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1 **Chapter X**

2 **Alternative DNA repair pathways to handle complex DNA damage generated by**
3 **oxidative stress and anticancer drugs**

4
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17

18 ABSTRACT

19 The clinical features of inherited human DNA repair deficient disorders such as
20 Cockayne syndrome and Fanconi anemia point to the complex nature of
21 endogenous oxidative DNA damage, which may include bulky adducts, inter-strand
22 DNA crosslinks (ICLs) and clustered lesions. Conversely, severe biological effects of
23 DNA crosslinking agents and ionizing radiation correlated with formation of ICLs and
24 double-strand breaks in DNA, respectively. These complex DNA damages are
25 postulated to be critical because they are more difficult to repair than singular
26 lesions. It anticipated that the removal of ICLs and clustered oxidized bases on both
27 strands would, if not tightly regulated, either inhibit certain steps of repair or produce
28 persistent chromosome breaks and thus be lethal for the cells. Genetic and
29 biochemical data indicate that the elimination of complex damages requires several
30 distinct DNA repair pathways including: base excision repair, nucleotide incision
31 repair, Poly(ADP-ribose) polymerases-mediated DNA strand break repair, global
32 genome and transcription coupled nucleotide excision repair, mismatch repair,
33 homologous recombination, non-homologous end joining, and translesion DNA
34 synthesis pathways. In this review, we describe the role of recently discovered
35 alternative DNA repair pathways in the removal of complex DNA lesions.

36

37

38 **X.1 Chemical nature of complex DNA damage**

39 Cellular DNA constantly undergoes chemical alterations, resulting in DNA lesions
40 that are cytotoxic, miscoding or both and are believed to be at the origin of
41 mutagenesis and cell lethality.^{1,2} DNA damage can be classified by their nature:
42 spontaneous *versus* induced; by their structure: bulky *versus* nonbulky, base *versus*
43 sugar damage, singular *versus* complex damage (Figure 1). Complex DNA damage
44 (CDD) such as bulky DNA adducts, inter-strand DNA crosslinks (ICLs) and clustered
45 lesions (including double-strand breaks, DSBs) have complex character/structure as
46 compared to singular lesions such as randomly distributed abasic sites, deaminated,
47 alkylated and oxidized DNA bases.

48 [Insert Figure X.1 here]

49 Two of the most important features of CDD are their bulky character and presence of
50 more than one modification within one turn of DNA helix. Although CDD, such as
51 ICLs and DSBs, typically constitute relatively small fraction of the total DNA damage
52 induced by DNA crosslinking agents and ionizing radiation, respectively, they are
53 expected to play disproportionately important biological roles, since if not repaired,
54 these CDD could lead to a number of serious consequences, such as gross
55 chromosomal rearrangements and genome instability.

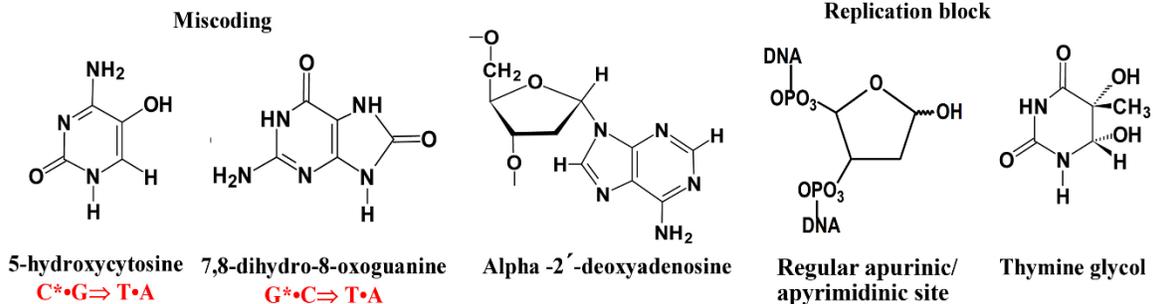
56 **X.1.2. Repair-resistant bulky DNA adducts**

57 **X.1.2.1. 8,5'-Cyclopurine-2'-deoxynucleosides**

58 Reactive oxygen species (ROS), produced during oxidative cellular metabolism and
59 by ionizing radiation, damage cellular DNA leading to formation of CDD. Bulky DNA
60 adducts such as diastereoisomeric (5'*S*)- and (5'*R*)-8,5'-*cyclo*-2'-deoxyadenosine

Figure 1

A. Singular DNA base damage



B. Complex DNA damage

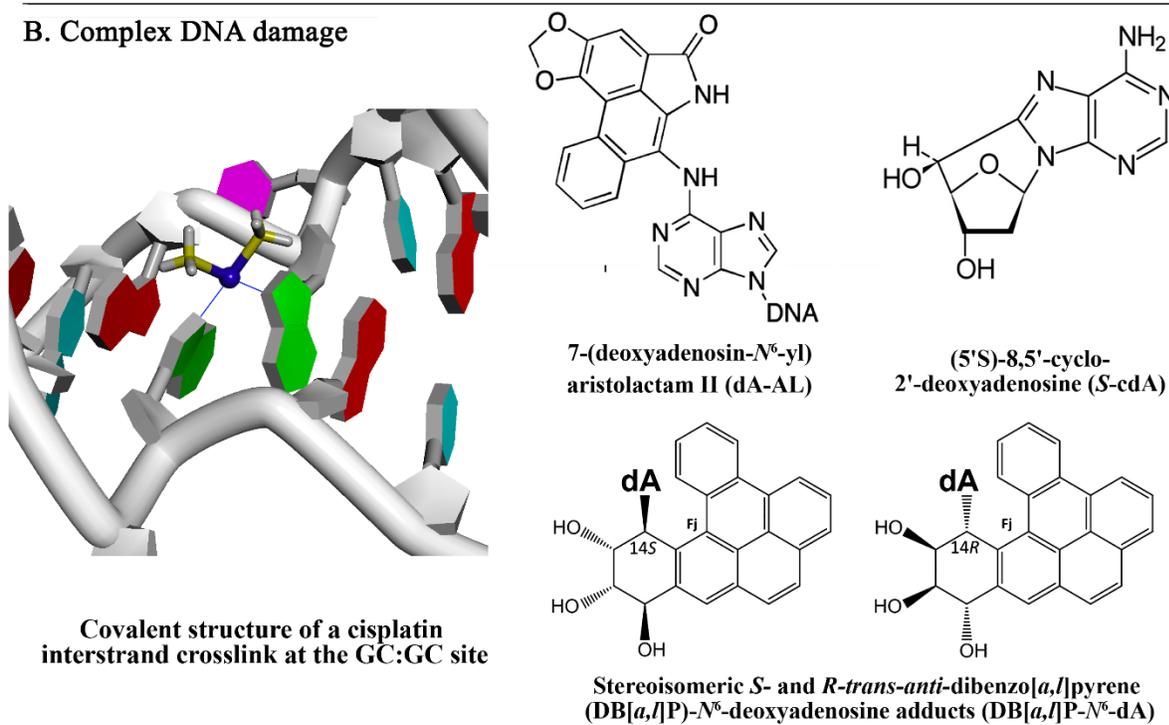


Figure 1. Schematic presentation of singular and complex DNA damage. **(A)** Chemical structures of some singular non-bulky DNA base lesions. **(B)** Chemical structures of complex DNA lesions including cisplatin-ICL and bulky repair-resistant DNA base lesions.

61 (cdA) and 8,5'-*cyclo*-2'-deoxyguanosine (cdG) are generated by hydroxyl radical
62 attack at C5' sugar by H-abstraction resulting in the formation of a covalent bond
63 between the C5'- and C8-positions of the purine nucleoside³ (Figure 1B). These DNA
64 adducts are helix-distorting lesions and strong blocks for both DNA replication and
65 transcription.^{4,5} It was demonstrated that the global genome nucleotide excision
66 repair (GG-NER) pathway can remove cdA adducts with an efficiency comparable to
67 that of UV-induced cyclobutane pyrimidine dimers, but exhibits higher activity in
68 excising the *R*-isomer.^{4,6} Indeed, cdG and cdA adducts accumulate in keratinocytes
69 from NER-deficient xeroderma pigmentosum (XP) complementation group C (XPC)
70 and Cockayne syndrome (CS) complementation group A (CSA) patients exposed to
71 X-rays and potassium bromate (KBrO₃)^{7,8} and also in organs of CSB^{-/-} knockout
72 mice.⁹ Noteworthy, cdA and cdG lesions accumulate in age dependent manner in
73 nuclear DNA of wild-type mice, implying the difficulties in the removal of these
74 complex DNA lesions.¹⁰ Also, the *S*-cdA diastereoisomer present at higher levels in
75 organs from control non-exposed mice because they are removed by GG-NER much
76 less efficiently than the corresponding the *R*-cdA diastereomer.^{10,11}

77 **X.1.2.2. Bulky DNA adducts induced by environmental carcinogens**

78 Bulky DNA adducts that are generated by exposure to environmental carcinogens
79 cause mutations that can drive malignant transformation of affected cells. As we
80 described above, bulky DNA adducts are removed in the GG-NER pathway;
81 however, certain bulky lesions that cause a minimal decrease, or an enhancement in
82 the stabilities of the DNA duplex, can be very resistant to the repair machinery.
83 Benzo[*a*]pyrene (B[*a*]P) is a polycyclic aromatic hydrocarbon (PAH) and well known
84 carcinogen, which upon metabolic activation to diol epoxide can interact with
85 guanines in DNA to form bulky B[*a*]P-*N*²-dG adducts. In contrast, dibenzo[*a,h*]pyrene

86 (DB[a,l]P), another PAH that is present in about ten-fold lower concentration in the
87 environment, is 100 times more tumorigenic than B[a]P in rodent model systems.¹²⁻¹⁷
88 The reactive diol epoxide intermediates of DB[a,l]P react with either *N*⁶-adenine or
89 *N*²-guanine in DNA to form stable DB[a,l]P-*N*⁶-dA and DB[a,l]P-*N*²-dG adducts¹⁸⁻²⁰
90 (Figure 1B). Remarkably, Kropachev et al., demonstrated that the *S* and *R*
91 diastereoisomers of DB[a,l]P-*N*²-dG adduct are 15 and 35 times more efficiently
92 removed by GG-NER, respectively, as compared to the stereochemically identical
93 DB[a,l]P- *N*⁶-dA adducts.²¹ This observation suggests that the higher genotoxic
94 activity of DB[a,l]P, as compared to B[a]P, might be due to the generation of repair-
95 resistant and thus persistent DB[a,l]P-derived adenine adducts in exposed cells.

96 Other bulky DNA lesions, that cause little or no distortion and small
97 thermodynamic destabilization of DNA duplex, also exhibit NER resistance including
98 aristolactam-DNA adducts generated by the exposure to aristolochic acid^{22,23} (Figure
99 1B), 2-acetylaminofluorene AAF-*N*²-dG adduct,^{24,25} 3-benzanthrone derived DNA
100 adducts,²⁶ and stable Fapy-dG adducts derived from aflatoxin B1.²⁷ Thus, despite
101 their bulky character, certain highly mutagenic DNA lesions can escape DNA
102 damage surveillance and repair. These repair-resistant DNA damage present a
103 challenge for the cell since their can persist in the genome and lead to the
104 transcription and replication blockages and mutations.

105 X.1.3. Formation and chemical nature of DNA crosslinks

106 Bifunctional alkylating agents, such as nitrogen mustard (HN2), psoralen and
107 mitomycin C (MMC), can generate a covalent bond between nucleotides on opposite
108 strands of a DNA duplex resulting in formation of ICLs. ICLs are highly lethal DNA
109 lesions that block DNA replication, transcription and recombination by preventing

110 strand separation. Due to their high cytotoxicity, ICL-inducing agents are widely used
111 against hyperplastic diseases, such as cancer and psoriasis. 8-methoxypsoralen (8-
112 MOP) is a planar, tricyclic compound that intercalates into DNA duplex preferentially
113 at 5'-TpA sites. Upon photoactivation, 8-MOP primarily photoalkylates DNA by
114 cycloaddition to the 5,6-double bond of a thymine generating monoadducts (MA) and
115 ICLs.^{28,29} Although, the yield of psoralen MAs to pyrimidine bases is three-fold higher
116 than that of ICLs, the latter class of damage appears to have more severe biological
117 effect.³⁰ Derivatives of HN2 induce a variety of DNA lesions, and only 5% among
118 them constitute cross-links between the N7 position of guanine bases on opposite
119 DNA strands.³¹ The platinum compounds such as cis-diamminedichloroplatinum (II),
120 also known as cisplatin, reacts with guanines and induces mainly DNA diadducts:
121 65% d(GpG) intra-strand cross-links, 25% d(ApG) intra-strand cross-links and 5–8%
122 ICLs between the guanines in the sequence d(GpC). The structures of HN2 and
123 cisplatin-induced ICLs revealed that these agents induce dramatic distortions to the
124 DNA helix,³² whereas MMC and psoralen-induced ICLs cause relatively minor
125 distortions.³³ Endogenous sources such as aldehydes formed as products of lipid
126 peroxidation and nitrous acid can also form ICLs in non-treated cells.³⁴ In addition to
127 ICLs, formaldehyde, ionizing and UV radiations can crosslink proteins to an
128 undisrupted DNA strand generating a DNA-protein crosslink (DPC).³⁵

129 **X. 2 DNA glycosylase-mediated repair of complex DNA lesions**

130 **X. 2. 1. DNA glycosylase-mediated removal of bulky DNA lesions**

131 Bulky DNA adducts and ICLs are highly toxic for cells and accumulation of
132 endogenously occurring ICLs have been linked to premature aging.^{36,37} Although the
133 GG-NER pathway is thought to be main pathway for the removal of ICLs and bulky

134 lesions that disrupt DNA helix conformation,^{38,39} most of singular DNA lesions
135 generated by oxidative stress are eliminated by two distinct pathways: base excision
136 and nucleotide incision repair (BER) and (NIR), respectively.^{40,41}

137 BER is initiated by a DNA glycosylase that recognizes and excises damaged
138 bases by hydrolysis of the glycosidic bond. In general, substrate specificities of DNA
139 glycosylases and AP endonucleases are limited to structurally small non-bulky DNA
140 base damage that have little impact on thermodynamic stability of DNA duplex.

141 Majority of DNA glycosylases bind to damaged DNA and flip out of the duplex the
142 aberrant nucleotide into the enzyme's active site pocket, while inserting residues in
143 the void left in the helix to stabilize the DNA substrate conformation.⁴²⁻⁴⁴ In general,
144 nucleobase binding pockets are too small to accommodate large size DNA adducts
145 and ICLs; however, several lines of evidence argue that DNA glycosylases are able
146 to accommodate bulky DNA adducts despite steric constraints to fit these lesions
147 into their active sites. Specifically, it was demonstrated that DNA glycosylases Fpg,
148 T4 endonuclease V (T4 pyrimidine dimer glycosylase) and NEIL1 can recognize and
149 efficiently excise very bulky DNA adducts such as imidazole ring opened form of
150 guanine-C8-N-hydroxy-2-aminofluorene adduct, cyclobutane dimer, psoralen-
151 thymine monoadduct, and aflatoxin-Fapy-guanine adducts (AFB₁-FapyGua),
152 respectively.⁴⁵⁻⁴⁸ Structural studies revealed that T4 endonuclease V kinks the DNA
153 helix by about 60° and flips out the opposing adenine base complementary to the 5'
154 pyrimidine of the thymine dimer out of the DNA base stack, thus avoiding steric
155 problems.⁴⁶ Whereas, Fpg flips bulky *N7*-substituted Fapy-dG derivatives guanine
156 lesion out of the DNA helix to the binding pocket, but enables *N7*-bulky group to stay
157 outside.⁴⁹ This latter mechanism might be also employed by human NEIL1 DNA

158 glycosylase when acting upon large voluminous DNA lesions such as psoralen-
159 thymine, protein-DNA crosslinks and AFB₁-FapyGua.^{47,48,50}

160 Recent studies by Eichmann laboratory revealed a new family of DNA
161 glycosylases, which utilizes a non-base-flipping mechanism to recognize bulky DNA
162 base damage and a certain type of ICL.⁵¹⁻⁵³ DNA glycosylases AlkC and AlkD
163 originally discovered in *Bacillus cereus*⁵⁴ and constituting a distinct DNA
164 glycosylases superfamily⁵⁵ can excise positively charged N³- and N⁷-alkylpurines
165 with high specificity without inserting a damaged base into active site pocket.⁵¹ AlkD
166 traps damaged bases in a sheared, base-stacked conformation, directly contacting
167 the deoxyribose with catalytic active site residues, but not the nucleobase like other
168 DNA glycosylases. AlkD is capable of excising bulky DNA adducts such as
169 pyridyloxobutyl (POB) adducts, arising in DNA as a result of cigarette-smoke
170 carcinogen – nitrosamine ketone, and N³-yatakemycinadenine (YTMA) produced
171 by extremely cytotoxic alkylating product yatakemycin (YTM).^{51,56,57} The ability of
172 AlkD to resolve bulky YTM and POB DNA modifications located in any grooves is
173 presumed to be due to the absence of steric restraint. Nevertheless, the presence of
174 other classic alkylpurine DNA glycosylases in these bacteria points to the fact that
175 catalytic activity of AlkD is limited to positively charged DNA lesions that can be
176 easily removed naturally.^{51,58} AlkC DNA glycosylase contains immunoglobulin (Ig)-
177 like domain that was not found in the known repair proteins, which along with HEAT-
178 like repeats (HLR), allows the enzyme to almost completely wrap around DNA
179 duplex and excise damaged nucleobases. AlkC acts through non-base-flipping
180 mechanism similar to AlkD; however, it generates sharp kink in DNA duplex to gain
181 access to the modified base through inserting its catalytic residues into the exposed
182 space.⁵⁵

183 Azinomycin B (AZB) is a genotoxic non-ribosomal peptide-polyketide
184 secondary metabolite produced by soil-dwelling bacteria including *Streptomyces*
185 *sahachiroi* and *Streptomyces griseofuscus*. AZB generates ICLs in DNA duplexes at
186 5'-GNC or 5'-GNT sequence contexts by nucleophilic addition at the N7 positions of
187 purines in a major groove.⁵⁹ The classical repair mechanisms used to resolve ICLs in
188 bacterial cells consists of coordinated function of NER machinery and homologous
189 recombination (HR).⁶⁰ Nevertheless, recently it has been found that AlkZ DNA
190 glycosylase of *Streptomyces sahachiroi* is capable of unhooking AZB-induced ICLs
191 through the cleavage of N-glycosidic bond on both sides of the complementary DNA
192 strands.^{58,61,62} The existence of unusual alternative DNA repair mechanisms in toxin-
193 producing cells may provide insight for potential mechanisms implicated in drug
194 resistance in cancer cells.⁵³

195 X. 2. 2. DNA glycosylases mediated repair of inter-strand DNA crosslinks

196 Genetic and biochemical evidences suggest that in vertebrates, the repair of ICLs
197 proceeds in DNA replication-dependent manner and coordinated by Fanconi anemia
198 (FA) proteins. The removal of an ICL goes through formation of a DSB as a result of
199 the unhooking via dual incisions on both sides of the lesion by the scaffolding protein
200 SLX4 and structure-specific endonuclease XPF/ERCC1.^{63,64} The resulting unhooked
201 ICL is processed by translesion synthesis (TLS) specific DNA polymerases yielding a
202 three-stranded DNA repair intermediate composed of a short oligomer covalently
203 bound to the duplex. It thought that this crosslinked fragment or unhooked ICL is
204 removed by the classic GG-NER pathway.^{38,39} However, Couve et al., revealed that
205 the oxidative DNA glycosylases *Escherichia coli* (*E. coli*) Nei and human NEIL1
206 excise with high efficiency the psoralen-induced bulky unhooked ICL fragment within
207 a three-stranded DNA structure⁶⁵ (Figure 2A). Three Nei-like (NEIL) DNA

Figure 2

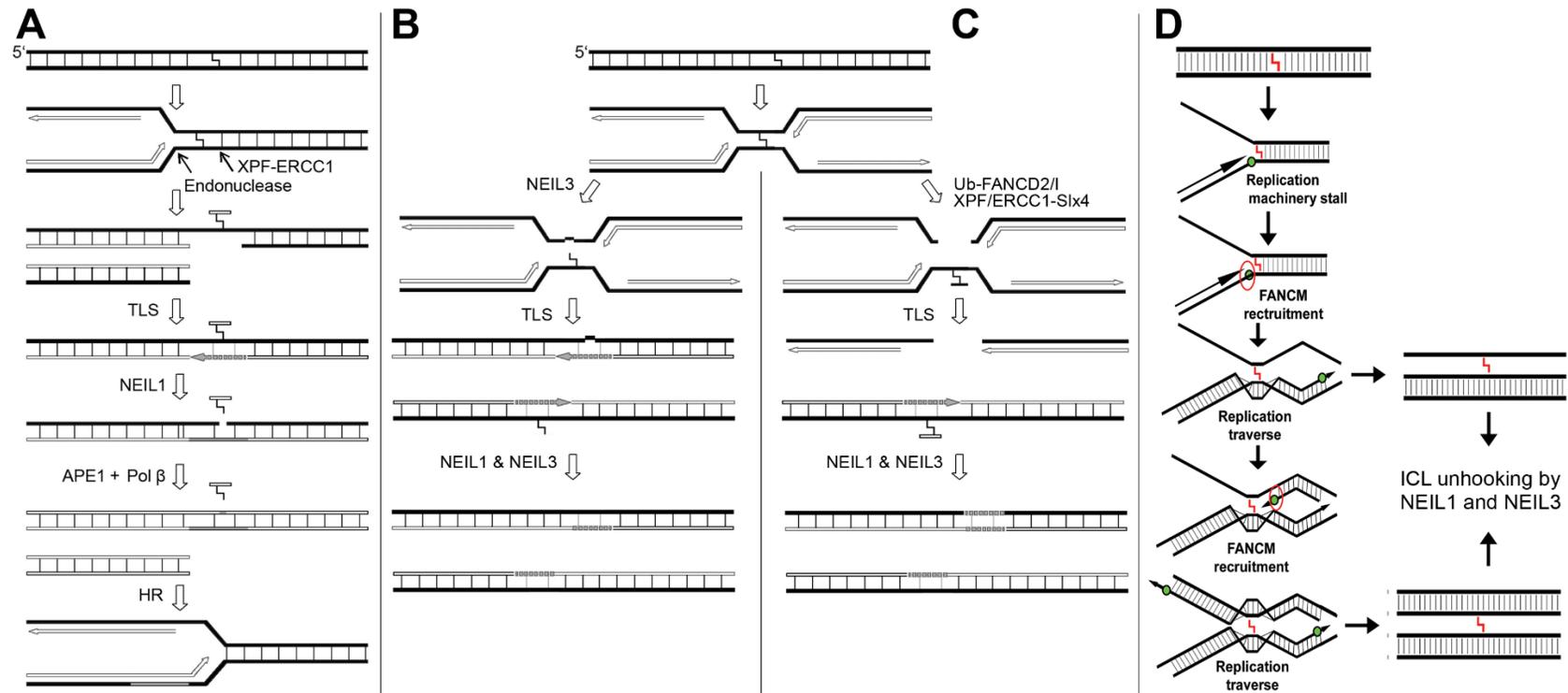


Figure 2. DNA replication-coupled repair of inter-strand DNA crosslinks. **(A)** NEIL1 excises unhooked crosslinked oligomer in three-stranded DNA repair intermediate. **(B)** NEIL3- and **(C)** Fanconi anemia-mediated repair of ICL. **(D)** The hypothetical mechanism of the FANCM-mediated DNA replication fork bypass of ICL and NEILs-mediated repair of three- and four stranded crosslinked DNA structures.

208 glycosylases are present in mammalian cells; these proteins show structural
209 homology to the Fpg and Nei proteins of *E. coli* and remove oxidised bases from
210 DNA.⁶⁶ While NEIL1 and NEIL3 appear to be cell cycle regulated, with expression
211 peaking in S phase and late S/G2 respectively, NEIL2 is constitutively expressed
212 throughout the cell cycle.^{67,68} All three proteins have DNA glycosylase and β or β/δ -
213 lyase activities, with an unusual preference for single-stranded DNA and other DNA
214 open structures generated during DNA replication and transcription.

215 [Insert Figure X.2 here]

216 Reconstitution of the repair of plasmids containing a single ICL in *Xenopus*
217 eggs extracts showed that the removal of ICL was coupled to DNA replication and
218 involved convergence of two replication forks on the lesion, with the formation of an
219 X-shaped DNA structure⁶⁹ (Figure 2B). Subsequently, it has been proposed that ICL
220 repair requires the convergence of two forks on the lesion since, when only one fork
221 was stalled at the ICL in egg extracts, no ICL repair took place.⁷⁰ On the other side,
222 Huang et al., investigated the collision of replication forks with fluorescently labelled
223 psoralen-ICLs in mammalian cells using DNA combing.⁷¹ They observed that during
224 S phase the majority of psoralen ICLs (around 60%) were processed through a
225 replication-traverse pathway, in which the ICLs are left unrepaired, but traversed by
226 the replication machinery to allow DNA synthesis to resume on the other side. In
227 these scenarios of fork traverse and the former one of dual fork convergence, a
228 similar X-shaped DNA structure is generated around the ICL, which is critical to
229 initiate ICL repair.⁷² Noteworthy, these unrepaired psoralen-induced ICLs are
230 subsequently removed during a post-replication repair without the generation of
231 DSBs.⁷¹ In the following work, Semlow et al., have demonstrated that NEIL3 from
232 *Xenopus laevis* can excise psoralen and abasic site ICLs in DNA in X-shaped

233 double-stranded DNA structures via an incision-independent repair mechanism,
234 suggesting that this activity may be a principal role of NEIL3 in rapidly dividing cells⁷³
235 (Figure 2B). More recent biochemical studies by Martin et al., showed that human
236 NEIL3 cleaves psoralen-induced ICL in single-stranded, three-stranded and four-
237 stranded DNA substrates to generate unhooked DNA fragments containing either an
238 abasic site or a psoralen-thymine monoadduct. In addition, Nei and NEIL1 also
239 excised a psoralen-induced four-stranded DNA substrate to generate two unhooked
240 DNA duplexes with a nick, while NEIL3 targeted both DNA strands in the ICL
241 substrate without generating single-strand breaks. Based on these observations,
242 Martin et al., proposed a model for the mechanism of ICL repair in mammalian cells
243 that implicates the FANCM-catalysed replication traverse of an ICL site, without
244 preceding DNA incision and the generation of either three- or four-stranded DNA
245 structures composed of the duplex cross-linked either to single-stranded or duplex
246 DNA⁷⁴ (Figure 2C). These putative ICL lesion bypass products can then be repaired
247 by the Nei-like DNA glycosylases-initiated BER pathway without generation of highly
248 toxic DSB (Figure 2C).

