Published in final edited form as:
Trends Biochem Sci. 2011 May ; 36(5): 262–271. doi:10.1016/j.tibs.2011.01.006.

Tidying up loose ends: the role of polynucleotide kinase/phosphatase in DNA strand break repair

Michael Weinfeld[1], **Rajam S. Mani**[1], **Ismail Abdou**[1], **R. Daniel Acetuno**[2], and **J.N. Mark Glover**[2]

[1]Department of Oncology, University of Alberta, and Cross Cancer Institute, Edmonton, Alberta, Canada

[2]Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada

Abstract

The termini of DNA strand breaks induced by internal and external factors often require processing before missing nucleotides can be replaced by DNA polymerases and the strands rejoined by DNA ligases. Polynucleotide kinase/phosphatase (PNKP) serves a critical role in the repair of DNA strand breaks by catalyzing the restoration of 5'-phosphate and 3'-hydroxyl termini. It participates in several DNA repair pathways through interactions with other DNA repair proteins, notably XRCC1 and XRCC4. Recent studies have highlighted the physiological importance of PNKP in maintaining the genomic stability of normal tissues, particularly developing neural cells, as well as enhancing the resistance of cancer cells to genotoxic therapeutic agents.

Polynucleotide kinase/phosphatase: an essential enzyme for the repair of damaged DNA termini

Damage to cellular DNA is considered to be a significant factor in aging [1], cancer etiology and treatment [2–4], and neurological disorders [5]. DNA damage, in the form of base modification, base loss and strand breaks, can be triggered by intracellular agents, primarily reactive oxygen species (ROS), as well as by exogenous agents. As a result, cells have evolved a battery of repair pathways to counter the mutational and cytotoxic consequences of DNA damage [6]. Strand breaks can be produced by various mechanisms, including cleavage by physical and chemical means such as ionizing radiation (IR) and ROS, and by enzymatic processes, such as those mediated by topoisomerases and DNA repair endonucleases. Thus, strand breaks do not constitute a homogeneous class of lesions and, in addition to classification based on cleavage of one or both DNA strands, strand breaks can be further subdivided based on the nature of their termini (Box 1). This review focuses on the enzyme polynucleotide kinase/phosphatase (PNKP) and its role in the repair of DNA strand breaks. PNKP possesses both 5'-kinase and 3'-phosphatase activities that are frequently required for processing of single- and double-strand break (SSB and DSB) termini [7–10]. PNKP, like several other DNA repair proteins, has become of increasing clinical interest owing to the identification of small molecule inhibitors of these enzymes

Corresponding author: Weinfeld, M. (mweinfel@ualberta.ca).

that sensitize cells to IR or chemotherapeutic agents [11, 12]. In addition, mutations that lead to alterations in PNKP, similar to mutations in genes encoding other strand break repair proteins, have recently been associated with a severe autosomal recessive neurological disorder [13].

Molecular architecture of PNKP

PNKP is a multi-domain enzyme that consists of an N-terminal FHA (forkhead-associated) domain and a C-terminal catalytic domain composed of fused phosphatase and kinase sub-domains. The FHA domain is linked to the catalytic domain through a flexible polypeptide segment and acts to selectively bind acidic casein kinase 2 (CK2)-phosphorylated regions in XRCC1 [14] and XRCC4 [15], which are key scaffolding proteins in the repair of DNA SSBs and DSBs respectively. The DNA repair factors aprataxin and APLF (aprataxin and PNKP-like factor) also contain FHA domains that similarly bind CK2-phosphorylated XRCC1 and XRCC4, which could lead to the coordinated regulation of these proteins in terms of their binding to the phosphorylated scaffolding factors [16–18].

The PNKP catalytic domain bears overall similarity to phage T4 polynucleotide kinase, which also contains contiguous kinase and phosphatase domains [19, 20]. The T4 enzyme differs, however, in that it lacks an FHA domain and that the kinase sub-domain lies N-terminal to the phosphatase sub-domain.

The crystal structure of murine PNKP [21] showed that the two catalytic active sites are positioned on the same side of the protein (Fig. 1a, b). The murine and T4 kinase sub-domain share a similar overall structure of a bipartite active site cleft with separate ATP and DNA binding sites. The ATP binding site is defined by the Walker A (P-loop) and B motifs conserved in various kinases, as well as an aspartic acid (Asp396 in murine PNKP) that activates the 5'-OH for attack on the ATP γ-phosphate. Notably, the DNA binding site differs significantly between the mammalian and phage enzymes. The phage PNK DNA binding cleft forms a narrow channel leading to the conserved catalytic aspartic acid residue that accommodates single-stranded, but not double-stranded, substrates [22]. By contrast, the mammalian enzyme preferentially phosphorylates 5'-hydroxyl termini within nicked, gapped or DSBs with single-stranded 3' overhanging ends, whereas single-stranded 5'-termini or blunt double-stranded ends are phosphorylated less efficiently [23, 24]. The selective recognition of the larger, double-stranded DNA substrates is effected by a broad DNA recognition groove composed of two distinct positively charged surfaces. The effect of amino acid substitutions in these surfaces on kinase activity, together with structural information from small angle X-ray scattering (SAXS) experiments, indicates that DNA substrates bind across these surfaces in a defined orientation [24] (Fig. 1b).

The phosphatase sub-domain adopts a haloacid dehalogenase (HAD) fold [25], characteristic of many phosphatases including that of T4 PNK. In general, the mechanism employed by these enzymes is dependent on Mg^{2+} and proceeds via a catalytic aspartate and acyl-phosphate intermediate. Mammalian PNKP readily acts on a variety of 3'-phosphate ends, including those within nicks, gaps, or DSBs, as well as single-stranded termini. Two narrow channels surrounded by largely positively charged loops provide access to the phosphatase active site (Fig. 1b). Interestingly, neither of these channels is wide enough to accommodate double-stranded substrates, which suggests either a requirement for remodeling of the phosphatase substrate binding surface or an unwinding of the DNA.

