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**REVIEW**

# How Does a Cell Repair Damaged DNA?

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**Abstract**—DNA in living cells is constantly subjected to different chemical and physical factors of the environment and to cell metabolites. Some changes altering DNA structure occur spontaneously. This raises the potential danger of harmful mutations that could be transmitted to offspring. To avoid the danger of mutations and changing genetic information, a cell is capable to switch on multiple mechanisms of DNA repair that remove damage and restore native structure. In many cases, removal of the same damage may involve several alternative pathways; this is very important for DNA repair under the most unfavorable conditions. This review summarizes data about all known mechanisms of eukaryotic DNA repair including excision repair (base excision repair and nucleotide excision repair), mismatch repair, repair of double-strand breaks, and cross-link repair. Special attention is given to the regulation of excision repair by different proteins—proliferating cell nuclear antigen (PCNA), p53, and proteasome. The review also highlights problem of bypassing irremovable lesions in DNA.

*Key words*: DNA damage, DNA repair, lesion bypass

DNA stores genetic information transmitted from generation to generation of living organisms. It is vitally important to preserve this genetic material intact. In the case of DNA damage, a cell must restore its native structure or the danger of mutations leading to various abnormalities (pathologies) appears. Rapid restoration of DNA structure is especially important during formation of reproductive cells and in embryonal development, because active synthesis of new DNA strands significantly increases the probability of appearance and repetition of mistakes. In this review, I consider the origin of DNA damages and mechanisms that eukaryotic cells employ to correct these damages.

## DNA DAMAGES

DNA is constantly subjected to spontaneous changes and also to changes induced by various physicochemical factors and even by cell metabolites. These changes rather often include apurinization, i.e., removal of purine bases (formation of so-called AP-sites) [1] and deamination of bases [2]. Products of deamination of cytosine, adenine, and guanine produce nitrogenous bases (uracil, hypoxanthine, and xanthine, respectively) that are not typical for primary structure of DNA. Ultraviolet irradiation results in dimer formation involving two neighboring pyrimidine bases of the same DNA strand. Ionizing radiation causes several types of damages: opening of purine ring, base fragmentation, oxidation of AP-site, and also single and double strand breaks [3]. Some chemical agents induce cross-links of DNA strands [4]. Reactive oxygen species (e.g., $OH^{\cdot}$, $O_2^{\cdot-}$, $H_2O_2$, lipid peroxides, etc.) cause damages to bases and deoxyribose and induce formation of new covalent bonds (cross-links) [5]. In DNA molecules, GG sequences represent "hot-points" for oxidative damage, resulting in formation of an oxidative derivative of guanine, 8-oxoG [6]. Reactive oxygen species may also induce breaks in the sugar-phosphate bonds. If AP-sites in both DNA strands are positioned opposite each other or deoxyribose fragmentation occurs near these sites double-strand breaks appear [7]. Allele recombination and replication errors may lead to appearance of mismatch (unpaired) nucleotides in complementary strands, which impair DNA structure.

Thus, all known DNA damages can be classified into several main groups: modified and damaged bases, AP-sites, cross-links between neighboring pyrimidines, mismatch (unpaired) nucleotides, inter-strand cross-links, and single and double strand breaks. Figure 1 schematically shows types of DNA damages and factors inducing these damages.

Cell can remove all these damages and restore DNA structure during repair processes. Removal of diverse damages requires involvement of various repair mechanisms. Although not all mechanisms have been exhaustively studied, it is known that they function according to some general principles. Repair of any damage begins

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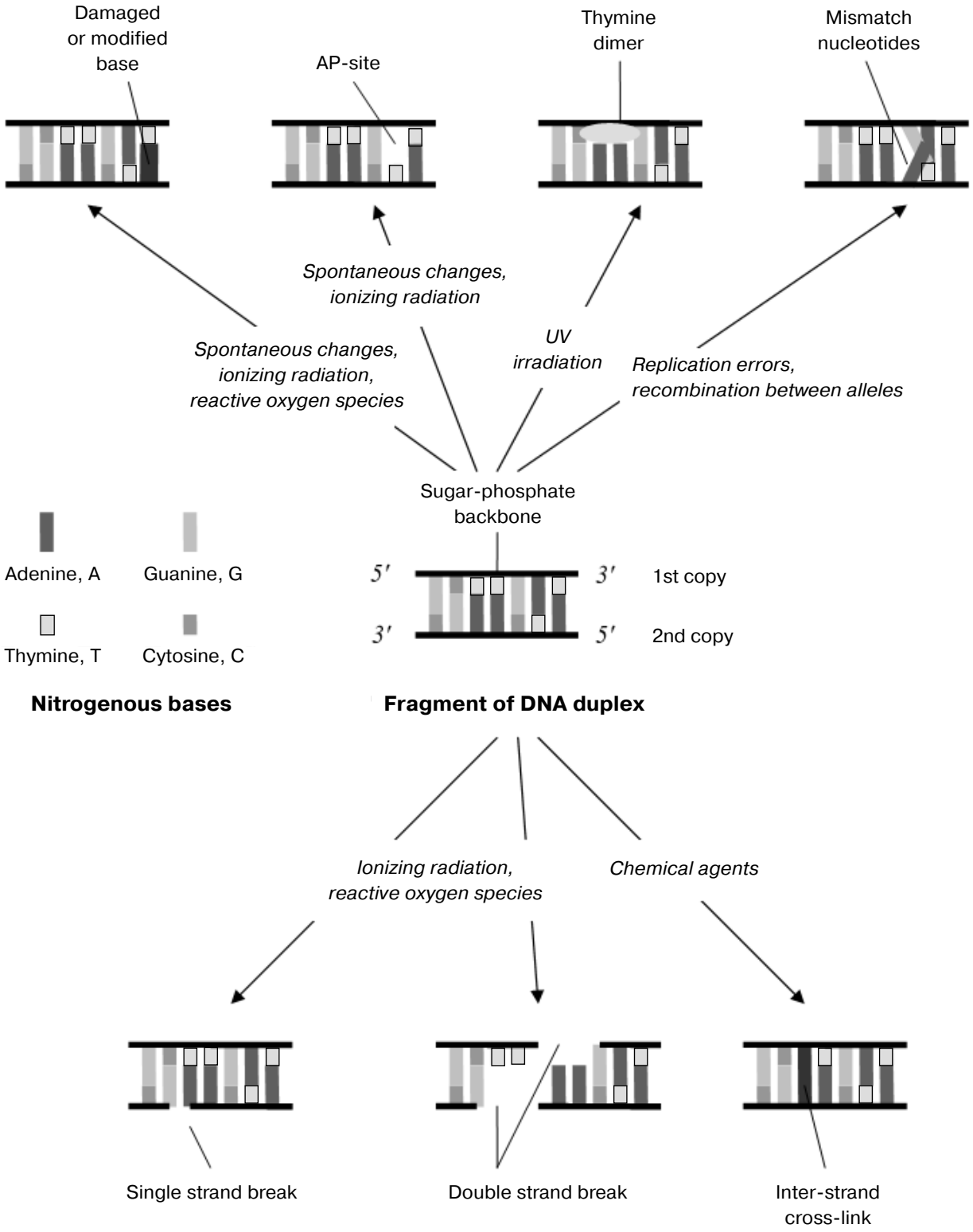


**Fig. 1.** Types of DNA damages.

with "diagnostics" of damage by one or several protein factors "arriving" at the sites of DNA damage. The next step usually includes removal (excision) of the damaged site; this involves a series of enzymatic reactions. After the excision, DNA polymerase synthesizes the "right" fragment and DNA ligase finishes repair process by joining DNA fragments into the whole strand. This is the common scenario of repair of various DNA damages.

## BASE EXCISION REPAIR

Most damages in DNA structure (modified and damaged bases, AP-sites, and single strand breaks) are removed by means of base excision repair (BER). This explains why this type of repair is the most studied. There are two pathways of BER, "short-patch" BER and "long-patch" BER. The former involves excision of only one (damaged) nucleotide, whereas the latter involves excision of a longer DNA stretch, which may include from 2 to 10 nucleotides [8-10] (Fig. 2).

What factors determine selection of the BER pathway? First of all, it is the type of DNA damage. Repair of modified bases, such as 8-oxoG, uracil, and also normal AP-sites usually involves short-patch BER [10]. Abnormal AP-sites (reduced or oxidized) and single-strand breaks are repaired via long-patch BER [11-13]. Removal of hypoxanthine, the product of adenine deamination, may involve either pathway of BER. The cellular level of DNA polymerases is another crucial factor influencing selection of the BER pathway. For example, wild type mouse embryonal fibroblast cell lines preferentially repair AP-sites via short-patch BER (in 80% of cases), whereas cells with a damaged gene encoding DNA polymerase β repair AP-sites only via the long-patch pathway [10].