249 Thus, the ICL repair in eukaryotic cells is coupled to DNA replication and
250 proceeds *via* two alternative mechanisms: (i) Fanconi anemia system incision-
251 dependent pathway, which engenders replication fork collapse and generation of
252 DSBs; and, (ii) the incision-independent pathway in which NEIL1 and NEIL3 DNA
253 glycosylases mediate ICL unhooking without formation of highly genotoxic DNA
254 repair intermediates. Although, an ICL covalently links the two strands of the DNA, it
255 is important to highlight that the level of distortion of the DNA helix and the choice of
256 the DNA repair pathway depends on the chemical nature of ICL. Cisplatin- and
257 nitrogen mustard-generated ICLs are preferentially processed via the incision-

258 dependent Fanconi anemia coordinated network,^{39,69} whereas, psoralen and abasic
259 site induced ICLs processed rather via NEIL3-catalyzed BER pathway.^{73,74}
260 Nonetheless, NEIL3^{-/-} knockout mouse embryonic fibroblasts (MEF) exhibit modest
261 sensitivity to cisplatin,⁷⁵ implying that some cisplatin-ICLs might be removed in the
262 BER pathway, which can serve as a back-up repair system for FA and NER.
263 Importantly, it has been proposed that NEIL3-mediated repair of ICLs would be more
264 mutagenic as compared to that of Fanconi anemia.⁷³

265 **X. 2. 3. Aberrant repair of interstrand DNA crosslinks**

266 Cisplatin [*cis*-diamminedichloroplatinum(II)] is well known for its capacity to trigger a
267 strong DNA damage response signal, which may eventually result in irreversible
268 apoptosis. Studies of the roles of BER enzymes in the repair of ICLs have produced
269 conflicting results, suggesting involvement of multiple pathways and mechanisms
270 depending on the cellular and structural contexts. For example, Kothandapani et al.,
271 have shown that inhibition of BER by methoxyamine (MX), a specific inhibitor of
272 APE1-catalyzed cleavage of AP sites, along with the depletion of DNA polymerase β
273 (Pol β) and uracil-DNA glycosylase (UNG) enhances the resistance of cells to
274 cisplatin.⁷⁶ Using synthetic oligonucleotides with a single site-specific cisplatin-ICL,
275 the authors demonstrated that the accelerated and preferential deamination of
276 flipped out cytosine residues next to cross-linked guanines, can induce formation of
277 uracil residues adjacent to ICL. These uracils are excised by UNG to generate AP
278 sites, which then are cleaved by APE1, with following Pol β -catalyzed gap-filling DNA
279 repair synthesis. The authors proposed that initiation of BER adjacent to cisplatin ICL
280 sites could interfere with the NER and HR pathways and lead to the inhibition of ICL
281 removal and increasing cisplatin cytotoxicity. Both repair and redox domains of APE1
282 appear to require for mediating cisplatin toxicity in breast cancer.⁷⁷ Several small

283 inhibitors developed against APE1, Pol β and PARP1 are currently studying in anti-
284 cancer therapy [see (see Chapters X,X,X (Kelley, Curtin, Madhusudan))]. Thus,
285 understanding the roles of BER proteins in the repair of complex DNA lesions might
286 be important to counteract the resistance of cancer cells.⁷⁸

287 **X.3. The apurinic/aprimidinic (AP) endonuclease–initiated nucleotide incision** 288 **repair pathway for oxidative DNA damage**

289 **X.3.1. Substrate specificity of AP endonucleases as multifunctional enzymes**

290 Recognition and removal of non-bulky damage of nitrogenous bases proceed via the
291 BER pathway initiated by DNA glycosylases.⁷⁹⁻⁸¹ In general, the BER pathway
292 includes sequential actions of two enzymes for DNA incision: a DNA glycosylase and
293 an AP endonuclease (Figure 3, pathways 1-3). Noteworthy, DNA glycosylases
294 generate highly genotoxic intermediates in DNA, e.g. AP-sites and blocking 3'-end
295 groups, which should be removed in order to initiate the DNA repair synthesis and
296 ligation. AP endonucleases are key participants in the BER process, since all three
297 types of products generated by DNA glycosylases^{82,83} are repaired by these
298 enzymes. An AP endonuclease hydrolyses the phosphodiester bond located 5' to the
299 AP-site and introduces a break into the deoxyribosephosphate backbone (Figure 3,
300 pathway 1), in addition, it removes the remaining 3'-blocking groups: either the 3'
301 phospho α,β -unsaturated aldehyde (Figure 3, pathway 2) or the 3'-terminal
302 phosphate group (Figure 3, pathway 3).

303 [Insert Figure X.3 here]

304 Previously, it was thought that the main biological function of AP endonucleases was
305 the hydrolytic cleavage 5' at AP-site and subsequent formation of SSB with a 5'-

Figure 3

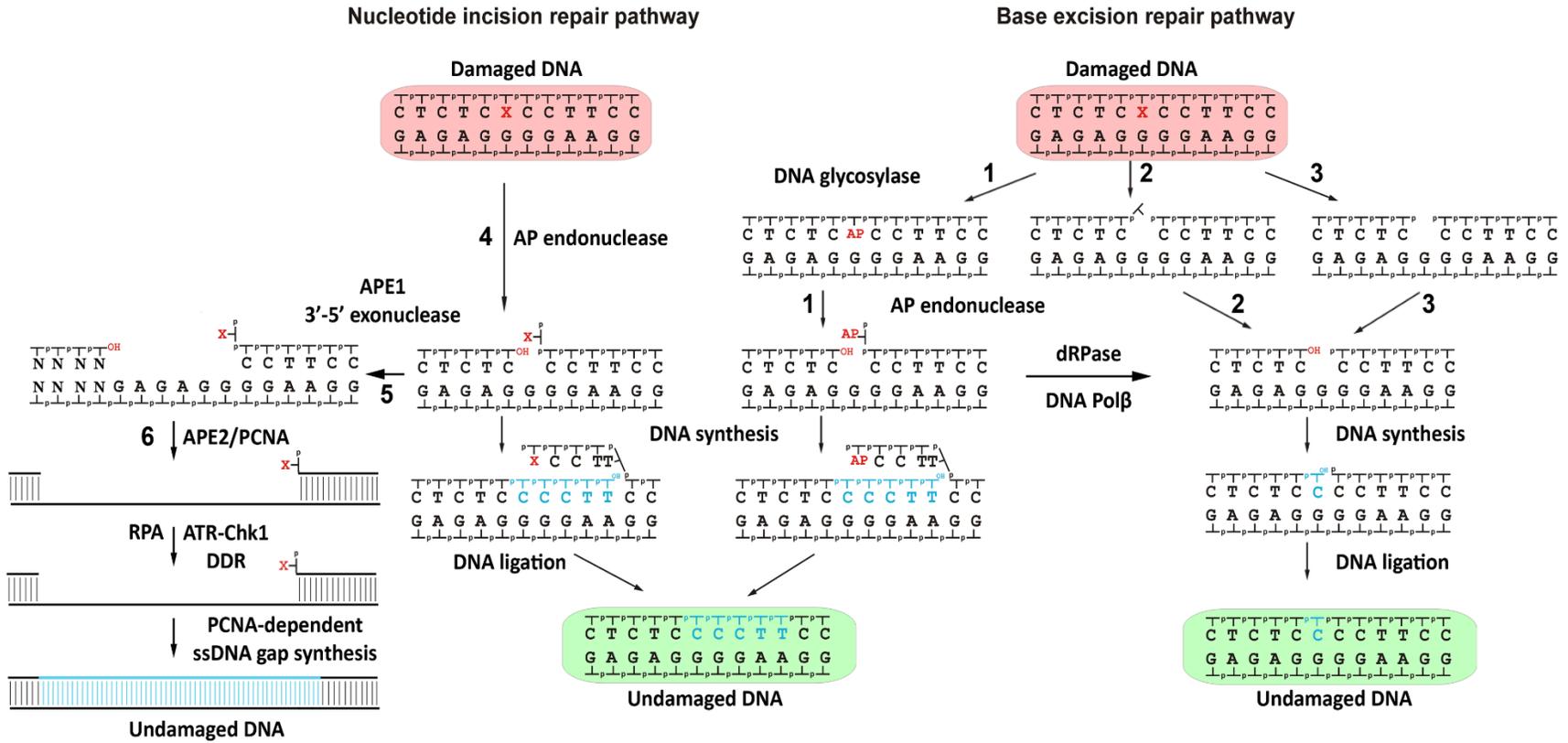


Figure 3. The scheme of BER and NIR pathways.

306 deoxyribosephosphate and a 3'-hydroxyl group.^{84,85} However, certain AP
307 endonucleases can recognise not only AP-sites, but also damaged nucleotides
308 containing a modified base (Figure 3, pathway 4).⁸⁶ This DNA glycosylase–
309 independent removal of damaged bases was named the NIR pathway.⁴¹ Afterwards,
310 a number of studies have shown that AP endonucleases recognise a variety of
311 structurally diverse types of lesions from bulky benzene-derived DNA adducts⁸⁷ up to
312 small oxidatively damaged pyrimidines.⁸⁸ Noteworthy, the genetic dissection of BER
313 and NIR functions of an AP endonuclease revealed that the NIR pathway is essential
314 for protecting cells from potentially lethal oxidative DNA lesions, which cannot be
315 removed by BER.⁸⁹

316 A comparison of amino acid sequences among AP endonucleases suggests that
317 there are two structural families, judging by similarities to two prokaryotic enzymes:
318 exonuclease III (Exo III, also referred as Xth)⁹⁰ and endonuclease IV (Endo IV, also
319 referred as Nfo).⁹¹ Mammalian cells possess two AP endonucleases (APE1 and
320 APE2) that are homologues of the bacterial Xth protein.⁹²⁻⁹⁴ APE1 contains a highly
321 efficient AP endonuclease activity; however, its 3'-end cleansing including 3'→5'
322 exonuclease activities is substantially lower than that of Xth.^{84,93,95-98} In addition to
323 AP-sites, APE1 recognises a variety of damaged DNA bases, including α -anomers of
324 2'-deoxynucleosides (α A, α C and α T),⁹⁹ oxidized pyrimidines,⁸⁸ and uracil.¹⁰⁰
325 Contrary to APE1, the 3'→5' exonuclease activity of APE2 is higher than its AP
326 endonuclease activity and may represent the main biological function of this
327 enzyme.¹⁰¹ Of note, several other Xth-like AP endonucleases such as prokaryotic
328 enzymes (*Mycobacterium tuberculosis* MtbXthA and *Neisseria meningitidis* NApe
329 and NExo) and a plant enzyme (*Arabidopsis thaliana* atAPE1L) lack the NIR
330 function.¹⁰²⁻¹⁰⁴

331 Furthermore, recently, a novel AP endonuclease, referred as endonuclease Q
332 (EndoQ), which does not belong to Xth and Nfo-family, was identified in the
333 hyperthermophilic euryarchaeon *Pyrococcus furiosus*. EndoQ is involved in the NIR
334 pathway for uracil and hypoxanthine residues in DNA.^{105,106} Noteworthy, amino acid
335 sequence analysis indicates that putative EndoQ homologs are present in most of
336 Archaea, but only in a few groups in bacteria.¹⁰⁵ A homolog of EndoQ in the gram-
337 positive bacterium *Bacillus pumilus*, exhibits NIR activity towards uracil residues.¹⁰⁷
338 EndoQ enzymes generate a nick immediately 5' to uracil, hypoxanthine, xanthine,
339 and AP site in DNA.^{105,107} Notably, the endonuclease activity of *P. furiosus* EndoQ
340 towards a uracil is identical to that of *E. coli* Xth acting upon AP-site.¹⁰⁶

341 It should be noted that the same catalytic mechanism of 5'-phosphodiester bond
342 hydrolysis was proposed for members of both the Xth family^{92,108-111} and Nfo family
343 enzymes.^{91,112,113} Despite differences in the structure and metal requirements among
344 these AP endonucleases, a comparison of their active-site structures surprisingly
345 reveals strong geometric conservation of the catalytic reaction.^{114,115} Nonetheless,
346 there is still a debate in the literature about the functional role of certain amino acid
347 residues in the active site of enzyme.¹¹⁶⁻¹¹⁹ In addition, it has been shown that during
348 the endoribonuclease action of human APE1, the Mg²⁺ ion is not required, thus
349 pointing to an alternative catalytic mechanism in the case of RNA substrates.¹²⁰

350 **X. 3. 2. A putative physiological role of APE1-catalyzed NIR and 3'→5'** 351 **exonuclease functions**

352 Both human APE1 and its homolog APE2 contain 3'→5' exonuclease activity^{101,121}
353 and this function is evolutionary conserved in Xth- and Nfo-families of AP
354 endonucleases. Importantly, previous studies have established that *E. coli* Nfo and

355 human APE1-catalyzed NIR and 3'→5' exonuclease activities are functionally and
356 genetically coupled and that the repair of oxidatively damaged base in the DNA
357 glycosylase-independent NIR pathway can generate a small single-stranded (ss) gap
358 5' to the lesion in duplex DNA.^{88,122,123} On the other hand, it has been shown that
359 *Xenopus* and human APE2 can exonucleolytically process single-strand DNA break
360 (SSB) in the 3'→5' direction to generate an extended ssDNA gap, which in turn
361 activates ATR-Chk1 DNA damage response (DDR) pathway.^{124,125} More recently,
362 using *Xenopus* egg extracts Lin et al., demonstrated that APE1's exonuclease
363 function is required for APE2-mediated 3'-resection of SSB and ATR-Chk1
364 signalling.¹²⁶ Strikingly, *Xenopus* APE1-D306A mutant, which corresponds to human
365 NIR-deficient APE1-D308A variant,¹²³ is unable to trigger ATR-Chk1 DDR in egg
366 extracts,¹²⁶ implying the role of NIR function in the generation of extended ssDNA
367 gaps at oxidized DNA bases. Taken together, these observations led us to propose a
368 schematic model in which the removal of oxidized bases in DNA glycosylase-
369 independent NIR pathway generates extended ssDNA gap via coupled action of
370 APE1 and APE2/PCNA complex (Figure 3, pathways 5-6). The resulting gapped
371 ssDNA structure bound with RPA, triggers ATR-Chk1 DDR pathway in the
372 replication-independent manner, which in turn enables the PCNA-directed gap filling
373 by high-fidelity DNA polymerases (Figure 3, left panel). It is tempting to speculate
374 that the NIR pathway may take place in actively transcribed open chromatin regions
375 to ensure high-fidelity DNA repair synthesis, whereas, the classic BER pathway
376 occurs in heterochromatin and proceeds mainly via DNA polymerase β -dependent
377 short-patch pathway (Figure 3, pathways 2 and 3).

378 X. 3. 3. Conformational dynamics of enzyme–substrate complexes

379 Despite the active research into functional features of AP endonucleases, it is
380 currently unknown how the active site of one enzyme can recognise substantially
381 different (by structure and by nature) damaged and undamaged nucleotides. Certain
382 important features of catalytic complex formation are still unclear: the sequence of
383 the enzymatic events; the nature of interactions at the initial stage of substrate
384 recognition; formation of which contacts leads to the discrimination between a
385 substrate and 'non-substrate'; and which interactions ensure substrate specificity of
386 the enzyme. Therefore, a major contribution to the understanding of the mechanisms
387 underlying specific enzyme–substrate interactions has been done in studies utilizing
388 pre-steady-state kinetics and thermodynamics of the enzymatic process with
389 registration of conformational transformations of the interacting molecules.

390 Conformational transformations of AP endonucleases were recorded by means of a
391 change in the intrinsic fluorescence of their tryptophan residues.¹²⁷⁻¹³² In nucleic
392 acids, conformational transitions can be detected using fluorescent analogues of
393 heterocyclic bases.^{119,133-141} It has been reported that the interaction of APE1 with a
394 substrate includes at least two stages: DNA binding and recognition of an AP-site,
395 which result in the formation of a catalytically-competent complex. The irreversible
396 stage of catalytic hydrolysis of the 5'-phosphodiester bond at the AP-site takes place
397 in this complex. The last stage of the kinetic mechanism is characterised by an
398 equilibrium process of enzymatic-complex dissociation from the reaction product.
399 Thermodynamic analysis of the fast stages of recognition and cleavage of DNA
400 containing an abasic site¹³² has shown that there are two steps, DNA binding and F-
401 site recognition that cause the formation of a catalytically competent complex.

402 **X. 3. 4. The mechanism of substrate specificity towards damaged nucleotides,**
403 **with human APE1 as an example**

404 During the last three decades, extensive characterization of DNA repair enzymes
405 provided insight into how damaged DNA bases were located and recognised among
406 numerous unmodified bases.^{142,143} Structural data indicate that conformational
407 changes in the enzyme and DNA play an important part in the recognition of specific
408 substrates and may control the substrate specificity of enzymes.¹⁴⁴⁻¹⁵⁴

409 Nevertheless, a recent report uncovered substantial differences in the mechanisms
410 of specific substrate recognition by many well-studied DNA glycosylases and by
411 human APE1.¹⁵⁵ This study clarified the key steps of the mechanism underlying
412 APE1–DNA interaction that enable high-specificity recognition of structurally different
413 damaged DNA containing ϵ A, α A, 5,6-dihydrouridine, and an F-site. Accordingly,¹⁵⁵
414 the substrate specificity of APE1 should depend on the efficiency of damaged-
415 nucleotide eversion from the duplex during enzyme-induced DNA bending. The
416 ability of an AP site or damaged nucleotide to flip out of DNA and to penetrate the
417 enzyme pocket during the formation of contacts between the enzyme and DNA may
418 be the main contributing factor for substrate specificity of APE1. This conclusion
419 means that the model of a mutual enzyme–substrate induced fit, as determined for
420 DNA glycosylases,¹⁵⁶ is now transformed into the model of conformational selection
421 in the case of APE1 (Figure 4). In this model, any nucleotide, which everts from the
422 duplex in the response to a disturbance induced by the enzyme and enters into the
423 active site, will be a substrate for the enzyme. This model of substrate specificity is
424 well consistent with the ability of APE1 to act as an exonuclease and
425 endoribonuclease because of the structural features of RNA that help with the
426 eversion of intact nucleotides at the junction of the single-stranded parts, hairpins,
427 duplexes, and loops. Moreover, it is reasonable to propose that non-B-form DNA and
428 some structures formed due to DNA sequence flexibility can bind to APE1 and lead

429 to undamaged-nucleotide recognition with subsequent topology-specific DNA
430 cleavage.

431 [Insert Figure X.4 here]

432 **X. 4. Poly(ADP-ribose) polymerase catalysed covalent modification of DNA** 433 **strand break extremities and its role in DNA repair**

434 Endogenous and exogenous DNA damage are rapidly sensed and activate
435 evolutionarily-conserved signaling pathways, known collectively as DDR, whose
436 components can be separated into four functional groups: damage sensors, signal
437 transducers, repair effectors, and arrest or death effectors.¹⁵⁷ Single-stranded,
438 double-stranded and complex closely spaced clustered DNA strand breaks that
439 represent either an initial DNA damage or DNA repair intermediates generated
440 during DNA damage processing by cellular DNA repair enzymes are one of the
441 fastest and most strong inducers of DDR. DNA break-sensing molecules, poly(ADP-
442 ribose) polymerases (PARPs) are the key element of the DDR in mammalian cells.
443 PARPs catalyze transfer of mono- or poly-ADP-ribose (MAR or PAR, respectively)
444 moieties on proteins,¹⁵⁸ DNA^{159,160} and RNA¹⁶¹ by using nicotinamide adenine
445 dinucleotide (NAD⁺) as a substrate. It has been shown that these post-translational,
446 post-replicative and post-transcriptional modifications play roles not only in DNA
447 damage repair,¹⁶² but also in a large array of cellular processes including regulation
448 of gene expression, apoptosis and autophagy (reviewed in¹⁶³⁻¹⁶⁶).

449 The PAR polymer designates a chain of ADP-ribose (ADPr) units, where each unit is
450 linked to other one through glycosidic ribose–ribose 1''→2' bonds.¹⁶⁷ This polymer
451 can vary in length from a few to 200 – 400 ADPr units *in vivo* and *in vitro*.¹⁶⁸ The
452 PAR can be branched at a frequency of 1 branch per 20-50 subunits of the linear

Figure 4

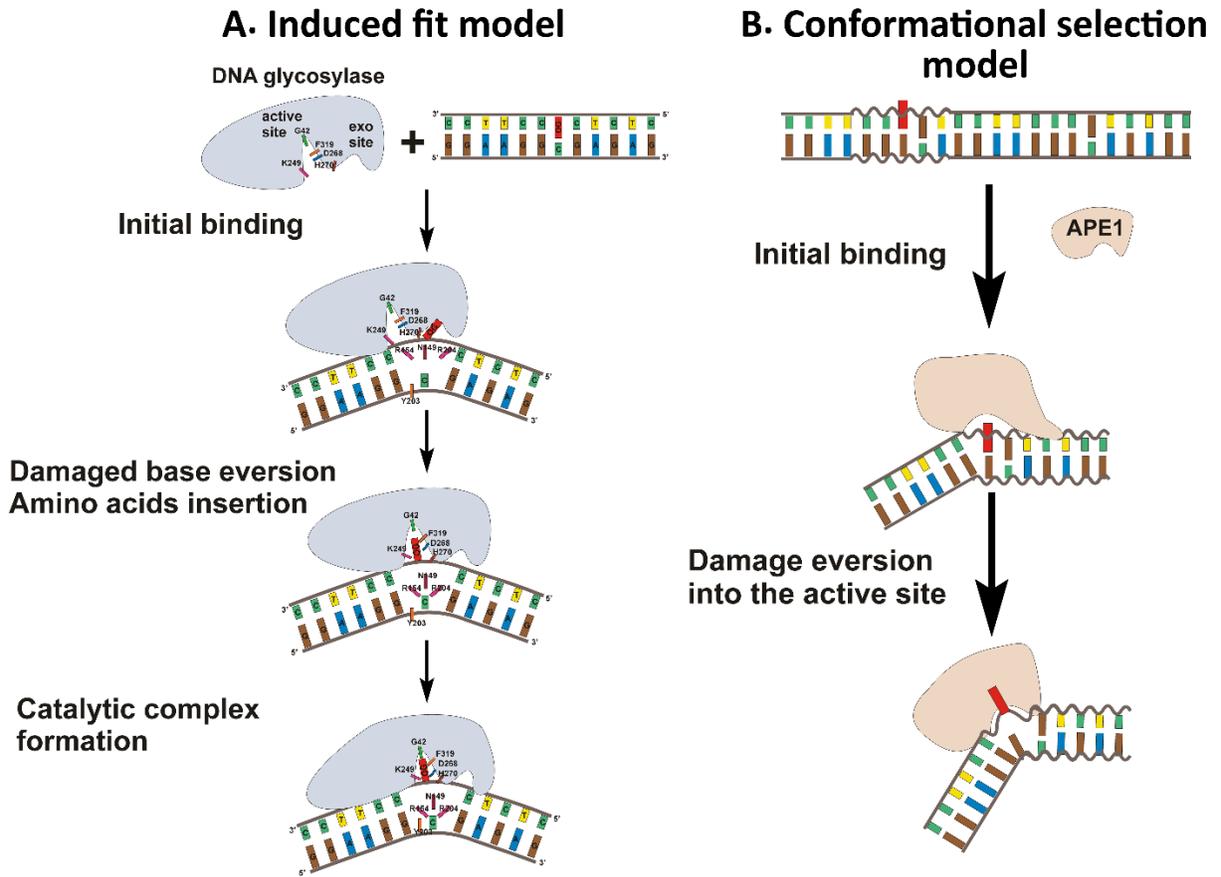


Figure 4. Mechanisms of DNA damage recognition by DNA glycosylases,^{90,104} (A) and AP endonucleases^{78,102} (B).

453 polymer. Branched PAR polymer can have a very complex structure, including
454 helicoidal secondary structures which are, at some extent, similar to RNA and
455 DNA.¹⁶⁹ PAR (free or attached to proteins) that is synthesized upon genotoxic stress
456 may undergo biphasic decay, with most (~85%) of PAR having a half-life of about 40
457 s and the remaining catabolized within approximately 6 min. In contrast, the
458 constitutive fraction of PAR have a much longer half-life (~7.7 h).¹⁷⁰ A tight regulation
459 of the level of the polymer in the cell by ADPr-protein hydrolases, that reverse the
460 reaction by hydrolyzing the protein–ADPr bond or the bonds between two ADPr units
461 of PAR is important, since its accumulation have strong cytotoxic effects.¹⁷¹

462 Family of PARPs, also known as diphtheria toxin-like ADP-ribosyltransferases
463 includes 18 known members¹⁶⁸ that are divided into 4 sub-families as DNA
464 dependent PARPs, Tankyrases, CCCH PARPs and macroPARPs.¹⁷² Among PARPs
465 only PARP1, PARP2 and PARP3 are activated in DNA dependent manner, which
466 signals the presence of DNA strand breaks.¹⁷³ PARP1-3 structures consist of
467 multiple independent domains connected by flexible linkers.¹⁷⁴ Binding and
468 recognition of sites of DNA strand breaks induce the reorganization of PARPs
469 domains structures, promoting extensive inter-domain contacts and allosteric
470 activation, by unfolding of an auto-inhibitory helical domain (HD), which then allows
471 NAD⁺ binding necessary for ADP-ribosylation activity.¹⁷⁴⁻¹⁷⁷ PARP1 is the most
472 ubiquitous and abundant PARP, which responsible for majority of the PARylation
473 activity in a cell in response to DNA damage and involved in multiple DNA repair
474 pathways, including BER, HR, NHEJ and NER.^{166,178,179} PARP2 plays partially
475 redundant functions with PARP1 and accounts for about 10% of DNA damage-
476 induced cellular PARylation activities, which are essential for cell survival. Depletion
477 of PARP1 or PARP2 results in hypersensitivity to ionizing radiation, oxidative stress

478 and alkylating agents, whereas double-knockout mice show early embryonic
479 lethality.¹⁸⁰ PARP3 is characterized by PARylation activity and act synergistically
480 with PARP1 in response to genotoxic drugs and X-irradiation generating DSBs.¹⁸¹⁻¹⁸³
481 Importantly, PAR attached to a protein rapidly degraded by PAR glycohydrolase
482 (PARG), except for the last PAR moiety, which remains bound to the protein. PARG
483 specifically hydrolyses the ribose–ribose bonds and it is the main enzyme in
484 mammalian cells. Disruption of PARG gene in mice results in embryonic lethality and
485 PARG-deficient cells exhibited increased cell death and impaired repair of DNA base
486 damage and strand breaks,¹⁸⁴ indicating that accumulation of the PARylated
487 macromolecules is highly toxic to the cell.

488 The widespread presence of PARP proteins in eukaryotes and their protein
489 modification activity are possibly due to the fact that in eukaryotic cells, DNA is tightly
490 packed into a composite of DNA and proteins referred to as chromatin. Chromatin
491 structure imposes restriction upon DNA-protein interactions, and several studies
492 have demonstrated that tight chromatin packaging restrict the ability of DNA repair
493 machinery to access the sites of DNA damage.^{185,186} PARPs mediated ADP-
494 ribosylation of histones and other nuclear proteins induce recruitment and
495 modification of chromatin remodeling factors, including chromatin insulator protein
496 CTCF (CCCTC-binding factor) and chromodomain-helicase-DNA-binding protein 1-
497 like (ALC1), and provide an effective chromatin remodeling mechanism necessary
498 for the recruitment of repair proteins to DNA damage sites and efficient repair of
499 DNA strand breaks (reviewed in^{164,179,187,188}). Moreover, covalently attached ADP-
500 ribose polymer with a complex branched structure confers negative charge to
501 PARPs and histones, resulting in decrease in DNA binding and electrostatic
502 repulsion of these proteins from DNA.^{189,190} However, in the case of severe

503 genotoxic stress, hyperactivation of PARP results in strong reduction of NAD⁺ levels,
504 which in turn, induce release of apoptosis-inducing factor (AIF) from mitochondria
505 and lead to apoptosis in a caspase-independent manner.¹⁹¹

506 Recently, it has been demonstrated that *in vitro* PARP1-3 can reversibly ADP-
507 ribosylate termini of DNA molecules containing multiple closely spaced DNA strand
508 breaks (DSB and SSB), producing a covalent PAR/MAR–DNA adducts, pointing to
509 the underlying complex nature of the PARP-dependent DNA strand break signaling.
510 ^{159,192,193} Mechanistic studies showed that PARP1 and PARP2 can covalently modify
511 DNA duplexes by PARylation of 3'- and 5'-terminal phosphates and 2'-OH termini of
512 modified nucleotides at DNA strand breaks.¹⁵⁹ Contrary to ADP-ribosylation of
513 proteins, PAR and MAR DNA modifications can be effectively and completely
514 removed by PARG.^{159,192,193} MACROD2, TARG1 and ARH3 hydrolases can also
515 remove the MAR adduct from DNA, leaving phosphorylated termini.¹⁹³ PARP2 and
516 PARP3 can ADP-ribosylate DNA termini in long linear and circular DNA duplexes,
517 which more closely resemble the cellular DNA context.¹⁹² Most importantly, under
518 certain configuration of strand breaks in duplex DNA, PARPs can switch their
519 substrate specificity from auto-ADP-ribosylation to DNA-ADP-ribosylation.¹⁹² DNA
520 PARylation experiments in cell-free extracts and immunoblotting experiments with
521 purified genomic DNA after genotoxic treatment suggest that certain types of
522 complex DNA strand breaks in chromosomal DNA can be ADP-ribosylated by
523 PARPs in the cellular response to DNA damage.¹⁹²

524 The three-dimensional structures of PARP1 bound to DNA with a SSB
525 revealed that PARP1 binds and bends DNA duplex at an SSB site through
526 cooperative interactions of two N-terminal zinc finger domains (F1 and F2) with
527 exposed DNA bases and adjacent minor grooves and then drives stepwise assembly
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528 of the remaining F3, WGR (Trp-Gly-Arg) and catalytic (CAT) domains leading to
529 PARP1 activation.^{174,176} In contrast, PARP2 and PARP3 use only short N-terminal
530 and WGR domains for binding to damaged DNA.^{181,194} However, the resolved crystal
531 structures of assembled PARP-DNA complexes still do not provide sufficient insight
532 into the observed PARPs specificity for the phosphorylated DNA termini. In these
533 complexes, the DNA-binding site of PARP1 is far from its catalytic (CAT) domain in
534 activated PARP1-DNA complex and sterically protected from ADP-ribosylation by the
535 PARP1 protein itself. However, another proximal DNA strand breakage site in the
536 same PARP-DNA complex might interact with the CAT domain taking into account
537 high flexibility of domain linkers. A putative mechanistic model of DNA strand break-
538 oriented ADP-ribosylation of DNA termini by PARP1-3 shown in [Figure 5](#) has been
539 proposed based on the observed dependence of DNA termini covalent modification
540 on the distance between the phosphorylated DNA terminus as an acceptor and the
541 DNA strand break as an activator.¹⁹² The following rules have been established for
542 the efficient ADP-ribosylation of 5' or 3' upstream DNA termini in duplex DNA
543 containing multiple strand breaks: (i) the distance between phosphate residue and
544 SSB should not exceed ~2 helix turns of DNA duplex; and (ii) the relative position of
545 the acceptor phosphate on DNA helix to the DNA-bound and activated PARPs.¹⁹²

546 [Insert Figure X.5 here]

547 Specific requirements for closely spaced or clustered DNA strand breaks in the
548 PARP-dependent DNA ADP-ribosylation open an important question: whether such
549 DNA strand breaks can be formed for example by ionizing radiation, and if there are
550 some DNA processing pathways that may generate strand breaks in a non-random
551 manner? It is quite possible that DNA duplexes containing DSB and proximal SSB

Figure 5

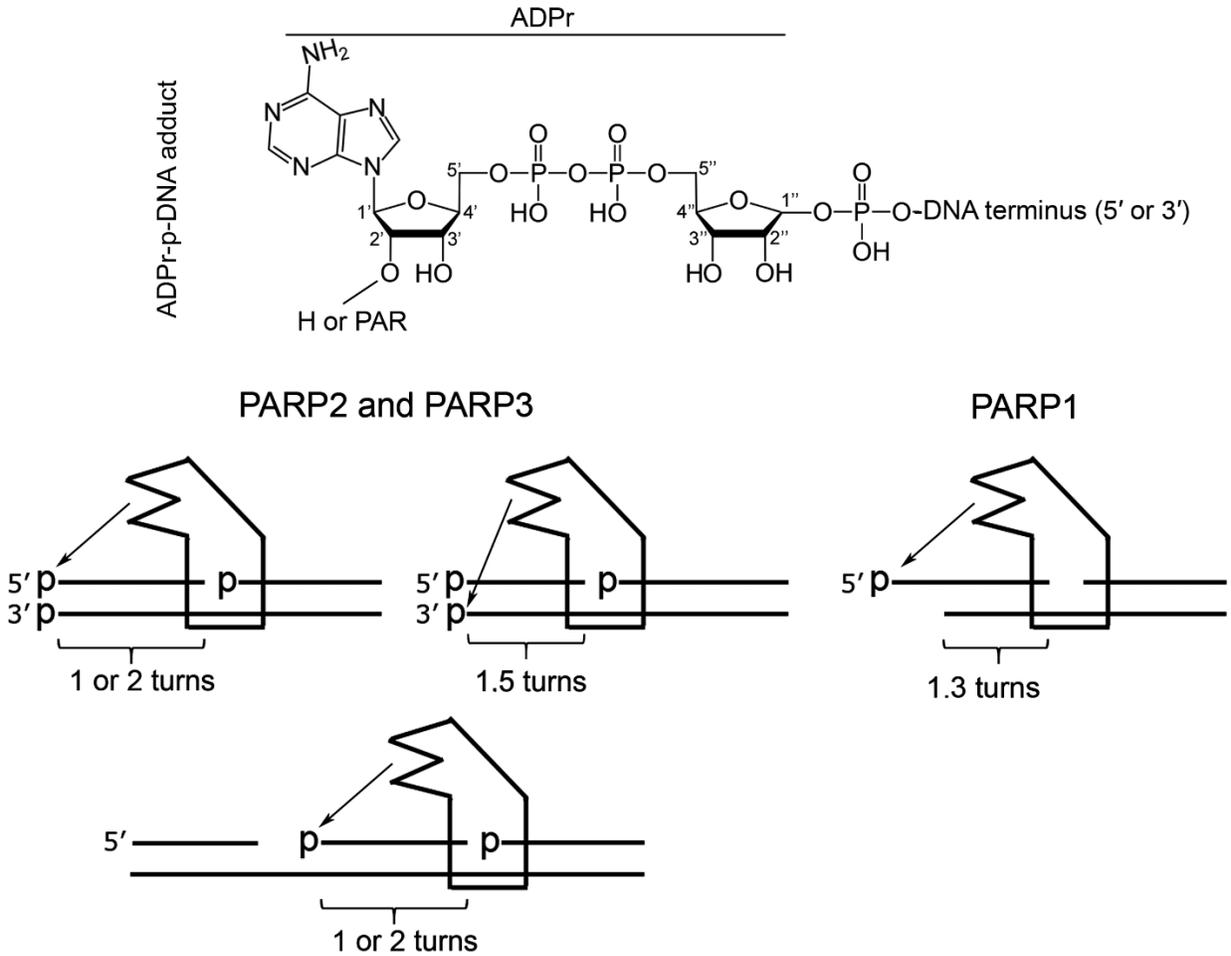


Figure 5. The mechanistic model of DNA strand break-oriented DNA termini ADP-ribosylation by PARP1, PARP2 and PARP3.

552 can be generated during DSB repair by HR and NHEJ repair pathways. Intriguingly,
553 it has been found that the MRN–CtIP complex generated an internal nick located 20
554 nucleotides downstream of 5'-termini of a DSB,¹⁹⁵ and that this configuration of
555 closely-spaced DNA strand breaks is prone to covalent DNA ADP-ribosylation by the
556 PARP1-3 proteins.

557 Importantly, not only DNA strand breaks activate PARPs catalyzed ADP-ribosylation.
558 PARP1 can also be activated by DNA hairpins, cruciform structures, stalled
559 replication forks, stably unpaired regions and other non-B-conformations of
560 DNA.^{196,197} Thus, the presence of a single SSB or DSB near to some of non-B DNA
561 structures or specific chromatin configurations could potentially trigger ADP-
562 ribosylation of DNA strand breaks termini. Moreover, the pool of PARPs activators
563 has been enlarged during different studies showing that PARPs can be activated
564 independently of DNA by protein-protein interactions, post-translational modifications
565 and by specific type of RNA structures (summarized in [Table 1](#)).^{163,172,198-200} In
566 addition to PARP1 and Tankyrase, a new PARPs family member has been shown to
567 be activated by a DNA damage independent way. During genotoxic stress, PARP2 is
568 activated by binding to RNA via its SAP domain, in nucleoli, and this stimulates PAR
569 formation.²⁰¹ On the other hand, PARP1 inactivation was observed upon binding to
570 the histone variant macroH2A1.1,²⁰² also the deacetylation and sumoylation of
571 PARP1 inhibit its self-PARylation.¹⁹⁹

572 [Insert Table x.1 here]

573 The phenomenon of ADP-ribosylation of a terminal phosphate group at the DNA
574 strand breaks opens important question about possible functional role of this new
575 modifying activity of PARPs. It is generally accepted that covalently attached PAR

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949 **Table 1. Mediators of PARP1-3 activation**

Type of activation	Activator	Activated PARP	References
Nucleic acids binding	non-B DNA structures (hairpins, cruciforms, and loops)	PARP1	197,204
	DNA breaks: nicks, gaps, flaps, DSB	PARP1-3	174-177,205,206
	small nucleolar RNA	PARP1	207
	Short rRNA and other single-stranded RNAs	PARP2	201
	PAR polymer	PARP2	208
Protein-Protein interactions	Nucleosomes and histones	PARP1	209,210
	Phosphorylated extracellular signal-regulated kinases, ERK		211
	Nicotinamide mononucleotide adenylyltransferase 1, NMNAT1		212
	CTCF		213
	TET1		214
	TOPO2		215
	Bromodomain adjacent to zinc finger domain 2A, BAZ2A/TIP5		216
	Human tyrosyl tRNA synthetase, TyrRS		217

Post-translational modifications	phosphorylation at Ser372 and Thr373 (by extracellular signal-regulated kinases, ERK1/2)	PARP1	218
	phosphorylation at Tyr907 (by c-Jun N-terminal kinase, JNK1/ MAPK8)		219
	phosphorylation at Ser782, Ser785, and Ser786 (by cyclin-dependent kinase, CDK2)		220
	acetylation at Lys498, Lys505, Lys508, Lys521, and Lys524 (by E1A binding protein, p300/ CREB-binding protein CBP)		221
	acetylation of 1-214 and 477-525 aa regions (by P300/CBP-associated factor, PCAF)		222
	MARylation by PARP3		223
	MARylation at Lys 521 (by Sirtuin 6, SIRT6)		224
	phosphorylation at Thr420, Thr622, Thr656 (by checkpoint kinase 2, CHK2)		225
	methylation at K508 (by Histone-lysine N-methyltransferase, SET7/9)		226

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576 polymer confers negative charge to PARPs, histones and other PARylated proteins,
577 resulting in decrease in DNA binding and electrostatic repulsion of these proteins
578 from DNA.¹⁹⁰ Thus, PARylated proteins together with PAR-recruited complexes of
579 DNA repair factors might be also withdrawn together from the site of DNA damage.
580 In contrast, the PAR chain covalently attached to DNA termini should stably recruit
581 those factors directly to the site of DNA damage. Importantly, it has been recently
582 demonstrated that DNA PARylated at 5'-phosphate at 1 nt gap is recognized as 5'-
583 adenylated DNA substrate and can be ligated in the absence of ATP by DNA ligase
584 I, IIIa and by other DNA ligases, producing ligated double-stranded DNA molecule
585 with an aberrant AP site. This AP site can be further processed by BER-associated
586 APE1.¹⁶⁰ In line with these results, it has been suggested that PARP2 and PARP3
587 are involved in the final ligation step of NHEJ, because 5' phosphorylated nicks are
588 particularly efficient activators of auto-ADP-ribosylation activity of PARP2 and
589 PARP3, but not PARP1. We speculate that DNA ADP-ribosylation can promote
590 retention of the DSB ends until either the full repair complex put in place or required
591 ATP concentration for DNA ligation reached. Similarly, in the case of SSB repair
592 PARP-mediated ADP-ribosylation can promote ligation of a gap without a
593 polymerase synthesis and ATP. Of note, PARP-mediated extensive PAR synthesis
594 can block the glycolysis and lead to ATP loss and the inhibition of hexokinase 1
595 activity.²⁰³

596 We hypothesize that the role of the ADP-ribosylation of DNA strand break termini is
597 different from that of proteins ([Figure 6](#)) since it: (i) can be used as a temporary block
598 of the processing of strand breaks and by this protect them from non-specific
599 degradation and aberrant error-prone end joining; (ii) inhibits the NHEJ via inhibition
600 of the binding of Ku to DSB ends; (iii) promotes direct aberrant ligation of ADP-

Figure 6

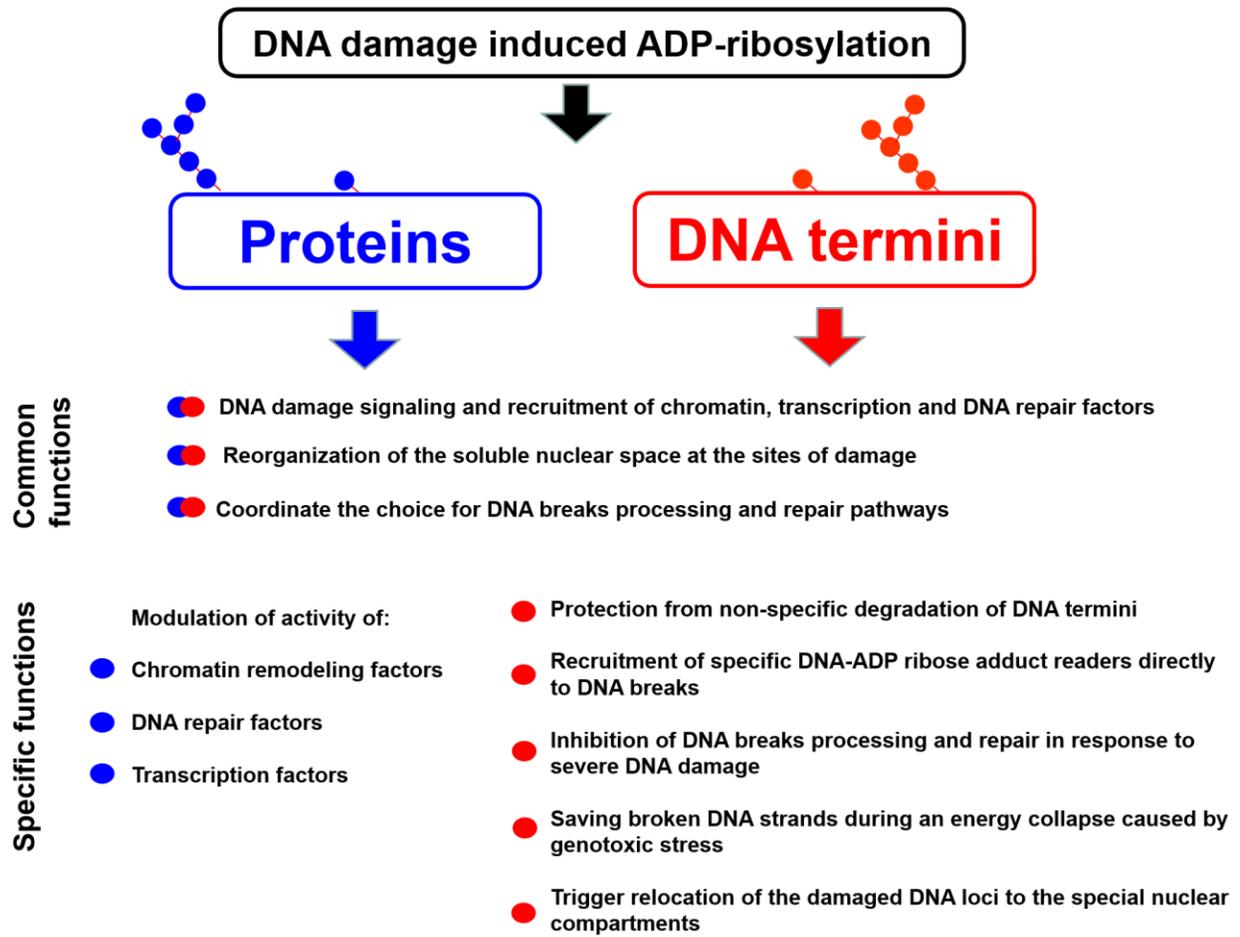


Figure 6. Putative and confirmed roles of the PARPs-catalyzed ADP-ribosylation in response to DNA damage.

601 ribosylated DNA breaks during bioenergetic collapse in order to avoid degradation
602 and de novo formation of toxic DSBs; (iv) initiates and stimulates DNA strand
603 resection by MRN complex to promote the alternative DNA repair pathways; (v)
604 enables an efficient apoptotic signal if not removed; (vi) triggers relocation of the
605 damaged DNA loci to the special nuclear compartments; (vii) provides precise
606 PAR/MAR-guided recruitment and assembly of the DNA repair factors; and (viii)
607 coordinates the choice of the DNA breaks signaling, processing and repair
608 pathways.

609 [Insert Figure X.6 here]

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953 **FIGURE AND TABLE CAPTIONS**

954 *Figure 1. Schematic presentation of singular and complex DNA damage. (A)*

955 *Chemical structures of some singular non-bulky DNA base lesions. (B) Chemical*
956 *structures of complex DNA lesions including cisplatin-ICL and bulky repair-resistant*
957 *DNA base lesions.*

958 *Figure 2. DNA replication-coupled repair of inter-strand DNA crosslinks. (A) NEIL1*
959 *excises unhooked crosslinked oligomer in three-stranded DNA repair intermediate.*

960 *(B) NEIL3- and (C) Fanconi anemia-mediated repair of ICL. (D) The hypothetical*
961 *mechanism of the FANCM-mediated DNA replication fork bypass of ICL and NEILs-*
962 *mediated repair of three- and four stranded crosslinked DNA structures.*

963 *Figure 3. The scheme of BER and NIR pathways.*

964 *Figure 4. Mechanisms of DNA damage recognition by DNA glycosylases^{90,104} (A)*
965 *and AP endonucleases^{78,102} (B).*

966 *Figure 5. The mechanistic model of DNA strand break-oriented DNA termini ADP-*
967 *ribosylation by PARP1, PARP2 and PARP3.*

968 *Figure 6. Putative and confirmed roles of the PARPs-catalyzed ADP-ribosylation in*
969 *response to DNA damage.*

970

971 *Table 1. Mediators of PARP1-3 activation*

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