PNKP function is modulated by interaction with the DNA repair scaffold proteins XRCC1 and XRCC4, which is mediated by binding of the PNKP FHA domain to phosphorylated motifs on XRCC1 and XRCC4 (Fig. 1c). FHA domains are well-studied phospho-peptide binding modules that adopt a β-sandwich fold. A series of loops protrude from one side of

the β-sandwich and provide a peptide binding surface with a marked preference for targets containing a phospho-threonine residue [26]. Whereas XRCC1 and XRCC4 are structurally unrelated, they contain similar motifs that are phosphorylated by CK2 and serve as the binding sites for the PNKP FHA domain. A cluster of CK2 phosphorylation sites between residues 515 and 526 in XRCC1 is required for interactions with PNKP and amino acid substitutions within this region lead to a significant reduction in the efficiency of SSB repair [27]. Likewise, a primary CK2 site in XRCC4, Thr233, is required both for PNKP binding and for efficient repair of DSBs *in vivo* [15]. Alignment of these sites reveals significant conservation of sequence around the primary threonine phosphorylation site (Thr519 in human XRCC1, Thr233 in human XRCC4) (Fig. 1d). Phosphorylation of a conserved serine at the -1 position with respect to the primary phospho-threonine in the XRCC1 peptide enhances interactions with PNKP ~10-fold and the structure of this complex reveals a dynamic interaction of this residue with either Arg35 or Arg44 of the PNKP FHA domain [28]. A tyrosine residue is conserved at the -4 position in both XRCC1 and XRCC4 and stacks against Pro37 in the FHA domain (Fig. 1e). Similar to other FHA domains [26], an asparagine residue at +3 is recognized by a hydrogen bonding interaction with Asn97 of the FHA domain; however this interaction is not conserved in the complex with XRCC4, where the +3 position residue is a glutamic acid. Both peptides are highly acidic, and long-range electrostatic interactions between these residues and the largely positively charged peptide-binding surface probably also contribute to binding specificity. Interestingly, in XRCC1, a secondary threonine phosphorylation at the +4 position also contributes to binding selectivity, most likely through the recruitment of a second PNKP FHA domain in a cooperative interaction [28].

PNKP and single-strand break repair (SSBR)

SSBR is a multi-enzyme pathway that employs different players depending on the causative agent. In the case of IR-induced strand-breaks, which typically involve the loss of at least one nucleotide, the initial process of damage recognition and correction of the strand break termini is primarily carried out by poly(ADP-ribose) polymerase (PARP), XRCC1, AP endonuclease I (APE1) and/or PNKP, although other proteins, such as tyrosyl-DNA phosphodiesterase (TDP1) and aprataxin might provide backup functionality [29]. Subsequent replacement of nucleotides and strand resealing can occur by either a short patch pathway involving DNA polymerase β and DNA ligase III or a long patch pathway employing DNA polymerase δ and/or ε, the FEN1 endonuclease and DNA ligase I. In response to IR, APE1 removes 3'-phosphoglycolates whereas PNKP hydrolyses 3'-phosphate groups, given that the 3'-phosphatase activity of APE1 is considerably weaker than that of PNKP [30]. PNKP also ensures that 5'-OH termini are phosphorylated. However, the phosphatase activity of PNKP is significantly more active than the kinase activity, so in strand breaks with both 3'-phosphate and 5'-OH termini, the phosphatase activity takes precedence [31]. The importance of the phosphatase activity in the rapid repair of hydrogen peroxide-induced SSBs in mammalian cells was illustrated by the failure of overexpression of phosphatase-defective PNKP, unlike wildtype PNKP, to compensate for *Xrcc1* deficiency [27]. Similarly, a small molecule inhibitor of PNKP phosphatase activity dramatically retarded SSBR in irradiated human cells [32]. The physiological importance of the 5'-kinase activity has yet to be established.

Several key questions remain regarding the chain of events in SSBR, including the temporal order of protein addition to the damaged substrate, the formation of protein complexes, and the role of post-translational modification. In the more commonly accepted model for repair of radiation-induced SSBs, the breaks are recognized by PARP, which catalyzes the polymerization of chains of ADP-ribose onto acceptor chromatin proteins and itself. This attracts the scaffold protein, XRCC1, probably together with tightly bound DNA ligase III,

which in turn recruits PNKP or APE1 to restore the requisite terminal groups for DNA polymerase β to add the missing base and DNA ligase III to rejoin the strand. Examination of protein–protein interactions indicated direct interactions between XRCC1 and PNKP, as well as DNA polymerase β and DNA ligase III [33], suggesting several possible interactive partnerships including a tetrameric complex between all four proteins, which would be in agreement with the model described above; however, this does not rule out other models. Indeed, evidence exists that counters the concept that XRCC1 recruits either PNKP or APE1 to the strand break [34]. Experiments that tracked the temporal association of SSBR proteins in HeLa cell extracts through crosslinking the proteins to DNA substrates during the course of incubation revealed that for substrates bearing either 3'-phosphoglycolate termini or 3'-phosphate termini, APE1 and PNKP, respectively, were recruited to the strand breaks before XRCC1/DNA ligase III. Furthermore, immunodepletion of APE1 or PNKP reduced the binding of XRCC1 to the respective substrates, implying that APE1 and PNKP recruit XRCC1 to sites of oxidative damage rather than *vice versa*. By contrast, PNKP foci were observed in the nuclei of hydrogen peroxide-treated cells expressing XRCC1, but not in cells lacking XRCC1 [14]. It has, therefore, been argued that although XRCC1 might not be required for the initial recruitment of PNKP or APE1, it facilitates the focal accumulation and stimulation of these enzymes at sites of chromosomal damage [29].

XRCC1, which lacks inherent enzymatic activity, can enhance both the kinase and phosphatase activities of PNKP [33]. Insights into the mechanism underlying XRCC1-induced stimulation of PNKP came from fluorescence measurements of the binding between PNKP and substrates that mimic different strand breaks [35]. PNKP bound reasonably tightly to a nicked substrate with a 5'-OH terminus with a K_d of 0.25 μM. However, this was only 5–6 fold tighter than PNKP binding to the identical duplex bearing a 5'-phosphate, a finding which suggested that PNKP remains bound to the product of its kinase activity. Although the additional presence of XRCC1 did not influence the binding of PNKP to the non-phosphorylated substrate, PNKP interaction with the phosphorylated duplex was completely abrogated, indicating that XRCC1 displaced PNKP from the reaction product. This finding was confirmed by following the kinetics of product accumulation under limiting enzyme concentration: the addition of XRCC1 increased PNKP enzymatic turnover. Similar kinetic data were observed for PNPK phosphatase activity [36].

The interaction between PNKP and XRCC1 is rendered more complicated by CK2-mediated phosphorylation of XRCC1. (In addition to promoting the interaction with other proteins, XRCC1 phosphorylation stabilizes the XRCC1–DNA ligase III complex [37]). Multiple sites of CK2-mediated XRCC1 phosphorylation are observed *in vivo* and *in vitro*, clustered within three locations, residues 408–410, 461–488 and 518–525, although the 518–525 cluster appears to be most important [14, 16, 28, 36]. XRCC1 phosphorylation is required for recruitment of XRCC1 and PNKP to nuclear foci in hydrogen peroxide-treated or γ–irradiated cells, and to facilitate more rapid repair of SSBs, although the lack of XRCC1 phosphorylation does not appear to impact cell survival [14, 27]. Further analysis indicated that reconstitution of cells lacking functional XRCC1 with the triple mutant $XRCC1^{S518A;T519A;T523A}$, but not mutants within the other two clusters, failed to fully restore rapid SSBR, thereby implicating the interaction with PNKP [27]. However, efficient repair can be rescued by over-expression of PNKP [27], an observation that is consistent with a role for XRCC1 in increasing PNKP enzyme turnover, especially if the cell contains a limiting concentration of PNKP.

Phosphorylation of XRCC1 by CK2 stimulates the kinase and phosphatase activities of PNKP, measured *in vitro*, to a greater extent than non-phosphorylated XRCC1 [14, 36]. As with the stimulation by non-phosphorylated XRCC1, this results from enhanced enzymatic turnover of PNKP [36]. This presents an interesting conundrum because it is clear that

phosphorylated and non-phosphorylated XRCC1 bind PNKP at different sites and with different affinities, yet both stimulate PNKP by a similar mechanism. Whereas phosphorylated XRCC1 binds the FHA domain with a K_d of 4 nM, the non-phosphorylated protein binds the catalytic domain of PNKP with 10-fold weaker affinity [35, 36]. Evidence supporting the possibility of phosphorylation-independent interaction between PNKP and XRCC1 in human cells was provided by Luo et al. [16], who found that PNKP co-immunoprecipitated with the $XRCC1^{S518A/T519A/T523A}$ triple mutant expressed in human 293T cells. Although 85–90% of the cellular XRCC1 is phosphorylated [37], it is not clear if the key cluster of amino acids involved in interaction with the FHA domain is fully phosphorylated. Treatment of cells with hydrogen peroxide led to an increase in phosphorylation at the cluster and an approximately 3-fold increase in PNKP co-purifying with XRCC1 [14]. We therefore speculated [36] that cells enhance CK2-mediated phosphorylation of XRCC1 and its subsequent interaction with the PNKP FHA domain in response to challenge by hydrogen peroxide or radiation in order to deal with relatively high levels of DNA damage in a timely manner. By contrast, unstressed cells can cope with the comparatively low level of endogenous DNA damage using non-phosphorylated XRCC1, or XRCC1 with a limited degree of phosphorylation, to stimulate PNKP through binding to the catalytic domain.

PNKP depletion in human cells and *Pnk1* deletion in fission yeast renders cells sensitive to camptothecin [13, 38, 39]. The repair of these strand breaks is also mediated by XRCC1, which forms a complex with TDP1, DNA ligase III and PNKP [40, 41]. Mutation of *TDP1* is responsible for the neurodegenerative disorder, spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) [42]. In addition to their reduced capacity to repair camptothecin-induced SSBs, SCAN1 cells also display slow repair of hydrogen peroxide-induced SSBs, suggesting that TDP1 is required to repair lesions generated by oxidative processes, and that these lesions might account for the neurodegeneration observed in SCAN1 [41]. This idea was supported experiments showing that for fission yeast in G0, Tdp1 and Pnk1 act sequentially to process the 3'-termini of naturally occurring SSBs [51].

PNKP and base excision repair (BER)

BER is responsible for the repair of most minor base modifications induced by IR, ROS and alkylating agents. The first step is removal of the modified base by DNA glycosylase followed by cleavage of the DNA at the newly formed apurinic/apyrimidinic site (AP) by APE1 [43]. Alternatively, the glycosylase might have AP lyase activity that hydrolyzes the AP site [43]. The involvement of PNKP in the BER pathway became evident after the discovery of the nei endonuclease VIII-like 1 (NEIL1) and NEIL2 mammalian DNA glycosylases, which possess β,δ–AP lyase activity that generates 3'-phosphate termini [30, 44, 45]. Although neither of these glycosylases binds directly to PNKP, both are stably associated in larger complexes containing other BER components including PNKP [30, 45]. These glycosylases act on a variety of base lesions including thymine glycol, 5-hydroxyuracil and 8-oxoguanine [43], but can also cleave intact abasic sites, generated by glycosylases that do not possess AP lyase activity, and the pentenal moiety generated by the β-elimination AP lyases of other DNA glycosylases [30]. In this regard, the NEIL glycosylases would compete with APE1, and therefore form the basis of an alternative, APE1-independent, BER pathway. It is still not clear to what extent NEIL1- or NEIL2-catalyzed cleavage of abasic sites occurs in cells, but it could provide an explanation for the increased sensitivity of PNKP-depleted cells to the alkylating agent methyl methanesulfonate (MMS) [38]. The sensitivity to MMS was unexpected because the major lesions inflicted by this agent are N^7-methylguanine and N^3-methyladenine, with little if any direct strand scission [6]. However, given that N-methylpurine-DNA glycosylase (MPG), the human DNA glycosylase responsible for removing these methylated bases, does not

possess AP lyase activity [43], it is conceivable that NEIL1 or 2 might act upon the abasic sites generated by MPG to produce strand breaks with 3'-phosphate termini. Interestingly, down-regulation of aprataxin expression also renders cells sensitive to MMS [16].

PNKP and double-strand break repair (DSBR)

Of the two major DSBR pathways, there is clear evidence for PNKP participation in nonhomologous end joining (NHEJ) [15, 46, 47]. but the failure to influence IR-induced sister chromatid exchange by PNKP depletion suggests that PNKP is not involved in homologous recombination [47]. PNKP also plays a role in a back-up, XRCC1-dependent, DSB repair pathway [48]. Evidence for PNKP participation in NHEJ came initially from experiments using human cell-free extracts, showing that the PNKP kinase activity was required before ligation of linearized plasmid substrates bearing 5'-OH termini could occur [46]. Successful phosphorylation was dependent on XRCC4 and DNA-PK_{cs}. Analogous to the role of XRCC1 in linking PNKP to DNA ligase III, XRCC4 links PNKP to DNA ligase IV [15]. As discussed above, CK2-mediated phosphorylation of XRCC4 Thr233 promotes interaction with the PNKP FHA domain, and efficiently stimulates XRCC4-DNA ligase IV mediated ligation of a 5'-dephosphorylated plasmid substrate *in vitro* [15]. Expression of $XRCC4^{T233A}$ in place of wild type XRCC4 in an *Xrcc4*-deficient cell line reduced survival by approximately 30% following irradiation and slowed down the rate of DSB repair [15].

Combined biophysical and biochemical examination provided insight into the functional role of the XRCC4–PNKP interaction [49]. Although phosphorylation of XRCC4 promotes a tight affinity for PNKP (K_d = 4 nM), non-phosphorylated XRCC4 can also bind to PNKP, but in this case binding is to the catalytic domain of PNKP and the affinity is weaker (K_d = 90 nM). Non-phosphorylated XRCC4 stimulates PNKP enzymatic turnover from DSBs in a similar fashion to XRCC1 stimulation of PNKP turnover from SSBs. Surprisingly, the presence of phosphorylated XRCC4 fails to stimulate PNKP, and indeed appears to block PNKP-mediated DNA phosphorylation. However, the additional presence of DNA ligase IV in complex with phosphorylated XRCC4 reverses the inhibition and stimulates PNKP turnover. Quantification of the proteins in HeLa cells indicated a ratio of XRCC4:DNA ligase IV:PNKP of ~7:1:3, with approximately half of the XRCC4 constitutively phosphorylated at Thr233. It can thus be inferred that in cells only a fraction of XRCC4 can be complexed to DNA ligase IV, and the possibility exists for FHA-independent interaction between XRCC4 and PNKP. The latter was confirmed by XRCC4 co-immunoprecipitation with $PNKP^{R35A}$ expressed in cells depleted of endogenous PNKP. Taken together [15, 49], the data present a complicated picture with both phosphorylated and non-phosphorylated XRCC4 acting in several different combinations with PNKP and DNA ligase IV to accomplish two main functions: (i) the displacement of PNKP from processed strand break termini, which would utilize non-phosphorylated XRCC4 alone or XRCC4–DNA ligase IV complex, and (ii) the recruitment of DNA ligase IV to rejoin PNKP processed strands, which would be enhanced by tight binding between phosphorylated XRCC4 and the PNKP FHA domain (Fig. 2).

Another important function for PNKP in the NHEJ pathway, in combination with TDP1, is the processing of DSB 3'-phosphoglycolate termini, especially 3'-overhanging and blunt-ended termini [50, 51]. Such termini are produced by IR, bleomycin and enediyne compounds such as neocarzinostatin. Although APE1 can remove phosphoglycolate groups at SSB termini and recessed DSB termini, it loses its effectiveness on blunt-ended DSB termini and is completely ineffective on overhanging termini [52].

Physiological roles and clinical potential of PNKP

The increased interest in PNKP reflects its involvement in several DNA repair pathways that protect cells from endogenous and exogenous genotoxic agents. Disruption of NHEJ genes and SSBR/BER genes are known to cause neurological disorders with various symptoms; for example, microcephaly is seen in individuals with mutations in *LIG4* (which encodes DNA ligase IV) [53], whereas deletion of *Xrcc1* in mice causes seizures [54]. A recent report established that a severe neurological autosomal recessive disease (MCSZ) characterized by microcephaly, intractable seizures and developmental delay is caused by PNKP mutations [13]. Individuals from seven families were analyzed, and mutations were found in both the kinase and phosphatase domains. The collection of symptoms displayed by individuals with MCSZ is unique, and it has been argued that this reflects the involvement of PNKP in multiple DNA repair pathways [13].

Two other recent reports have linked PNKP to pathophysiological conditions. The first is an intriguing observation of elevated expression of PNKP in arthrofibrotic tissue, which suggests a role for PNKP in ameliorating the effects of ROS generated by macrophages [55]. The second shows that physiologically and environmentally relevant doses of cadmium and copper, which are known to elicit neurotoxic and carcinogenic effects, can inhibit PNKP [64].

The DNA repair capacity of tumour cells is regarded as an important factor in the clinical response to many antineoplastic agents. As a result, inhibitors of several DNA repair enzymes, including PNKP [12, 56], are being investigated for their ability to sensitize cells to radiation and chemotherapeutic drugs. A small molecule inhibitor of PNKP phosphatase activity was recently identified and shown to enhance the sensitivity of cells to IR and camptothecin [32, 57], the parent compound of two clinically important topoisomerase I poisons, irinotecan and topotecan, frequently used to treat colon and ovarian cancers, respectively.

Concluding remarks

PNKP is a key enzyme in the cellular processing of stand break termini and not surprisingly participates in several DNA repair pathways (summarized in Fig. 3). Further progress is required to clarify its regulation, interactions with other repair enzymes, and physiological role in neurons and other tissues. Because of its involvement in a variety of repair pathways, PNKP is now regarded as a therapeutic target in the treatment of cancer, so new inhibitory compounds will need to be identified and optimized for clinical use. Given the recent excitement over the efficacy of PARP inhibitors, especially their potential use as single agents against tumours deficient in proteins that have a synthetic lethal partnership with PARP [58], such as breast cancer 1, early onset (BRCA1) and BRCA2, a major emphasis should be placed on identifying synthetic lethal partners of PNKP.

Acknowledgments

We thank Dr. Susan Lees-Miller (University of Calgary) for critically reading the manuscript. This work was supported by grants from the Canadian Institutes of Health Research, the Canadian Cancer Society, the Alberta Cancer Foundation, and the National Institutes of Health. JNMG acknowledges the support of the Howard Hughes International Scholar program.