**Short-patch BER.** The first step of the short-patch BER includes recognition and removal of modified base by corresponding DNA N-glycosylase. This step leaves deoxyribophosphate, a structure similar to an AP-site. The next step includes nicking the sugar-phosphate backbone by AP-endonuclease type II at the 5′-end of this intermediate and removal of the 5′-terminal residue of deoxyribose-phosphate. The resulting gap (of one nucleotide) is correctly filled in by DNA polymerase and the break is linked by DNA ligase [8]. Good evidence exists that DNA polymerase β of low processivity is involved into short-patch BER. This was proved by: 1) studies of uracil repair in mammalian cell extracts in the presence of various DNA polymerase inhibitors [14]; 2) fractionation of cell extracts resulted in separation of activities repairing AP-sites or uracil [15-17]; 3) BER sensitivity to antibodies against DNA polymerase β [16, 18]; 4) reconstitution of repair processes using purified enzymes [19]; 5) mutation analysis [20]. DNA polymerase β plays a dual role in short-patch BER: it removes the 5′-terminal deoxyribose-phosphate residue of the AP-site via β-elimination due to AP-lyase (5′-deoxyribose-phosphatase) activity [21] and fills the gap of one nucleotide due to DNA polymerase activity. It was recently shown that short-patch repair of DNA containing uracil *in vitro* may involve recently discovered DNA polymerase λ. Besides DNA polymerase activity this enzyme exhibits 5′-deoxyribose-phosphate lyase (5′-dRp-lyase) but not AP-lyase activity [22]. This activity of DNA polymerase λ removes nucleotide residues containing modified bases (but not AP-sites as DNA polymerase β does). It is possible that in the case of necessity the cell can employ both types of DNA polymerases for both types of short-patch BER.

Replacement of one nucleotide gap by DNA polymerase β or λ by short-patch BER is completed with ligation of the break by DNA ligase I [23] or complex of protein Xrcc1 and DNA ligase III [24, 25]. Protein Xrcc1 lacks catalytic activity, but it binds to repair proteins such as DNA polymerase β and poly(ADP-ribose) polymerase (PARP) and promotes involvement of these proteins into BER [26, 27]. What is the putative role of PARP in short-patch repair? Perhaps PARP is a sensor recognizing DNA nicks [12]. Short-patch BER requires coordinative interactions between all protein components of this process. Undoubtedly, such concerted action significantly facilitates the repair process. Interaction between DNA polymerase β and AP-endonuclease and between DNA polymerase β and DNA ligase I has also been demonstrated [12, 26, 28]. Kinetic studies proved that AP-endonuclease accelerates removal of 5′-terminal deoxyribose-5′-phosphate by DNA polymerase β [28].

**Long-patch BER.** Certain kinds of DNA damage (such as reduced and oxidized AP-sites, single strand breaks) can be repaired only by long-patch BER. In some cases repair of modified bases and normal AP-sites also involves long-patch BER. Repair of single strand breaks obviously begins with PARP [29]. The authors proposed the following model for functioning of this protein. PARP recognizes a break and binds to DNA at the damaged site. This activates synthesis of poly(ADP-ribose) at a specific site of PARP; thus PARP undergoes automodification. The latter decreases PARP affinity to DNA and the damaged site becomes accessible to repair enzymes, which are obviously directed by PARP. Regeneration of the modified PARP includes degradation of poly(ADP-ribose) by a specific glycohydrolase.

Modified base long-patch BER (as well as short-patch BER) begins with corresponding DNA N-glycosylase, and repair of the AP-site involves AP-endonuclease [12]. It is suggested that these enzymes also act as factors recognizing such damages. After DNA nicking by AP-endonuclease, FEN1 nuclease removes from 2 to 10 nucleotides from the 5′-side of the AP-site [8, 11]. For repair of reduced and oxidized AP-sites this is the only possible way because abnormal AP-sites are not substrates

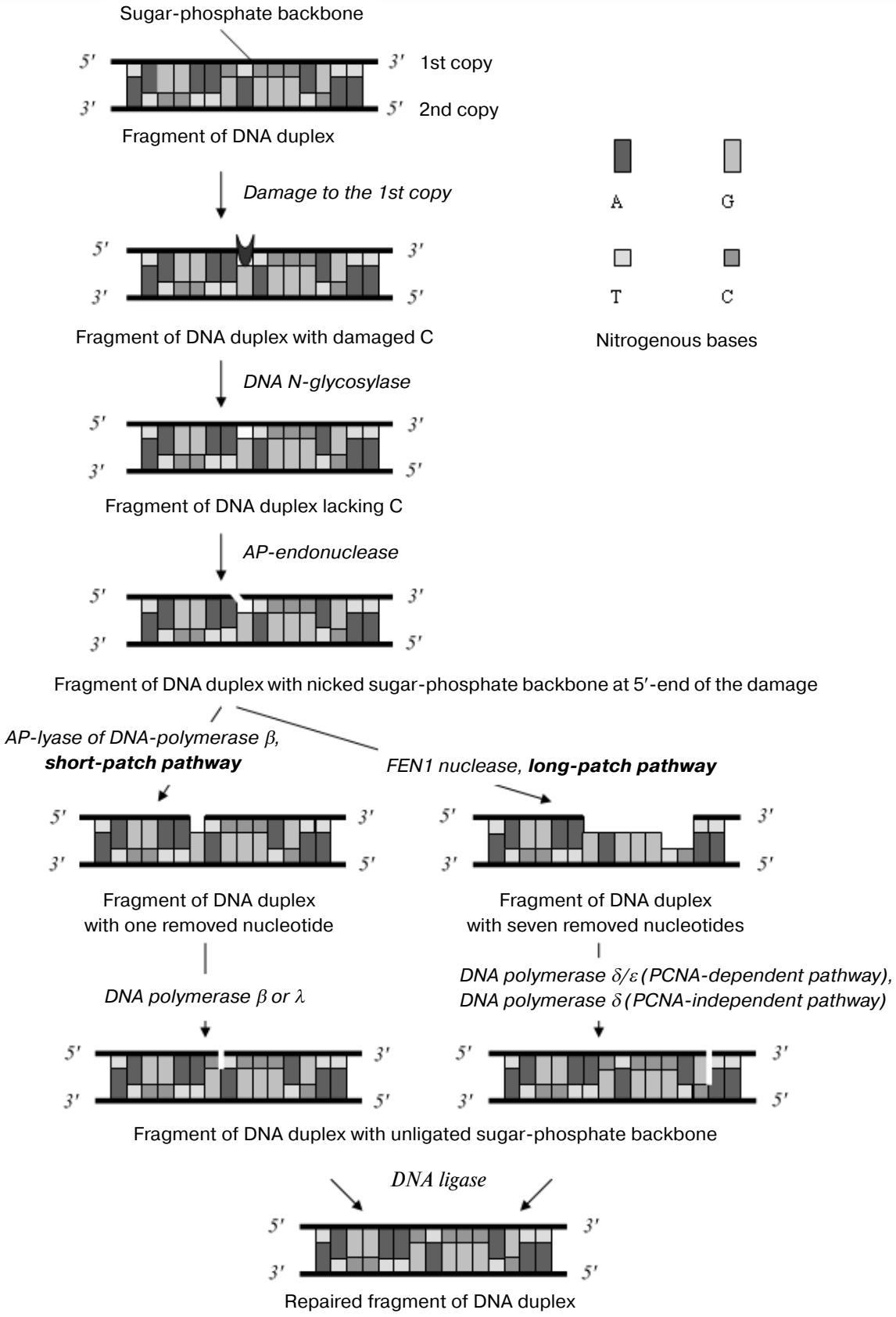


**Fig. 2.** Short-patch and long-patch pathways of base excision repair.

for 5′-phosphodiesterase activity of DNA polymerase β. Why are modified AP-sites not cleaved by DNA polymerase β as in the case of short-patch BER? Recently a mechanism for interaction between DNA polymerase β and oxidized AP-site has been proposed [30]. Oxidized AP-site appears as the result of effects of oxidative stress or radiation. Free radicals attack C1 carbon atom and this results in formation of 2-deoxyribonolactone (dL). Although AP-endonuclease could nick dL-containing DNA, subsequent dL excision by DNA polymerase β was strongly inhibited compared with normal AP-sites. This inhibition was accompanied by accumulation of cross-links between DNA and DNA polymerase β. Such cross-links were not detected in the case of unmodified AP-sites, but they were formed during reduction by sodium borohydride. Cross-linking of protein with dL involved Lys72 forming a Schiff base with the C1 aldehyde during removal of an unmodified AP-site. In the presence of dL, attack of C1 lactone by Lys72 occurs slower and apparently produces an amide compound. This explains why oxidized AP-sites cannot be removed via β-elimination by DNA polymerase β in the process of short-patch BER. They are removed by FEN1 in long-patch BER.

FEN1 is a nuclease exhibiting dual activity. It acts as: 1) 5′→3′-exonuclease cleaving mononucleotides and short oligonucleotides from the 5′-end of one strand of DNA duplex [31]; 2) endonuclease cleaving single strand "branches", known as "flap"-structures, possessing free 5′-end in DNA duplex [32]. The name FEN1 is an abbreviation reflecting the presence of both exo- and endonuclease activities: 5(**f**ive)′-**e**xo-**n**uclease and **f**lap-**e**ndo-**n**uclease [33]. Do exo- and endonuclease activities of FEN1 reflect different catalytic properties or does this enzyme exhibit the same activity with different substrates (double strand DNA with nick containing phosphorylated 5′-end and flap structure with free 5′-end which is not necessarily phosphorylated)? Certain evidence exists that the second suggestion is correct [33]. The 5′-OH end of a 5′-flap-structure is a substrate, which binds to FEN1, but 5′-OH end within a nick in a DNA duplex is not equivalent to that. Authors suggest that electrostatic pulsation of 5′-terminal phosphate within nick (FEN1 substrate) may create "breathing" 5′-end, which may acquire the configuration of a pseudo flap structure. It is also known that a flap one nucleotide in length is a substrate effectively utilized by FEN1 [34]. Anyway, both double stranded DNA and single strand flap structures are substrates for FEN1. This suggests the existence of two mechanisms employing FEN1 for removal of damaged DNA sites: 1) FEN1 forms gaps in DNA duplex which are filled by DNA polymerases; 2) FEN1 removes flap structures formed during preliminary DNA synthesis with strand displacement.

Which DNA polymerases are involved in DNA synthesis in long-patch BER? Experiments with model systems reconstituting long-patch repair of AP-sites using purified human cell proteins revealed the involvement of PCNA-dependent DNA polymerases δ and ε in reparative DNA synthesis [9, 35]. These DNA polymerases exhibit high processivity in the presence of PCNA (proliferating cell nuclear antigen); this protein retains polymerases on a DNA molecule until they synthesize a strand of requested length. Maximal length of newly synthesized strand reaches 7 [35] or 10 [9] nucleotides. In most cases, the length of newly synthesized DNA fragment does not exceed 2 [35] or 2-4 [9] nucleotides.

Certain evidence exists that DNA polymerase β may be involved in long-patch repair of AP-sites [11] and uracil containing DNA [36] as the enzyme synthesizing DNA. In long-patch BER, DNA polymerase β synthesizes DNA fragments of 2 to 6 nucleotides in length [11] in spite of low processivity of this enzyme on primer-templates and insensitivity to stimulation by PCNA. Involvement of DNA polymerase β becomes possible due to stabilization of enzyme–DNA complex by binding of the catalytic deoxyribose-phosphate lyase center of this enzyme to 5′-phosphate limiting a single strand gap [21, 37]. Recent data also suggest that newly discovered DNA polymerase λ may also fill small gaps (up to 5 nucleotides) in processive manner and possibly participates in reparative DNA synthesis during long-patch BER [38]. It is possible that DNA polymerase λ employs the same mechanism filling gaps as DNA polymerase β.

In eukaryotic cells, there is another pathway of long-patch BER employing DNA polymerase δ but without involvement of PCNA. Studying DNA repairs in embryonal development of bonefishes we found that in model systems DNA polymerase δ may fill rather long gaps (up to 10-13 nucleotides) in a processive manner. In spite of the absence of a deoxyribose-phosphatase catalytic site this enzyme forms tight complexes with DNA duplexes containing gaps of ~10 nucleotides due to its binding to the 5′-terminal site limiting the gap [39, 40]. We also confirmed involvement of DNA polymerase δ in long-patch BER during embryonal development of bonefishes in *in vivo* experiments studying the effect of oxidative stress on loach embryos. Exposure of embryos to treatment with superoxide radicals, generating mainly single strand breaks in DNA, was accompanied by increase in activity of DNA polymerase δ but not of replicative DNA polymerase α [41]. The described mechanism of DNA repair is especially important if we take into consideration that the long-patch pathway is responsible for removal of most DNA defects. This mechanism is unique because it can operate under extreme conditions at low level of PCNA and also under deficit of PCNA-independent DNA polymerases β and λ in the cell.

Long-patch BER is finished with the linking of DNA fragments with DNA ligase I [9, 35].

By analogy with short-patch BER, regulation of long-patch BER involves coordinated protein–protein interactions. For example, AP-endonuclease may interact with FEN1 [42]. This interaction activates by 1.5-2-

fold removal of reduced 5′-terminal AP-site by FEN1. Interestingly, PCNA can interact with all enzymes involved in long-patch BER. Besides the activating effect of PCNA on DNA polymerases δ and ε, PCNA can also stimulate FEN1 nuclease [11, 33, 43-47] and bind AP-endonuclease [42], DNA-ligase I [44, 48-50], DNA N-glycosylases [49, 51], DNA polymerase β [52], and DNA polymerase λ [53]. It was also demonstrated that PCNA binding to DNA polymerase δ, FEN1, DNA-ligase I, and two DNA N-glycosylases (UNG2 and MYH) involves the same consensus sequence found in these proteins [49]. It is suggested that PCNA plays a role of molecular adaptor facilitating interaction of these enzymes with sites of DNA repair. However, it should be noted that an activating effect of PCNA on some of these enzymes (AP-endonuclease, DNA N-glycosylase, DNA polymerases β and λ) has not been found.

## NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair (NER) is an effective tool for removal of cross-links formed between neighboring pyrimidines and various damages that form abnormal "overhangs" or impair helical structure [8]. The inherited disease xeroderma pigmentosum (XP) is related to impairments in NER. Patients with this disease are predisposed to altered pigmentation on open parts of their skin (susceptible to sunlight) and skin cancer. The mechanism of these impairments is well studied [3, 54, 55]. Seven protein products of *XP* genes (XPA-XPG) are involved in recognition and excision of damaged fragments from DNA. Mutation in one of these genes can result in the appearance of XP.

Figure 3 shows the scenario of removal of thymine dimers formed after ultraviolet treatment. What is the role of XPA-XPG proteins in this process? XPA protein recognizes DNA damage, interacts with other XP-proteins, and promotes their positioning at the site of DNA damage. XPB and XPD proteins are components of transcription factor IIH (TFIIH), which unwinds DNA at the site of subsequent excision. XPC protein promotes TFIIH stabilization at the damaged site, protein XPF nicks the 24th phosphodiester bond at the 5′-end of the damage, whereas XPG nicks the fifth phosphodiester bond at the 3′-end of the DNA damage [54]. The concerted action of these nucleases, called excinucleases, results in a gap formation of ~30 nucleotides in length.

Which DNA polymerases fill this gap? Inhibitory analysis has revealed that in the case of NER DNA polymerase δ or ε are involved in repair synthesis [56-60]. Using mammalian cell extracts, it was demonstrated that reparative synthesis of DNA in NER also requires PCNA [61, 62]. This is not surprising because processive filling the gap of 30 nucleotides by DNA polymerase δ is impossible without involvement of a processivity factor. *In vivo* experiments revealed that cell response to UV treatment includes redistribution of PCNA (without changes in its total amount) [63-65]. Besides PCNA, reparative synthesis by DNA polymerase δ also requires replication factor C (RFC), whereas gap filling by DNA polymerase ε is optimal in the presence of PCNA, RFC, and replication protein A (RPA) [66].

Figure 4 shows a functional complex of RFC, PCNA, and DNA polymerase δ. Assembly of this complex occurs in a sequential manner. In the presence of ATP RFC (exhibiting ATPase activity) binds at a DNA site limiting a gap from the 5′-end. PCNA binds to RFC, and this results in initial formation of unstable complex RFC–DNA [67]. DNA and PCNA stimulate ATPase activity of RFC [68, 69]. ATP hydrolysis is accompanied by a conformation change in RFC from U-shaped (closed bi-finger) structure to C-shaped (open) structure, which retains PCNA between "opened fingers" [70]. The next step consists of ATP-dependent threading of PCNA onto DNA duplex due to transient opening of the PCNA ring. The resultant stable complex binds DNA polymerase δ, which effectively fills the long gap [67]. The mechanism of formation of functionally active complex containing DNA polymerase ε is similar to that we have just considered.

The role of PCNA in NER is not limited to stimulation of DNA polymerase activity. PCNA also binds XPG endonuclease; mutant forms of such endonuclease cannot bind PCNA and cannot carry out repair [71]. NER is completed by linking of DNA fragments catalyzed by DNA ligase [8].

It should be noted that NER occurs much faster in cells with actively transcribing genes [54]. DNA repair in such cells is known as nucleotide excision repair coupled to transcription. Besides the above-mentioned protein, this type of repair also involves CSA and CSB proteins. Mutations in their genes cause an inherited disease known as Cockayne's syndrome, which is characterized by delayed growth, appearance of cataract, dental caries, photosensitization, and dermatoses [54]. A putative repair mechanism employing CSA and CSB might include the following scenario: during transcription, RNA polymerase II meets DNA damage and binds CSA and CSB proteins. These proteins promote rapid positioning of NER enzymes at this site of DNA; this leads to DNA repair followed by subsequent continuation of transcription [72].

## REGULATION OF EXCISION REPAIR

PCNA and protein p53 are the major cell regulators responsible for cell fate. How are mechanisms underlying effects of PCNA and p53 related to each other? Cell response to DNA damage includes p53-dependent induction of a gene encoding p21, a protein inhibitor of

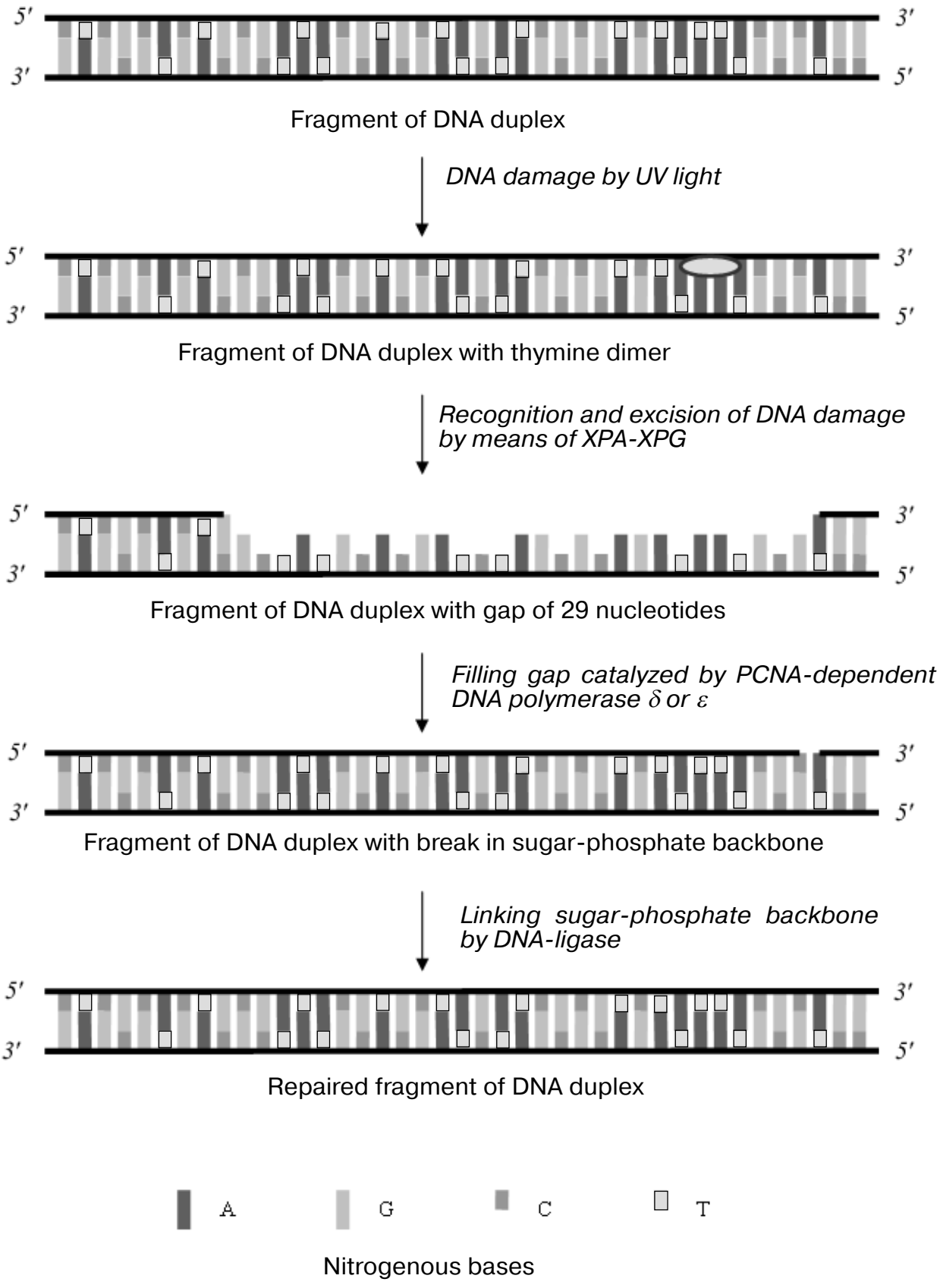


**Fig. 3.** Nucleotide excision repair.

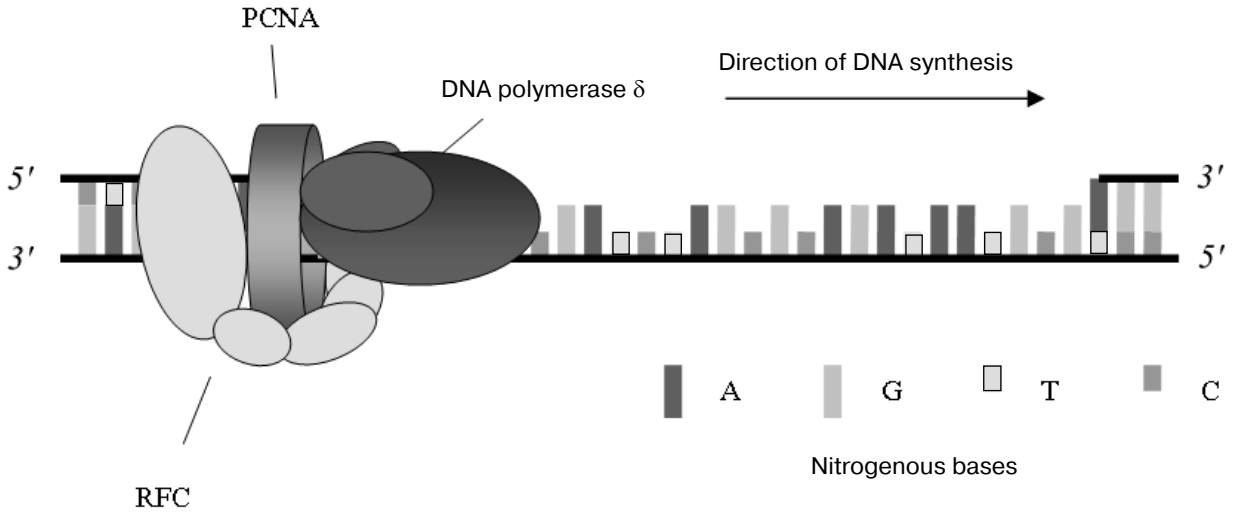


**Fig. 4.** Functionally active repair complex containing RFC, PCNA, DNA polymerase δ, and DNA with gap. DNA polymerase δ consists of four subunits with molecular masses of 125, 68, 55, and 12 kD. PCNA consists of three identical subunits with molecular mass of 36 kD, which form a ring structure; RFC consists of five subunits with molecular masses of 140, 40, 38, 37, and 36 kD.

cyclin-dependent kinases. Inhibition of DNA replication and cell cycle progression by p21 "gives" the cell a possibility for DNA repair [73, 74]. Inhibition of DNA replication by p21 includes blockade of activation of DNA polymerase δ and FEN1 and by PCNA [73, 75-77]; the mechanism of p21 action involves competition with these enzymes for the same binding site on the PCNA molecule [44, 77]. Interestingly, that this does not cause inhibition of the NER process, accompanied by PCNA-dependent synthesis of DNA by DNA polymerase δ [74, 78]. How is this possible? First, p21 prevents PCNA threading onto DNA duplex; second, p21 prevents binding of DNA polymerase δ to PCNA, which has already been bound to DNA [75]. The authors suggest that necessity of longer interaction between DNA polymerase δ and PCNA in replication than in repair may be accompanied by higher sensitivity to uncoupling effect of p21. However, from our viewpoint, the different effect of p21 on PCNA-dependent replication and PCNA-dependent repair *in vivo* could be logically explained also by redistribution of PCNA on DNA induced by various damaging treatments (e.g., UV irradiation). Excess of PCNA localized in damaged regions of DNA obviously neutralizes inhibitory effect of p21. Data on blockade of DNA repair by p21 in cell extract of HeLa cells exposed to UV and abolishing of this blockade by PCNA addition [79] seems to support this hypothesis.

Protein p21 inhibits PCNA-dependent BER *in vitro* [80]. The authors suggest that increased content of p21 *in vivo* may cause a switch of reparative synthesis by DNA polymerase δ for synthesis by p21-insensitive DNA polymerase β provided that this effect of p21 exists not only in *in vitro* systems but also *in vivo*. Our results indicate a possible switch of PCNA-dependent BER in this case for PCNA-independent process catalyzed by the same DNA polymerase δ [39, 40].

It should be noted that p21 plays another important role during DNA damage; p21 binding to PCNA prevents "non-planned" DNA hypermethylation by DNA-(cytosine-5) methyl transferase (MCMT) [81]. Under normal conditions, MCMT methylates newly synthesized DNA and this methylation occurs very rapidly, before DNA packaging into nucleosomes and inclusion of histone H1 into them (H1 later inhibits DNA methylation [81]). Rapid DNA methylation obviously involves PCNA, which binds to MCMT and facilitates attachment of this enzyme to DNA (without any influence on its catalytic activity). In the case of DNA damage p21 arrests methylation by competition with MCMT for PCNA during DNA repair.

Proteasome 26S, a multisubunit protein complex involved in ubiquitin-dependent protein degradation [82], is one of the regulators of reparative processes in cells. For example, proteasome 26S degrades XPC [83], which plays a certain role in NER (see section "Nucleotide excision repair"). Degradation of enzymes and factors involved in removal of DNA damages causes inhibition of repair. This usually occurs after restoration of DNA structure and has certain biological sense. Proteasome 26S or its constituent, 19S regulatory subunit, may function as molecular chaperones, which help proteins to acquire requested conformation. In this case, proteasome stimulates repair.

In the case of irremovable DNA damages, the cell may inhibit repair processes. Clearly, serious damages will drive the cell to trigger cell death by inhibiting replication

and repair. This may avoid carcinogenesis and protein p21 may simultaneously interrupt replication and DNA repair [80]. It is possible that proteasome also participates in this process; a putative role of proteasome consists of elimination of replicative and reparative proteins.

## MISMATCH REPAIR

Mismatch repair (MMR) is for correction of replication errors and for removal of unpaired nucleotides from heteroduplexes formed during allele recombination [8, 84, 85]. The simplest way for correction of replication error consists of immediate removal of wrongly inserted nucleotide by means of 3′→5′-exonuclease activity of DNA polymerases δ and ε [39, 86, 87]. The second way of MMR includes enzymes involved in excision repair and a group of additional proteins. Special proteins participating in this type of DNA repair are homologs of proteins Mut S and Mut L of *Escherichia coli*. Such proteins recognize unpaired nucleotide or loops including up to four nucleotides [8, 85]. Mutations in human genes encoding these proteins cause predisposition to cancer diseases [85]. Nucleases (possibly) including excision repair nucleases excise unpaired fragment of DNA strand of 100-1000 nucleotides in length. Excision occurs in both directions (from both 3′-and 5′-sides of the unpaired site) [85].

Which DNA polymerases fill forming gaps? There is limited information about DNA polymerases involved in DNA synthesis during MMR compared with information of DNA polymerases involved in excision repair. Since MMR is accompanied by formation of continuous gaps in a DNA strand, it is relevant to suggest that these gaps are filled by DNA polymerases capable of processive DNA synthesis (such as DNA polymerases δ and ε). Inhibition of DNA repair synthesis in human cell extracts by aphidicolin [88] supports this suggestion. However, these data do not rule out involvement of DNA polymerase α in MMR. *In vitro* study of MMR by purified human cell fractions in the presence of components of nuclear extracts lacking repair activity revealed involvement of fractions enriched with DNA polymerase δ in MMR [89]. However, the presence of DNA polymerases α and ε in nuclear extracts does not rule out the possibility of their involvement in MMR under these conditions. Thus, information on the involvement of DNA polymerases in MMR is not sufficient to get a clear picture on the role of these enzymes in MMR.

It should be noted that like excision repair, MMR depends on PCNA. It is clear that the latter is required for DNA synthesis by PCNA-dependent DNA polymerases δ and ε. Earlier stages of MMR preceding DNA synthesis also require this protein. For example, PCNA interacts with homologs of Mut S and Mut L proteins including MSH2, MLH1 [90], MSH3, MSH6 [91], and heterodimers MSH2–MSH3 and MSH2–MSH6 [92, 93]. It is possible that such interaction is required for recognition of DNA strand that should be repaired [90]. It is also possible that PCNA is required for FEN1 activation because certain evidence exists that FEN1 homolog is involved in MMR in *Schizosaccharomyces pombe* [94].

## DOUBLE STRAND BREAK REPAIR

Double strand breaks may be induced by various factors (e.g., ionizing radiation, mechanic stress) or in cases when replicative DNA polymerases meet a single strand break in a DNA molecule. Double strand breaks can exist in DNA molecules as intermediates during normal biological processes such as recombination in developing lymphoid cells [95, 96]. Lack of ability for double strand break repair results in genome destabilization, mutations, and appearance of malignant tumors. In some cases, double strand breaks induce apoptosis. In eukaryotes, two major pathways are responsible for repair of double strand breaks of DNA. These include homologous recombination (or recombination repair) and non-homologous end joining [13, 96, 97]. If homologous DNA duplex containing at least a sequence complementary to one broken end is available, recombination repair is possible. In *Saccharomyces cerevisiae* yeast, recombination repair depends on protein Rad52 and it is the dominating type of repair. Repair by non-homologous end joining is possible in *S. cerevisiae* in the case of mutations in *RAD52* [13]. In mammals repair of double strand breaks of DNA mainly involves non-homologous end joining. In *Drosophila* fly, both pathways equally contribute to double strand break repair [13].

**Recombination repair.** According to a model of recombination repair, protein Rad52 binds with overhanging short DNA ends formed during double strand breaks and protects them against exonucleases [98]. Rad52 also stimulates binding of protein Rad51 to broken DNA ends; this promotes "insertion" of the broken ends (or one of them) into homologous DNA duplex followed by recombination. Repair of double strand breaks via homologous recombination is accompanied by DNA synthesis. Mutation analysis revealed that in *S. cerevisiae* cells this repair pathway depends on processivity factors, PCNA and RFC, even if insertion of broken ends into homologous DNA duplex is accompanied by DNA synthesis including up to 30 nucleotides [99]. This synthesis involves PCNA-dependent polymerases δ and ε. However, quite unexpectedly, termination of repair of double strand breaks in a cell with mutant replication DNA polymerase α was inhibited. Using these data the authors proposed the following model: insertion of repaired broken ends into homologous DNA duplex creates a modified replication fork in which synthesis of leading and lagging strands occurs on a donor template.

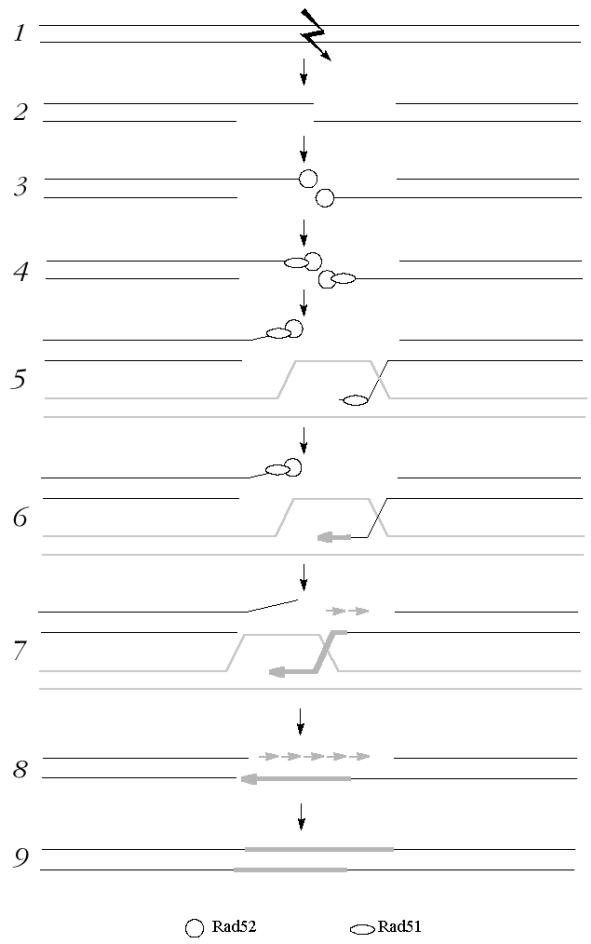


**Fig. 5.** Repair of double strand breaks by homologous DNA recombination in *S. cerevisiae*. Thin gray lines and bold gray lines designate homologous DNA duplex and newly synthesized DNA strand, respectively. (For convenience, only sugar-phosphate backbone without complementary bases is shown.) *1*) Action of damaging factors on DNA; *2*) formation of double strand breaks; *3*) Rad52 binding to overhanging ends of broken DNA duplex; *4*) Rad52 stimulation of Rad51 binding to broken ends of DNA duplex; *5*) insertion of one of the overhanging ends into homologous DNA duplex; *6*) onset of synthesis of leading strand of DNA by PCNA-dependent polymerase δ or ε. One strand (shown by thin gray line) is used as a template; *7*) synthesis of leading (solid bold gray line) and lagging (broken gray line) DNA strand in the resulting replication fork. The modified replication fork was formed by one of strand of the homologous duplex (lower thin gray line) and growing newly synthesized DNA strand (solid bold gray line). The leading strand is elongated by PCNA-dependent DNA polymerase δ or ε; synthesis of lagging strand involves DNA polymerase α and possibly PCNA-dependent DNA polymerase δ or ε; *8*) termination of DNA synthesis in the replication fork, removal of non-complementary branched site of DNA by nuclease; *9*) ligation of separate fragments of newly synthesized lagging strand by DNA ligase and termination of repair.

Replication terminates on reaching the second end of a double strand break. The other mechanism of homologous recombination accompanied by sequential synthesis of two leading strands in the fungus *Ustilago maydis* was also proposed [100]. Figure 5 summarizes literature data on repair of double strand breaks in *S. cerevisiae* by homologous recombination of DNA.

The other pathway of double strand break repair is related to homologous recombination. It includes annealing of single strand sites of DNA [101]. In this case, broken ends of DNA are subjected to degradation by specific exonuclease in both directions from the break until exposure of complementary sequences. This is accompanied by annealing complementary sites and eliminating non-homologous tails of DNA. This repair pathway also depends on Rad52 protein [98]. It should be noted that the first of these variants of repair recombination is conservative, whereas the latter is non-conservative due to loss of information contained in DNA sequences between annealed complementary sites.

**Non-homologous end joining.** Genetic and biochemical studies revealed proteins involved in repair of double strand breaks of DNA by non-homologous end joining. These include Ku protein, DNA-ligase IV complex, and protein product of *XRCC4* [102, 103]. These proteins are conservative in eukaryotes including yeast and mammals. This type of repair in vertebrates also requires DNA-dependent protein kinase (DNA-PK) [104, 105]. Although this involves direct linkage of broken ends (without any template), the repair process should be maximally accurate. Accuracy is achieved by Ku protein. This heterodimer comprises Ku70 and Ku80 subunits with molecular masses of 70 and 80 kD, respectively.

Recently, crystal structures of human heterodimeric Ku protein and of complex of Ku protein and DNA fragment of 55 nucleotides have been investigated [106]. Ku heterodimer forms a ring surrounding DNA duplex (Fig. 6). Although Ku protein does not contact DNA bases and forms only a small number of bonds with sugar-phosphate backbone, it spatially fits into the contours of major and minor grooves so that DNA helix is positioned in the protein ring in a certain manner. Such configuration of Ku–DNA complex is obviously needed for maintenance of structure of overhanging broken ends suitable for subsequent repair steps. Ku heterodimer not only aligns broken DNA ends but also plays the role of the factor recognizing double strand breaks and initiating their repair [107]. After binding to DNA, Ku protein then binds DNA-PK, which apparently plays a regulatory role [108, 109]. During the repair process, each broken end binds separate molecule of Ku heterodimer; the two heterodimers then associate with formation of a bridge, which binds other repair proteins [110]. Subsequent repair steps include filling gaps formed in the result of linkage of non-homologous DNA ends (this involves DNA polymerases), eliminating overhanging ends by nucleases (in the case of necessity) and linkage of repaired fragments by DNA ligase IV. Recently, DNA polymerase μ has been found in some eukaryotic species; this is an enzyme putatively responsible for filling DNA

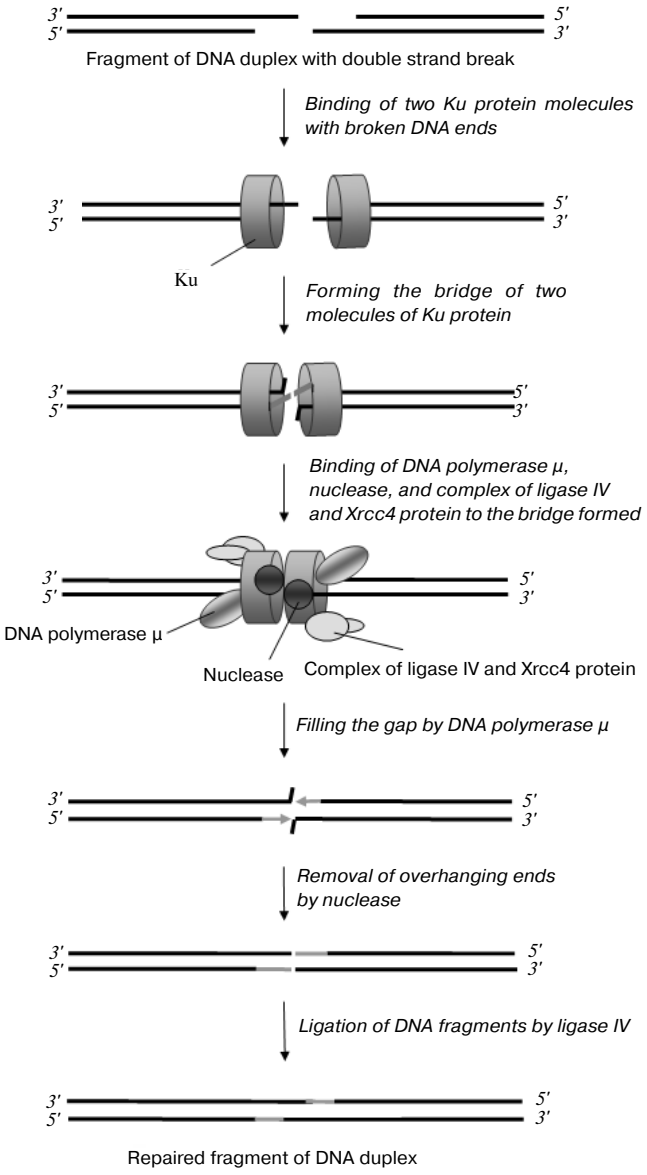


**Fig. 6.** Repair of double strand breaks by linking of non-homologous DNA ends. Newly synthesized DNA fragments are shown in gray.

gaps formed after non-homologous end joining during repair of double strand breaks [111].

## CROSS-LINK REPAIR

Some chemical agents, such as drugs for cancer chemotherapy (cisplatin, mitomycin, psoralen) induce formation of DNA cross-links [112]. Prokaryotes and eukaryotes can remove DNA cross-links but the mechanisms underlying this effect are not well understood. The most studied is cross-link repair in *E. coli*. Let us consider this type of repair in prokaryotes first; this will help better understanding of similar processes in eukaryotic cells. Genetic analysis revealed that removal of cross-links in *E. coli* involves reactions of nucleotide excision repair and also recombination [113]. Such proposed mechanism is based on experimental data obtained in *in vitro* systems using purified prokaryotic enzymes [114-117]. The initial step includes nicking one DNA strand on both sides of the damage by excision repair nuclease. This results in formation of an oligomer of 11 nucleotides bound to complementary strand with the cross-link [114]. This is a "recombinogenic" structure and RecA-dependent reaction forms triple strand intermediate with homologous third strand [115, 116]. This intermediate is recognized by components of excision repair system and in the second round (which includes double nicking) results in release of cross-linked oligomers [116, 117]. Thus, one strand is repaired by recombination, whereas the gap in the second strand is filled by DNA polymerase using the first strand as the template.

As in the case of *E. coli*, cross-link repair in *S. cerevisiae* depends on enzymatic systems of nucleotide excision repair and recombination [118-121]. However, mechanisms of cross-link repair in higher eukaryotes differ from those in yeast, and these mechanisms are less studied. It is known that nucleotide excision repair in man begins with nuclease excision repair, which represents coordinated activity of six repair factors (see section "Nucleotide excision repair"). One of these factors, heterodimer XPF-ERCC1, exhibiting endonuclease activity cuts DNA strand from 5′-end of the damage at a distance of 20 ± 5 nucleotides, whereas the other factor XPG cuts DNA strand from 3′-end at the distance of 6 ± 3 nucleotides [3, 55]. These nucleases are also involved in cross-link repair in human DNA. They cut two phosphodiester bonds on one DNA strand, but from one rather than from both sides of the damage [122]. This results in gap formation of 22-28 nucleotides near the 5′-side of the cross-link (Fig. 7).

Why in this case can excision repair nuclease not cut the DNA strand in the usual manner, on both sides of the damage? Some authors believe that this is due to the DNA structure in the region flanking the cross-link at the 3′-side. Unwinding of DNA duplex precedes DNA nicking. Unwinding at the 3′-side of the damage at the distance of 6 ± 3 nucleotides is impossible due to overlapping of the unwinding region with the cross-link, whereas initiation of unwinding is possible at the distance of 20 ± 5 nucleotides from the 5′-side of the damage. It is possible that the DNA duplex unwinds in the direction from 5′- to 3′-end of the excised oligonucleotide and terminates at the 5′-end of the cross-link. In the resultant "bubble" structure the XPG endonuclease active site is located directly at the 5′-end of the cross-link where the 3′-cut occurs; this is accompanied by a 5′-cut of about the 27th phosphodiester bond from the 5′-side of the cross-link catalyzed by XPF-ERCC1 [122]. Since effectiveness

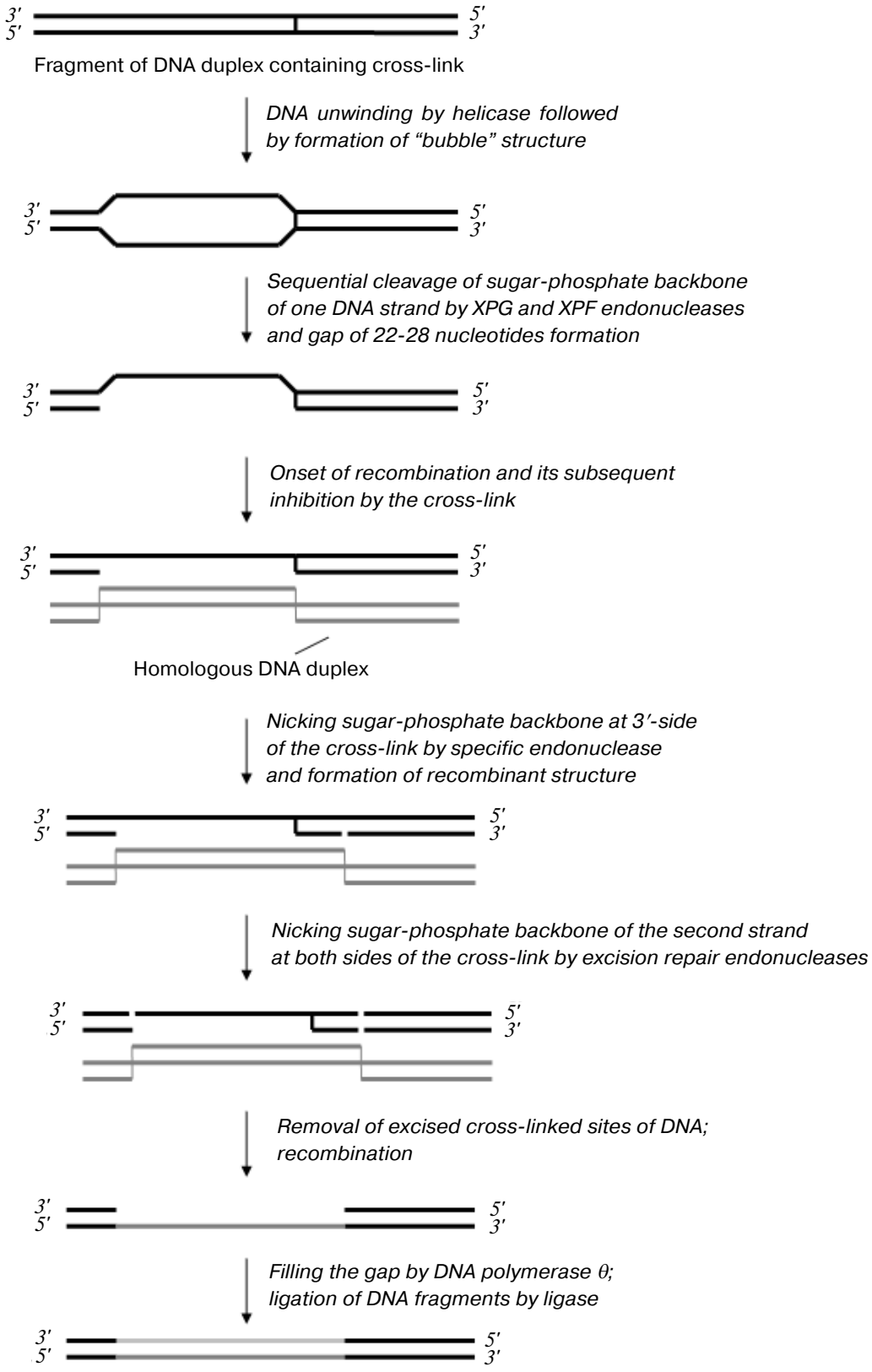


**Fig. 7.** Hypothetic scheme of DNA cross-link repair.

of gap formation at the 5′-side of the cross-link and removal of the DNA damage is nearly the same, some authors suggest that gap formation represents the first step in cross-link repair.

How does the gap of 22-28 nucleotides from the 5′-end of the cross-link in one DNA strand result in removal of such type of DNA damage? Authors have proposed a few hypothetical models for such repair.

1. The gap is filled by repair DNA-polymerases, but the nick cannot be ligated due to the cross-linked neighborhood. The second strand is subjected to the treatment by the same enzymatic system; this results in duplex formation with breaks at the 5′-sides of the cross-link in both strands. The cross-link is then removed by recombination.

2. Double nicking involves only one DNA strand. The forming gap initiates recombination, which is blocked at the stage of strand transfer. A specific endonuclease acts on the intermediate; this results either in nicking of the same strand from the other side of the cross-link (Fig. 7) or to nicking of the second strand. In the former case repair involves mechanisms similar to those in *E. coli*, and the latter case includes double strand break repair.

3. A gap in one of DNA strand leads to formation of a structure susceptible to exonuclease. The latter hydrolyzes a site of the same strand but from the other side of the gap. The extended gap contains a nucleotide cross-linked to the second chain. Such structure may be subjected to recombination or filling by DNA polymerases, which can bypass irremovable template damage.

Interestingly, a protein containing domains of helicase and DNA-polymerase has recently been found in higher eukaryotes [111]. Mutations in the gene encoding this protein significantly increased sensitivity of the organism to agents inducing DNA cross-links [13]. Since both helicase and DNA polymerase activities are required for a certain stage of repair related to recombination, the authors suggest involvement of this protein in DNA cross-link repair. This protein was named DNA polymerase θ [111].

Involvement of DNA polymerases in eukaryotic nuclear DNA repair

| DNA polymerase | Type of repair | Functions of DNA polymerases |
|---|---|---|
| α | mismatch repair (?) | synthesis of DNA primers on gaps of 100-1000 nucleotides (?) |
| | double strand break repair by homologous recombination | synthesis of lagging DNA strand primers in modified replication fork |
| β | base excision repair, short-patch pathway | AP-site removal, filling the gap of one nucleotide |
| | base excision repair, long-patch pathway | synthesis of DNA fragment of 2-6 nucleotides |
| δ and ε | base excision repair, long-patch pathway | synthesis of DNA fragment of 2-10 nucleotides |
| | nucleotide excision repair | filling a gap of ~30 nucleotides in length |
| | mismatch repair | filling a gap of 100-1000 nucleotides in length; mismatch repair due to 3′→5′ exonuclease activity |
| | double strand break repair by homologous recombination | DNA synthesis in modified replication fork |
| θ | cross-link repair | filling gap of several dozens of nucleotides in length |
| λ | base excision repair, short-patch pathway | removal of nucleotide with damaged base; filling one nucleotide gap |
| | base excision repair, long-patch pathway | synthesis of short DNA stretch of several nucleotides in length |
| μ | double strand break repair by non-homologous end joining | filling gaps of various length |

Thus, an overview of currently available data clearly indicates that not all DNA repairs have been studied in detail. The major attention has been given to studies of BER pathways. This is not surprising because this type of repair is often employed for both spontaneous DNA damage and damage induced by various chemical and physical factors. Significant progress achieved in recent years in studies of DNA-polymerases has revealed previously unknown BER pathways and filled some gaps in our understanding of the mechanisms responsible for double strand break repair and cross-link repair. The table summarizes information on functions of DNA-polymerases in various types of DNA repair.

## BYPASS OF IRREMOVABLE DNA DAMAGES

In spite of effective functioning of numerous mechanisms involved into eukaryotic DNA repair, cells cannot remove all types of DNA damage. In such cases replication is interrupted at damage sites of template and recently discovered DNA polymerases ζ [123], η [124, 125], κ [126, 127], ι [128, 129], μ [130], and REV1 [131, 132] continue DNA synthesis. DNA polymerases ζ, η, and μ can bypass thymine–thymine cyclobutane dimers induced by UV radiation [123, 124, 133]. DNA polymerases η and μ insert an AA pair opposite this damage. DNA polymerase η is the main DNA polymerase bypassing thymine–thymine dimers [134, 135]. The mechanisms of its action are now intensively studied [136-139]. It has been demonstrated that switch of DNA replication for synthesis versus thymine–thymine dimers catalyzed by DNA polymerase η involves its binding to monoubiquitinylated PCNA.

DNA polymerases κ [127], μ [133], η [125], and REV1 [131, 132] bypass such damages as 8-oxoG and AP-sites. DNA polymerases κ and REV1 insert mainly dAMP and dCMP, respectively, against all types of damage. DNA polymerase η inserts dCMP, dAMP, and dGMP at relative efficiency 100 : 56 : 14 against 8-oxoG; this enzyme inserts dGMP (or more rarely dAMP) against an AP-site. DNA-polymerase REV1 can bypass O(6)-methylguanine by inserting dCMP against it [132]. Interestingly, DNA polymerase ζ "works in cooperation" with DNA polymerase η [125] or REV1 [131], their concerted action elongating a bypassed damaged DNA site by several nucleotides.

Thus, involvement of these DNA polymerases in DNA synthesis at damaged template sites sometimes preserves DNA, but in other cases mutations appear ("error-free" and "error-prone" synthesis, respectively) [127]. DNA-polymerase REV1 is the most accurate enzyme among them; being a deoxycytidyl transferase, it is specific to G-templates (i.e., to DNA sites containing damaged dGMP residues) [131, 132].

Evidently, structural features of the above-considered DNA polymerases determine "bypassing possibility" in the case of DNA template damage. For example, study of crystal structure of DNA-polymerase η revealed that domain "four fingers and thumb" of this enzyme are rather small and look like "debris" of corresponding domains of replication and repair DNA-polymerases; this allows DNA-polymerase η "bypassing" such damage as thymine–thymine dimers [140].

It should be noted that now DNA polymerases bypassing DNA damage attract much attention; cells require the enzymatic machinery, which can synthesize DNA in situations when repair is ineffective or even impossible and "necessary risk" of accumulation of mutations in such cases is quite justifiable.

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## REFERENCES

1. Lindahl, T., and Nyberg, B. (1972) *Biochemistry*, **11**, 3610-3618.
2. Sakumi, T., and Sekiguchi, T. (1990) *Mutat. Res.*, **236**, 161-172.
3. Sancar, A. (1996) *Annu. Rev. Biochem.*, **65**, 43-81.
4. Kohn, K. W. (1996) *Cancer Res.*, **56**, 5533-5546.
5. Dizdaroglu, M. (1992) *Mutat. Res.*, **275**, 331-342.
6. Arkin, M. R., Stemp, E. D. A., Pulver, S. K., and Barton, J. K. (1997) *Chem. Biol.*, **4**, 389-400.
7. Povirk, L. F., Houlgrave, C. W., and Han, Y.-H. (1988) *J. Biol. Chem.*, **263**, 19263-19266.
8. Wood, R. D., and Shivji, M. K. K. (1997) *Carcinogenesis*, **18**, 605-610.
9. Pascucci, B., Stucki, M., Jonsson, Z. O., Dogliotti, E., and Hubscher, U. (1999) *J. Biol. Chem.*, **274**, 33696-33702.
10. Dogliotti, E., Fortini, P., Pascucci, B., and Parlanti, E. (2001) *Progr. Nucleic Acid. Res. Mol. Biol.*, **68**, 3-27.
11. Klungland, A., and Lindahl, T. (1997) *EMBO J.*, **16**, 3341-3348.
12. Wilson, D. M., III, and Thompson, L. H. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12754-12757.
13. Sekelsky, J. J., Burtis, K. C., and Hawley, R. S. (1998) *Genetics*, **148**, 1587-1598.
14. Dianov, G., Price, A., and Lindahl, T. (1992) *Mol. Cell. Biol.*, **12**, 1605-1612.
15. Matsumoto, Y., Kim, K., and Bogenhagen, D. F. (1994) *Mol. Cell. Biol.*, **14**, 6187-6197.
16. Singhal, R. K., Prasad, R., and Wilson, S. H. (1995) *J. Biol. Chem.*, **270**, 949-957.
17. Nealon, K., Nicholl, I. D., and Kenny, M. K. (1996) *Nucleic Acids Res.*, **24**, 3763-3770.
18. Wiebauer, K., and Jiricny, J. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5842-5845.
19. Kubota, Y., Nash, R., Klungland, A., Schar, P., Barnes, D., and Linfahl, T. (1996) *EMBO J.*, **15**, 6662-6670.
20. Sobol, R. W., Horton, J. K., Kuhn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K., and Wilson, S. H. (1996) *Nature*, **379**, 183-186.
21. Matsumoto, Y., and Kim, K. (1995) *Science*, **269**, 699-702.

22. Garcia-Diaz, M., Bebenek, K., Kunkel, T. A., and Blanco, L. (2001) *J. Biol. Chem.*, **276**, 34659-34663.
23. Prasad, R., Singhal, R. K., Srivastava, D. K., Molina, J. T., Tomkinson, A. E., and Wilson, S. H. (1996) *J. Biol. Chem.*, **271**, 16000-16007.
24. Caldecott, K. W., Tucker, J. D., Stanker, L. H., and Thompson, L. H. (1995) *Nucleic Acids Res.*, **23**, 4836-4843.
25. Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., and Frosina, G. (1997) *J. Biol. Chem.*, **272**, 23970-23975.
26. Tomkinson, A. E., Chen, L., Dong, Z., Leppard, J. B., Levin, D. S., Mackey, Z. B., and Motycka, T. A. (2001) *Progr. Nucleic Acid Res. Mol. Biol.*, **68**, 151-164.
27. Dianova, I. I., Sleeth, K. M., Allinson, S. L., Parsons, J. L., Breslin, C., Caldecott, K. W., and Dianov, G. L. (2004) *Nucleic Acids Res.*, **32**, 2550-2555.
28. Bennett, R. A. O., Wilson, D. M., III, Wong, D., and Demple, B. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 7166-7169.
29. Satoh, M. S., and Lindahl, T. (1992) *Nature*, **356**, 356-358.
30. DeMott, M. S., Beyret, E., Wong, D., Bales, B. C., Hwang, J. T., Greenberg, M. M., and Demple, B. (2002) *J. Biol. Chem.*, **277**, 7637-7640.
31. Lindahl, T., Gally, J. A., and Edelman, G. M. (1969) *Proc. Natl. Acad. Sci. USA*, **62**, 597-603.
32. Harrington, J. J., and Lieber, M. R. (1994) *Genes Dev.*, **8**, 1344-1355.
33. Wu, X., Li, J., Li, X., Hsieh, C. L., Burgers, P. M., and Lieber, M. R. (1996) *Nucleic Acids Res.*, **24**, 2036-2043.
34. Harrington, J. J., and Lieber, M. R. (1994) *EMBO J.*, **13**, 1235-1246.
35. Matsumoto, Y., Kim, K., Hurwitz, J., Gary, R., Levin, D. S., Tomkinson, A. E., and Park, M. S. (1999) *J. Biol. Chem.*, **274**, 33703-33708.
36. Horton, J. K., Prasad, R., Hou, E., and Wilson, S. H. (2000) *J. Biol. Chem.*, **275**, 2211-2218.
37. Prasad, R., Beard, W. A., Chyan, J. Y., Maciejewski, M. W., Mullen, G. P., and Wilson, S. H. (1998) *J. Biol. Chem.*, **273**, 11121-11126.
38. Garcia-Diaz, M., Bebenek, K., Sabariegos, R., Domiguez, O., Rodriguez, J., Kirchhoff, T., Garcia-Palomero, E., Picher, A. J., Juarez, R., Ruiz, J. F., Kunkel, T. A., and Blanco, L. (2002) *J. Biol. Chem.*, **277**, 13184-13191.
39. Sharova, N. P., Abramova, E. B., Dmitrieva, S. B., Dimitrova, D. D., and Mikhailov, V. S. (2000) *FEBS Lett.*, **486**, 14-18.
40. Sharova, N. P., Abramova, E. B., Dmitrieva, S. B., Dimitrova, D. D., and Mikhailov, V. S. (2001) *Biochemistry (Moscow)*, **66**, 402-409.
41. Peskin, A. V., Sharova, N. P., Dimitrova, D. D., Stolyarov, S. D., and Filatova, L. S. (1997) *Dokl. Ros. Akad. Nauk*, **355**, 262-265.
42. Dianova, I. I., Bohr, V. A., and Dianov, G. L. (2001) *Biochem.*, **40**, 12639-12644.
43. Li, X., Li, J., Harrington, J., Lieber, M. R., and Burgers, P. M. (1995) *J. Biol. Chem.*, **270**, 22109-22112.
44. Jonsson, Z. O., Hindges, R., and Hubsdher, U. (1998) *EMBO J.*, **17**, 2412-2425.
45. Gary, R., