAG1/2
209 complex and the DNA repair apparatus translate into physical interaction of key components.
210 Interestingly with regard to the analogy with PGR in ciliates, Raval et al. reported on the
211 interaction of RAG1 with the Ku70/Ku80 complex [55]. Moreover, an interaction between
212 RAG1 and another critical DNA repair factor, MDC1, was also reported [56]. The existence
213 of a physical link between RAG1/2 and the DNA repair machinery certainly accredits the
214 hypothesis of a DNA damage-repair coupling during V(D)J recombination.

215

216 **Meiotic recombination: Homologous Recombination is also concerned**

217 As opposed to NHEJ, homologous recombination uses DNA sequence homology on
218 an intact DNA template to repair the broken DNA molecule after a DSB. The repair template
219 can be located on the sister chromatid, on a homologous chromosome, or elsewhere in the
220 genome. The first step of homologous recombination is the resection of the 5' ends of the
221 DSB, first by the MRE11 complex, then by EXO1 and BLM/DNA2, which generates
222 protruding 3' ends that invade the repair DNA template, through the action of a RecA-related
223 recombinase, such as Rad51 [57]. In somatic cells, the preferred DNA repair template is the
224 sister chromatid and therefore, homologous recombination is restricted to the G2/M phases of
225 the cell cycle. During the meiotic prophase of sexually reproducing organisms, the
226 topoisomerase-like protein **Spo11** initiates **meiotic recombination** by introducing hundreds
227 of prDSBs along chromosomes. These prDSBs are important for recognition and pairing of
228 homologs and a few of them will be repaired by crossovers, generating a physical link
229 between homologs essential for their accurate segregation into the future gametes [58].

230 Meiotic recombination is a risky business for genome integrity of germ cells. Indeed,
231 the burden of prDSBs that are introduced by Spo11 during meiotic recombination is at high
232 risk of generating unwanted translocations or chromosome rearrangements, and their
233 formation is therefore highly controlled by the use of several processes specific to meiotic
234 cells. First, the DSB formation and repair steps both take place in a specific chromosome
235 compartment, **the chromosome axis**, from which chromatin loops emanate, and which will
236 be the place where the homologs become fully aligned within the synaptonemal complex
237 (Figure 3). The DNA sequences that are cleaved by **Spo11** are preferentially located on the
238 chromatin loops, although these sequences interact with proteins present along the
239 chromosome axis, implying a spatial folding of the loop towards the chromosome axis during
240 recombination. Spo11-accessory proteins link double-strand break sites to the chromosome
241 axis in early meiotic recombination [59]. This allows both to easily control the number of
242 prDSBs generated, and also to physically “isolate” the cleaved DNA sequence from non-
243 allelic/non-homologous sequences. This **physical tethering of the sequence to be cleaved**
244 onto the chromosome axis is facilitated by a specific histone modification, H3K4me3, which
245 bridges the DSB sequences to the DSB proteins located on the chromosome axis [60] thus
246 ensuring that **meiotic prDSBs are formed within the correct spatial context**. Another
247 regulation is exerted at the level of the “DSB forming complex”. Indeed, in all organisms
248 studied, the catalytic subunit Spo11 alone is not sufficient for DSB formation to take place,
249 and multiple other proteins (9 in budding yeast, at least 5 in mammals) are required [61-63].
250 Mainly discovered in budding yeast, these proteins now appear conserved among many
251 organisms, although they lack clear sequence homology, which renders them difficult to
252 identify. These proteins form several proposed subcomplexes that interact together to promote
253 DSB formation: a “core complex” composed of Spo11 (homolog of the catalytic TopoVI-A
254 subunit) and a homolog of the TopoVI-B subunit [64, 65], a “RMM” complex proposed to
255 interact directly with the chromosome axis, and other components that can vary depending on
256 the species [61]. Among the Spo11 protein partners required for DSB formation or
257 localization, several are also important for DSB repair: the **Mre11 complex** (in budding yeast,
258 the worm *C. elegans*, and maybe in mammals), Narya in the fruit fly *Drosophila*, and
259 PRDM9, which, is essential for their targeting to specific DNA sequences in humans and
260 mice, despite not being essential for DSB formation.

261 *The MRE11 complex*

262 The MRE11 complex is well known for the signaling and processing of DSBs [66]. Its
263 endonuclease activity is required to process meiotic DSBs, by removing the Spo11 protein
264 together with a short oligonucleotide from DSB ends [67]. In addition, at least in the budding
265 yeast *S. cerevisiae* and in the nematode *C. elegans*, this complex is integrated in the process
266 that is **required for prDSB formation by Spo11** [68, 69]. The functions of the MRE11
267 complex in DSB formation and repair are genetically separable, and its function for DSB
268 formation seems to involve mainly the Mre11 and Rad50 subunits [70, 71]. In mammals,
269 whether the MRE11 complex is needed for Spo11-induced DSB formation is not clear at the
270 moment, because members of the MRE11 complex are essential for viability, so only
271 hypomorphic mutants of the MRE11 complex could be tested in the mouse for their effect on
272 fertility, and they showed only defects in meiotic DSB repair [72]. Conditional knock out of
273 the Mre11 complex specifically in meiotic cells would be required to know if it is also
274 required for meiotic DSB formation in mammals. In budding yeast, components of the
275 MRE11 complex appear to directly interact with other DSB formation proteins [73], implying
276 a specialization of the MRE11 complex for the immediate signaling and processing of these
277 programmed meiotic DSBs. Likewise, in the plant *Arabidopsis thaliana*, although not strictly
278 required for meiotic DSB formation, a member of the MRE11 complex directly interacts with
279 a protein required for DSB formation ([74] and Mathilde Grelon, personal communication). It
280 is attractive to propose that incorporating the signaling and repair Mre11 complex in the step
281 of meiotic DSB formation allows the immediate processing of meiotic DSBs into the
282 homologous recombination pathway.

283 *Narya*

284 Another example of coupling between meiotic DSB formation and repair comes from
285 the fruit fly *Drosophila*, where a protein, **Narya**, fulfills functions both for formation and
286 repair of meiotic DSBs with a crossing over, to ensure proper homolog segregation and
287 successful meiosis [75]. Narya is a RING finger protein that is redundant with another related
288 protein, Nanya, for meiotic DSB formation and repair. It also interacts with Vilya, a third
289 protein of the family, required for DSB formation and interacting with MEI22, one of the
290 *Drosophila* DSB proteins [76]. Interestingly, a separation-of-function allele of Narya, in its
291 RING finger domain, shows that Narya is not only involved in DSB formation, but also
292 required for their repair as a crossover. In addition, the three proteins show two sequential
293 localization patterns; first, early with DSB sites and then, to crossover sites [75, 76]. This
294 illustrates again a double function for meiotic DSB formation and their repair into crossovers
295 within a single protein, therefore directly coupling these two steps of meiotic recombination.

296 ***PRDM9***

297 The histone methyltransferase **PRDM9**, responsible for targeting meiotic prDSBs to
298 its consensus DNA binding sequence in many organisms including mice and humans, is also
299 critical for meiotic prDSB repair. In its absence, Spo11 forms meiotic prDSBs at “default”
300 chromatin accessible locations within functional genomic elements, which are not well
301 repaired for unclear reasons [77, 78]. In addition, if PRDM9 is present on only one homolog
302 owing to a polymorphism affecting its consensus binding sequence, this also creates problems
303 in DSB repair [79]. This suggests that the symmetric binding of PRDM9 to both homologs,
304 thanks to its sequence specificity, facilitates the repair, perhaps by bringing close together into
305 the chromosome axis the two chromatid sequences that will experience the recombination
306 event (Figure 3). In favor of this hypothesis, PRDM9 physically interacts with several
307 components of the chromosome axis [80]. PRDM9 therefore represents yet another example
308 of coupling prDSB formation and repair within a single protein during programmed meiotic
309 recombination.

310

311 **Concluding remarks**

312 Besides meiosis, PGR, and V(D)J recombination, prDSBs have been identified during
313 signal-induced transcription in several experimental settings (see [81] for a recent review).
314 These activity-induced prDSBs occur primarily in early response genes and are introduced by
315 the topoisomerase II β . This is in particular the case in the response of MCF-7 cells to
316 estradiol [82] or activation through glucocorticoid receptors [83]. prDSBs also occur *in vivo*
317 and *in vitro* upon neuronal activity [84, 85]. Interestingly, in the case of the glucocorticoid
318 receptor-induced transcriptional activation, recruitment of Top2b and Ku70/86 via the BRG1
319 transcription activator-containing chromatin-remodeling complex is required at GR-
320 responsive promoters [83]. This suggests that a subset of DNA repair factors may be in place
321 before prDSBs, thus accrediting the hypothesis of a possible coupling of DNA damage and
322 repair during signal-induced transcription. Finally, beside Pgm in ciliates and RAG1/2, two
323 other domesticated transposases have been identified in human cells, the piggyBac
324 transposable element-derived 5 (PGBD5) [86] and THAP9, related to the *Drosophila* P-
325 element transposase [87], the exact functions of which are presently unknown. The
326 deregulated expression of PGBD5 in rhabdoid tumors in children participates in the
327 oncogenic transformation by promoting site-specific DNA rearrangements within tumor
328 suppressor genes [88]. Whether this deleterious activity of PGBD5 is counteracted in its

329 physiological context by a mechanism related to DNA damage-repair coupling is of course
330 speculative but represents an interesting issue to follow. If this were to be true, it would
331 reinforce the idea that DNA damage-repair coupling may represent an essential step in the
332 transposase domestication process.

333 Altogether coupling DNA damage and repair may have co-evolved with prDSBs to
334 ensure their efficient repair and thus avoid any associated genomic instability. Several
335 questions remain to be addressed: (i) what are the exact mechanisms governing this coupling,
336 (ii) given the oncogenic power of DSBs, what would be the consequences of losing this
337 coupling (see Outstanding Questions)?

338

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564

565 **Highlights**

566 • Several biological processes (meiosis, V(D)J recombination, PGR in ciliates, signal-
567 induced transcription) proceed via introduction of programmed DSBs (prDSBs).

568 • DSBs being the most toxic DNA lesions, as potentially oncogenic, prDSBs are likely
569 associated with very efficient, multi-layered DNA repair mechanisms. Coupling DNA
570 damage and repair is one critical layer.

571 • Ku80 is a critical factor to link DNA damage and repair during PGR in ciliates

572 • The C terminus of RAG2 may be responsible for the DNA damage-repair coupling
573 during V(D)J recombination as a safeguard against genome instability.

574 • During meiotic recombination, a specific pathway ensures that meiotic DSBs are
575 formed within the correct spatial chromosomal context.

576 • The MRE11 complex is required for the formation of prDSBs by Spo11 during
577 meiotic recombination.

578 • DNA damage-repair coupling may represent an essential step in the domestication
579 process of PiggyMac, RAG1/2 and other transposases.

580

581 **Outstanding Questions Box**

582 • What are the exact mechanisms and critical players of DNA damage-repair coupling
583 during prDSBs?

584 • What are the downstream consequences of uncoupling DNA damage-repair during
585 prDSBs driven processes (for example: genetic instability and tumor development,
586 cellular degeneracy)?

- 587 • Is DNA damage-repair coupling a general rule that applies to all biological processes
588 that proceed through prDSBs?
589

590 **Glossary**

- 591 • **DSBs:** DNA double strand breaks can be “accidental” as a result of environment insult
592 or “programmed” (prDSBs) as part of essential physiological processes (meiosis,
593 V(D)J recombination, PGR in ciliates).
- 594 • **HR:** The Homologous Recombination DNA repair pathway is one of the two main
595 mechanisms, with NHEJ, to repair DSBs. It operates exclusively in S phase of the cell
596 cycle when a sister chromatid is available as template.
- 597 • **Meiosis:** Meiosis is the process of chromosome segregation during the formation of
598 gametes. prDSBs are introduced by Spo11 during meiosis
- 599 • **MRE11 complex:** Composed of meiotic recombination 11 (MRE11), RAD50 and
600 Nijmegen breakage syndrome 1 (NBS1 or Nibrin, Xrs2 in budding yeast). Acts in the
601 sensing and signaling of DSBs. The endonuclease activity of MRE11 is essential for
602 the processing of protein-linked meiotic DSBs.
- 603 • **NHEJ:** The Non Homologous End Joining DNA repair pathway is one of the two
604 main mechanisms, with HR, to repair DSBs. It operates in all phases of the cell cycle.
- 605 • **PGR:** Programmed Genome Rearrangement that reproducibly eliminates large
606 fractions of germline DNA (25 to 95% according to species) during formation of the
607 Macronucleus (MAC) during the ciliate sexual cycle
- 608 • **PiggyMac (Pgm):** Domesticated transposase responsible for PGR in the ciliate
609 *Paramecium*.
- 610 • **RAG1 & RAG2:** The Recombination Activating Gene 1 & 2 constitute the
611 domesticated transposase initiating V(D)J recombination in immature B and T
612 lymphocytes.
- 613 • **SCID:** Severe Combined Immune Deficiency is a rare condition in humans (and
614 engineered mouse models) characterized by a profound defect in the
615 development/function of the adaptive immune system. Impaired V(D)J recombination
616 results in SCID.
- 617 • **Spo11:** Catalytic subunit of a topoisomerase-like complex that introduces prDSBs
618 during meiosis

- 619 • **V(D)J Recombination:** Lymphoid-specific somatic DNA rearrangement process of
620 immunoglobulin and T cell receptor (TCR) genes initiated by the RAG1/2 factors
621 aimed at generating the antigenic diversity (repertoire) of the adaptive immune
622 system.

623

624 **Figure Legends**

625 **Figure 1: Coupling between DSB formation and repair during programmed genome** 626 **rearrangements in ciliates**

627 **A)** In the ciliate *Paramecium tetraurelia*, vegetative cells harbor two MICs (black) and one
628 MAC (gray). During sexual reproduction, MICs undergo meiosis, a single meiotic product
629 divides and yields two gametic nuclei (black), while all others are degraded (light gray).
630 During conjugation, following reciprocal exchange of gametic nuclei between mating
631 partners, the resident and incoming nuclei fuse to give a diploid zygotic nucleus (black);
632 during autogamy (a self-fertilization process), the zygotic nucleus results from the fusion of
633 both gametic nuclei from the same cell. The zygotic nucleus then divides twice: two of the
634 resulting nuclei become the new MICs (black) and the other two differentiate into new MACs
635 (hatched purple and blue). Programmed DNA elimination takes place during MAC
636 development. Following mitosis of the new MICs, the new MACs segregate into each
637 daughter cell. Throughout the whole process, the old MAC is fragmented and is eventually
638 lost after a few cell divisions. **B)** In *Paramecium*, the presence of Ku is required for Pgm to
639 cleave DNA at IES ends, indicative of tight coupling between DSB formation and repair.
640 *Tetrahymena* intragenic IESs are excised by domesticated transposases (Tpb1 and Tpb6)
641 fused to a Ku80-like domain, the functional importance of which has not been established.

642

643 **Figure 2: Coupling between DSB formation and repair during V(D)J Recombination?**

644 **A)** Two complementary synapses (the XLF/XRCC4 filament and the RAG1/2 post cleavage
645 complex) ensure DNA end tethering during V(D)J recombination. The loss of both synapses
646 results in major genomic instability with the development of lymphomas. **B)** Synthetic
647 dysfunction of V(D)J recombination highlights two complementary axes during the repair
648 phase of V(D)J recombination. RAG2 participates in the ATM-PAXX-MRI axis,
649 complementary to the XLF-XRCC4 axis.

650

651 **Figure 3: Coupling between DSB formation and repair during programmed meiotic**
652 **recombination.**

653 During meiosis, homologous chromosomes experience homologous recombination, which
654 promotes their pairing, crossover and physical attachment through the chiasmata.
655 Chromosomes are organized around a protein axis (blue and red lines for maternal and
656 paternal chromosomes), from which chromatin loops emanate (gray). DSBs (yellow
657 lightening) are formed to initiate recombination, which culminates into at least one crossover
658 pair of homologs. Programmed DSBs occur at hotspot sequences, which become transiently
659 tethered to the chromosome axis where DSB proteins (including the catalytic subunit Spo11)
660 are located. Among these, the Mre11 complex is necessary both for the formation of DSBs by
661 Spo11 and for their repair, highlighting the specialized coupling between DSB formation and
662 repair during meiosis. Other examples of such coupling are discussed in the text.

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