References

1. Hoeijmakers JH. DNA damage, aging, and cancer. N Engl J Med. 2009; 361:1475–1485. [PubMed: 19812404]

2. Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. Cancer Res. 2008; 68:6863–6872. [PubMed: 18757397]
3. Paz-Elizur T, et al. DNA repair of oxidative DNA damage in human carcinogenesis: potential application for cancer risk assessment and prevention. Cancer Lett. 2008; 266:60–72. [PubMed: 18374480]
4. Madhusudan S, Middleton MR. The emerging role of DNA repair proteins as predictive, prognostic and therapeutic targets in cancer. Cancer Treat Rev. 2005; 31:603–617. [PubMed: 16298073]
5. McKinnon PJ. DNA repair deficiency and neurological disease. Nat Rev Neurosci. 2009; 10:100–112. [PubMed: 19145234]
6. Friedberg, ECW.; Siede, GC.; Wood, RD.; Schultz, RA.; Ellenberger, T. DNA Repair and Mutagenesis. ASM Press; 2006.
7. Pheiffer BH, Zimmerman SB. 3'-Phosphatase activity of the DNA kinase from rat liver. Biochem Biophys Res Commun. 1982; 109:1297–1302. [PubMed: 6301434]
8. Habraken Y, Verly WG. Further purification and characterization of the DNA 3'-phosphatase from rat-liver chromatin which is also a polynucleotide 5'-hydroxyl kinase. Eur J Biochem. 1988; 171:59–66. [PubMed: 2828058]
9. Jilani A, et al. Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3'-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. J Biol Chem. 1999; 274:24176–24186. [PubMed: 10446192]
10. Karimi-Busheri F, et al. Molecular characterization of a human DNA kinase. J Biol Chem. 1999; 274:24187–24194. [PubMed: 10446193]
11. Zhu Y, et al. Targeting DNA repair pathways: a novel approach to reduce cancer therapeutic resistance. Cancer Treat Rev. 2009; 35:590–596. [PubMed: 19635647]
12. Allinson SL. DNA end-processing enzyme polynucleotide kinase as a potential target in the treatment of cancer. Future Oncol. 2010; 6:1031–1042. [PubMed: 20528239]
13. Shen J, et al. Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. Nat Genet. 2010; 42:245–249. [PubMed: 20118933]
14. Loizou JI, et al. The protein kinase CK2 facilitates repair of chromosomal DNA single-strand breaks. Cell. 2004; 117:17–28. [PubMed: 15066279]
15. Koch CA, et al. Xrcc4 physically links DNA end processing by polynucleotide kinase to DNA ligation by DNA ligase IV. Embo J. 2004; 23:3874–3885. [PubMed: 15385968]
16. Luo H, et al. A new XRCC1-containing complex and its role in cellular survival of methyl methanesulfonate treatment. Mol Cell Biol. 2004; 24:8356–8365. [PubMed: 15367657]
17. Reynolds JJ, et al. Short-patch single-strand break repair in ataxia oculomotor apraxia-1. Biochem Soc Trans. 2009; 37:577–581. [PubMed: 19442253]
18. Clements PM, et al. The ataxia-oculomotor apraxia 1 gene product has a role distinct from ATM and interacts with the DNA strand break repair proteins XRCC1 and XRCC4. DNA Repair (Amst). 2004; 3:1493–1502. [PubMed: 15380105]
19. Wang LK, et al. Structure and mechanism of T4 polynucleotide kinase: an RNA repair enzyme. EMBO J. 2002; 21:3873–3880. [PubMed: 12110598]
20. Galburt EA, et al. Structure of a tRNA repair enzyme and molecular biology workhorse: T4 polynucleotide kinase. Structure. 2002; 10:1249–1260. [PubMed: 12220496]
21. Bernstein NK, et al. The molecular architecture of the mammalian DNA repair enzyme, polynucleotide kinase. Mol Cell. 2005; 17:657–670. [PubMed: 15749016]
22. Eastberg JH, et al. Recognition of DNA substrates by T4 bacteriophage polynucleotide kinase. Nucleic Acids Res. 2004; 32:653–660. [PubMed: 14754987]
23. Karimi-Busheri F, Weinfeld M. Purification and substrate specificity of polydeoxyribonucleotide kinases isolated from calf thymus and rat liver. J Cell Biochem. 1997; 64:258–272. [PubMed: 9027586]
24. Bernstein NK, et al. Mechanism of DNA substrate recognition by the mammalian DNA repair enzyme, Polynucleotide Kinase. Nucleic Acids Res. 2009

25. Koonin EV, Tatusov RL. Computer analysis of bacterial haloacid dehalogenases defines a large superfamily of hydrolases with diverse specificity. Application of an iterative approach to database search. J Mol Biol. 1994; 244:125–132. [PubMed: 7966317]
26. Durocher D, Jackson SP. The FHA domain. FEBS Lett. 2002; 513:58–66. [PubMed: 11911881]
27. Breslin C, Caldecott KW. DNA 3'-phosphatase activity is critical for rapid global rates of single-strand break repair following oxidative stress. Mol Cell Biol. 2009; 29:4653–4662. [PubMed: 19546231]
28. Ali AA, et al. Specific recognition of a multiply phosphorylated motif in the DNA repair scaffold XRCC1 by the FHA domain of human PNK. Nucleic Acids Res. 2009; 37:1701–1712. [PubMed: 19155274]
29. Caldecott KW. Single-strand break repair and genetic disease. Nat Rev Genet. 2008; 9:619–631. [PubMed: 18626472]
30. Wiederhold L, et al. AP endonuclease-independent DNA base excision repair in human cells. Mol Cell. 2004; 15:209–220. [PubMed: 15260972]
31. Dobson CJ, Allinson SL. The phosphatase activity of mammalian polynucleotide kinase takes precedence over its kinase activity in repair of single strand breaks. Nucleic Acids Res. 2006; 34:2230–2237. [PubMed: 16648365]
32. Freschauf GK, et al. Identification of a small molecule inhibitor of the human DNA repair enzyme polynucleotide kinase/phosphatase. Cancer Res. 2009; 69:7739–7746. [PubMed: 19773431]
33. Whitehouse CJ, et al. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell. 2001; 104:107–117. [PubMed: 11163244]
34. Parsons JL, et al. End-damage-specific proteins facilitate recruitment or stability of X-ray cross-complementing protein 1 at the sites of DNA single-strand break repair. Febs J. 2005; 272:5753–5763. [PubMed: 16279940]
35. Mani RS, et al. XRCC1 stimulates polynucleotide kinase by enhancing its damage discrimination and displacement from DNA repair intermediates. J Biol Chem. 2007; 282:28004–28013. [PubMed: 17650498]
36. Lu M, et al. Independent mechanisms of stimulation of polynucleotide kinase/phosphatase by phosphorylated and non-phosphorylated XRCC1. Nucleic Acids Res. 2010; 38:510–521. [PubMed: 19910369]
37. Parsons JL, et al. XRCC1 phosphorylation by CK2 is required for its stability and efficient DNA repair. DNA Repair (Amst). 2010; 9:835–841. [PubMed: 20471329]
38. Rasouli-Nia A, et al. Stable down-regulation of human polynucleotide kinase enhances spontaneous mutation frequency and sensitizes cells to genotoxic agents. Proc Natl Acad Sci U S A. 2004; 101:6905–6910. [PubMed: 15100409]
39. Meijer M, et al. Pnk1, a DNA kinase/phosphatase required for normal response to DNA damage by gamma-radiation or camptothecin in Schizosaccharomyces pombe. J Biol Chem. 2002; 277:4050–4055. [PubMed: 11729194]
40. Plo I, et al. Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. DNA Repair (Amst). 2003; 2:1087–1100. [PubMed: 13679147]
41. El-Khamisy SF, et al. Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. Nature. 2005; 434:108–113. [PubMed: 15744309]
42. Takashima H, et al. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. Nat Genet. 2002; 32:267–272. [PubMed: 12244316]
43. Hegde ML, et al. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. 2008; 18:27–47. [PubMed: 18166975]
44. Bandaru V, et al. A novel human DNA glycosylase that removes oxidative DNA damage and is homologous to Escherichia coli endonuclease VIII. DNA Repair (Amst). 2002; 1:517–529. [PubMed: 12509226]
45. Das A, et al. NEIL2-initiated, APE-independent repair of oxidized bases in DNA: Evidence for a repair complex in human cells. DNA Repair (Amst). 2006; 5:1439–1448. [PubMed: 16982218]

46. Chappell C, et al. Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. Embo J. 2002; 21:2827–2832. [PubMed: 12032095]
47. Karimi-Busheri F, et al. Human polynucleotide kinase participates in repair of DNA double-strand breaks by nonhomologous end joining but not homologous recombination. Cancer Res. 2007; 67:6619–6625. [PubMed: 17638872]
48. Audebert M, et al. Involvement of polynucleotide kinase in a poly(ADP-ribose) polymerase-1-dependent DNA double-strand breaks rejoining pathway. J Mol Biol. 2006; 356:257–265. [PubMed: 16364363]
49. Mani RS, et al. Dual Modes of Interaction between XRCC4 and Polynucleotide Kinase/Phosphatase: IMPLICATIONS FOR NONHOMOLOGOUS END JOINING. J Biol Chem. 2010; 285:37619–37629. [PubMed: 20852255]
50. Inamdar KV, et al. Conversion of phosphoglycolate to phosphate termini on 3' overhangs of DNA double strand breaks by the human tyrosyl-DNA phosphodiesterase hTdp1. J Biol Chem. 2002; 277:27162–27168. [PubMed: 12023295]
51. Zhou T, et al. Tyrosyl-DNA phosphodiesterase and the repair of 3'-phosphoglycolate-terminated DNA double-strand breaks. DNA Repair (Amst). 2009; 8:901–911. [PubMed: 19505854]
52. Suh D, et al. 3'-phosphodiesterase activity of human apurinic/apyrimidinic endonuclease at DNA double-strand break ends. Nucleic Acids Res. 1997; 25:2495–2500. [PubMed: 9171104]
53. O'Driscoll M, et al. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. Mol Cell. 2001; 8:1175–1185. [PubMed: 11779494]
54. Lee Y, et al. The genesis of cerebellar interneurons and the prevention of neural DNA damage require XRCC1. Nat Neurosci. 2009; 12:973–980. [PubMed: 19633665]
55. Freeman TA, et al. Reactive oxygen and nitrogen species induce protein and DNA modifications driving arthrofibrosis following total knee arthroplasty. Fibrogenesis Tissue Repair. 2009; 2:5. [PubMed: 19912645]
56. Bernstein NK, et al. Polynucleotide kinase as a potential target for enhancing cytotoxicity by ionizing radiation and topoisomerase I inhibitors. Anticancer Agents Med Chem. 2008; 8:358–367. [PubMed: 18473721]
57. Freschauf GK, et al. Mechanism of action of an imidopiperidine inhibitor of human polynucleotide kinase/phosphatase. J Biol Chem. 2010; 285:2351–2360. [PubMed: 19940137]
58. Megnin-Chanet F, et al. Targeting poly(ADP-ribose) polymerase activity for cancer therapy. Cell Mol Life Sci. 2010; 67:3649–3662. [PubMed: 20725763]
59. Henner WD, et al. gamma Ray induced deoxyribonucleic acid strand breaks. 3' Glycolate termini. J Biol Chem. 1983; 258:711–713. [PubMed: 6822504]
60. Buchko GW, Weinfeld M. Influence of nitrogen, oxygen, and nitroimidazole radiosensitizers on DNA damage induced by ionizing radiation. Biochemistry. 1993; 32:2186–2193. [PubMed: 8443160]
61. von Sonntag, C. Free-radical-induced DNA damage and its repair. Springer-Verlag: 2006.
62. Lennartz M, et al. Oxygen--effect on strand breaks and specific end-groups in DNA of irradiated thymocytes. Int J Radiat Biol Relat Stud Phys Chem Med. 1975; 27:577–587. [PubMed: 1081084]
63. Coquerelle T, et al. Strand breaks and 5' end-groups in DNA of irradiated thymocytes. Int J Radiat Biol Relat Stud Phys Chem Med. 1973; 24:397–404. [PubMed: 4542925]
64. Nikjoo H, et al. Quantitative modelling of DNA damage using Monte Carlo track structure method. Radiat Environ Biophys. 1999; 38:31–38. [PubMed: 10384953]
65. Sutherland BM, et al. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc Natl Acad Sci U S A. 2000; 97:103–108. [PubMed: 10618378]
66. Ward JF. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol. 1988; 35:95–125. [PubMed: 3065826]
67. Dahm-Daphi J, et al. Comparison of biological effects of DNA damage induced by ionizing radiation and hydrogen peroxide in CHO cells. Int J Radiat Biol. 2000; 76:67–75. [PubMed: 10665959]

68. Povirk LF. DNA damage and mutagenesis by radiomimetic DNA-cleaving agents: bleomycin, neocarzinostatin and other enediynes. Mutat Res. 1996; 355:71–89. [PubMed: 8781578]

69. Yang SW, et al. A eukaryotic enzyme that can disjoin dead-end covalent complexes between DNA and type I topoisomerases. Proc Natl Acad Sci U S A. 1996; 93:11534–11539. [PubMed: 8876170]

70. McCullough AK, et al. Initiation of base excision repair: glycosylase mechanisms and structures. Annu Rev Biochem. 1999; 68:255–285. [PubMed: 10872450]

71. Hazra TK, et al. Identification and characterization of a novel human DNA glycosylase for repair of cytosine-derived lesions. J Biol Chem. 2002; 277:30417–30420. [PubMed: 12097317]

72. Rosenquist TA, et al. The novel DNA glycosylase, NEIL1, protects mammalian cells from radiation-mediated cell death. DNA Repair (Amst). 2003; 2:581–591. [PubMed: 12713815]

73. Katafuchi A, et al. Differential specificity of human and Escherichia coli endonuclease III and VIII homologues for oxidative base lesions. J Biol Chem. 2004; 279:14464–14471. [PubMed: 14734554]

Text box 1 – Chemistry of strand break termini

(i) IR- and free radical-induced breaks

IR generates hydroxyl radicals, which produce strand breaks with a variety of end groups at 3' termini resulting from reaction at different carbon atoms within the deoxyribose group [59–61]. The two predominant end groups are phosphate and phosphoglycolate (Figure I). Whereas formation of the latter is dependent on the presence of oxygen, 3'-phosphate groups are produced under normoxia and anoxia [60]. At 5'-termini, the major end group is phosphate, but 5'-hydroxyl (5'-OH) termini have been observed at 10–14 % of strand breaks in irradiated mammalian cells [62, 63]. IR also generates complex lesions, containing two or more damaged bases or strand breaks in close proximity, as well as singly damaged sites, in a ratio of ~1:4 [64, 65]. Some of these complex lesions are frank DSBs and the ratio of SSB:DSB is ~25:1 [66]. Hydrogen peroxide produces strand breaks with similar termini to IR-mediated damage, but far fewer are frank DSBs [67]. The chemotherapeutic agent bleomycin also produces DSBs (ratio of SSB:DSB ~ 10:1) with 3'-phosphoglycolate termini [68]. The bi-stranded lesions are formed either directly opposite each other or with a l-base 5' stagger.

(ii) Camptothecin-induced breaks

In the process of relieving torsional strain, topoisomerase I creates a DNA nick with a 5'-OH terminus and a covalent 3'-phosphate-enzyme intermediate. Camptothecin prevents subsequent strand rejoining by the topoisomerase, thereby leaving a DNA-enzyme “dead-end” complex. Hydrolysis of the complex by tyrosyl-DNA phosphodiesterase (TDP1) creates nicks with 3'-phosphate and 5'-OH termini [40, 69].

(iii) Repair-endonuclease induced breaks

After removal of damaged bases by DNA glycosylases, the resulting abasic sites are cleaved by one of two classes of enzymes. An AP endonuclease, e.g. APE1, hydrolyses the phosphodiester bond 3' to the abasic site to give 3'-OH and 5'-deoxyribose phosphate termini. The latter can be converted to 5'-phosphate by DNA polymerase β[6]. Alternatively, many DNA glycosylases possess an AP-lyase activity, which cleaves the phosphodiester bond 5' to the abasic site by a β-elimination reaction to give an α,β-unsaturated aldehyde (4-hydroxy-2-pentenal) attached to a 3'-phosphate at one terminus and a 5'-phosphate at the other [70]. The pentenal moiety can then be removed either by an AP endonuclease to give 3'-OH or in some instances by further action (δ-elimination) by the AP-lyases to give 3'-phosphate [70]. Two mammalian DNA glycosylases possessing β,δ–lyase activity are NEIL1 and NEIL2 [44, 71–73]. These enzymes remove a spectrum of mutagenic and cytotoxic oxidative pyrimidine lesions and purine-derived formamidopyrimidines [44, 71–73].

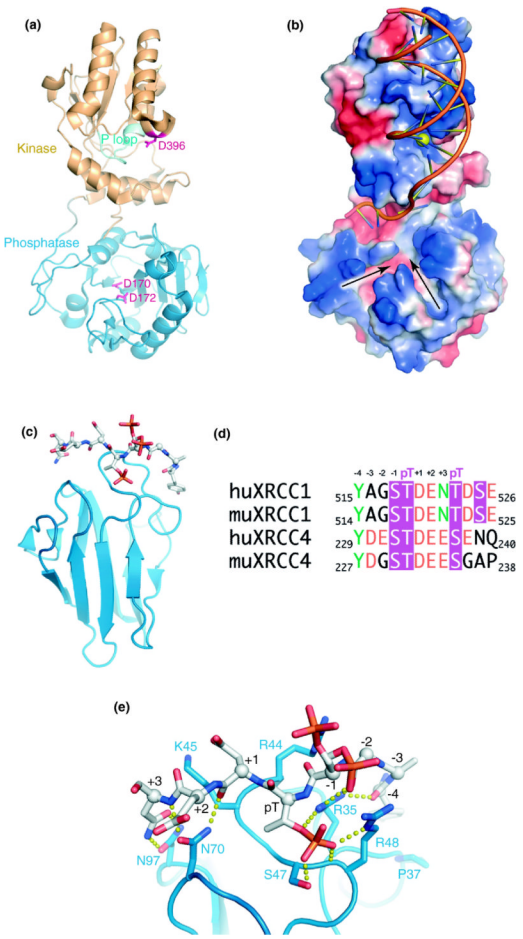


Figure 1.

Structure of Mammalian PNKP. Structural investigations have revealed the overall architecture of the PNKP catalytic domain, its interactions with kinase substrates, and its interactions with phosphorylated target proteins via its forkhead-associated (FHA) domain. (a) Overview of the catalytic domain of murine PNKP [21]. The kinase domain is orange with regions critical for catalysis (the P loop and Asp396) both highlighted. The phosphatase domain is blue with the catalytic Asp170 and Asp 172 highlighted. (b) A surface view of the murine PNKP catalytic domain in the same orientation as in (a), highlighting possible modes of substrate recognition. A minimal kinase substrate containing a 11-bp stem and a 5-nucleotide single-stranded 3'-overhang is shown bound to the catalytic domain as indicated by a combined SAXS-mutagenesis-molecular modeling study [24]. The position of the substrate 5'-OH is indicated by a yellow sphere. Two narrow channels that provide entrance to the phosphatase active site are indicated by arrows. (c) Overview of the structure of the human PNKP FHA domain bound to a doubly phosphorylated phosphopeptide derived from XRCC1 [28]. The FHA domain is in blue, the XRCC1 phosphopeptide in white sticks. (d) Amino acid sequence alignment of the regions of human (hu) and murine (mu) XRCC1 and XRCC4 bound by the PNKP FHA domain. Sites of CK2 phosphorylation are highlighted in purple, conserved acidic residues are in red, and conserved residues at −4 and +3 that also are selectively recognized by the FHA are colored green. (e) Detailed view of PNKP FHA–phospho-XRCC1 interactions. Residues contributing to key interactions are shown as sticks, with hydrogen bonding and salt bridging interactions indicated by yellow dotted lines. Note the presence of multiple conformers of pSer at the −1 position [28].

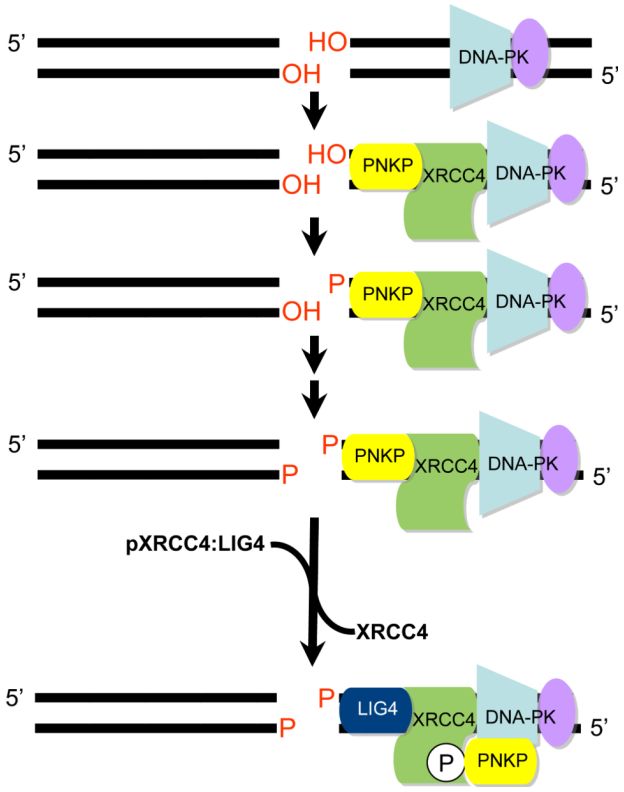


Figure 2.

Model of XRCC4 and PNKP participation in NHEJ. The model represents a double strand break with 5'-OH termini. After assembly of the DNA-PK complex composed of the Ku70–Ku80 heterodimer (purple) and DNA-PK_{cs} (light blue) at the strand break termini, XRCC4 (green) is recruited along with PNKP (yellow). (Identical protein recruitment and enzymatic activity would occur at each break terminus, but only one is shown for clarity.) Upon completion of phosphorylation of both termini, phosphorylated XRCC4–DNA ligase IV (green–dark blue) complex binds PNKP through the FHA domain and thereby displaces PNKP and non-phosphorylated XRCC4 from the strand break termini, so that other proteins, including DNA ligase IV, can gain access to the termini to complete the repair.

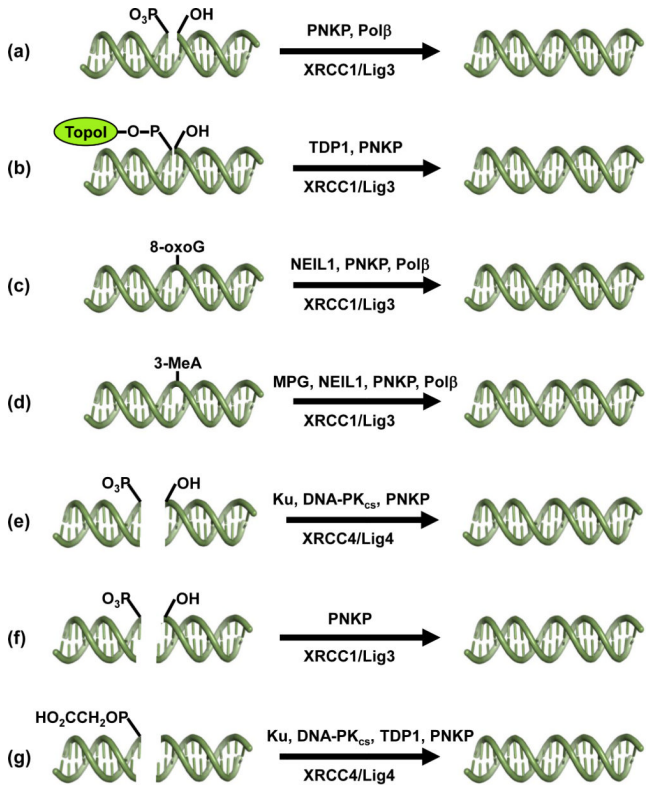
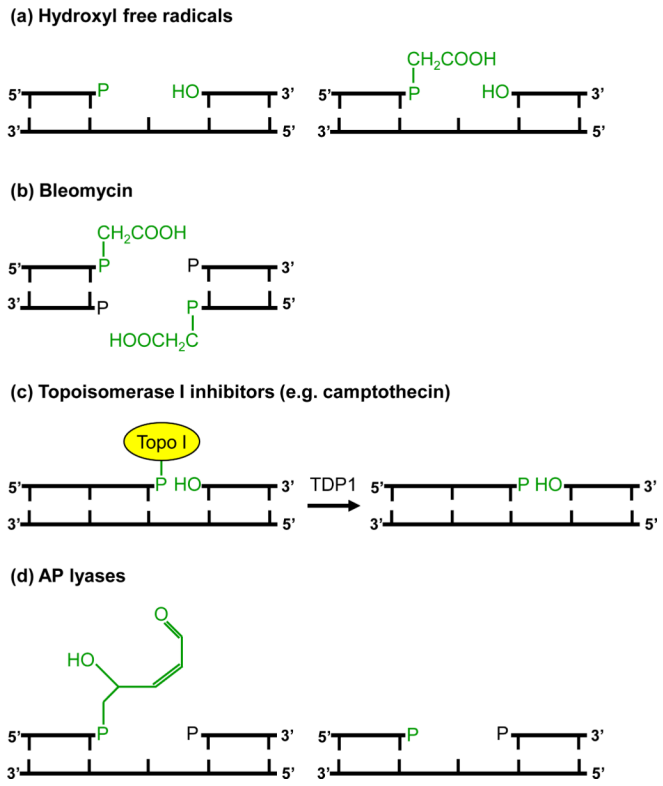


Figure 3.

Participation of PNKP in DNA repair pathways. The figure highlights the requirement of PNKP to process strand break termini at SSBs directly introduced by DNA damaging agents (a and b), at SSBs introduced in the course of BER (c and d), and at directly induced DSBs (e–g). (a) PNKP hydrolyzes 3'-phosphate and phosphorylates 5'-OH termini at a radiation-induced SSB prior to replacement of the missing nucleotide by DNA polymerase β and strand rejoining by DNA ligase III (LIG3). (b) Strand rejoining at a topoisomerase 1 (TopoI)–DNA "dead-end" complex first requires the hydrolysis of the covalent bond attaching topoisomerase I to the DNA 3'-phosphate group, followed by PNKP-mediated correction of the 3'-phosphate and 5'-OH termini and strand rejoining by DNA ligase III. (c) BER by DNA glycosylases that possess β,δ-AP lyase activity, such as NEIL1 and NEIL2, remove oxidized bases and cleave the abasic sites, leaving 3'-phosphate groups that are acted upon by PNKP. (d) DNA glycosylases that have no AP lyase activity, such as methylpurine-DNA glycosylase (MPG), create abasic sites that can be acted upon by NEIL1 or NEIL2, thereby generating strand breaks with 3'-phosphate termini. (e) The NHEJ pathway for DSB repair involves end-binding by the Ku heterodimer and DNA-PK_{cs} followed by end processing by PNKP (or other proteins, such as Artemis) and strand rejoining by DNA ligase IV (LIG4), which exists in complex with XRCC4 and possibly XLF (see Fig. 2 for a more detailed model of PNKP involvement in 5'-phosphorylation at DSB termini). (f) Alternative, XRCC1-dependent, DSB repair pathway. (g) Removal of phosphoglycolate groups at 3'-overhanging DSB termini entails hydrolysis of the glycolate group by TDP1 followed by dephosphorylation by PNKP as a component of the NHEJ pathway.



Box 1, Figure I.

Structures of DNA strand break termini requiring processing prior to ligation. (a) Hydroxyl radical attack of the deoxyribose can generate 5'-hydroxyl termini and 3'-phosphate (i) or 3'-phosphoglycolate (ii). (b) Bleomycin-induced DSB with 3'-phosphoglycolate termini. (c) Topoisomerase I (TopoI)–DNA “dead-end” complex generated by camptothecin. The enzyme, which is covalently attached to the DNA 3'-phosphate through a tyrosine residue, can be released from the DNA by TDP1. (d) DNA glycosylases with AP lyase activity cleave abasic sites. The products depend on the cleavage chemistry: β-elimination leads to the 4-hydroxy-2-pentenal moiety bound to the 3'-phosphate (i), whereas βδ-elimination produces a 3'-phosphate (ii). Phosphate groups are represented by the letter P.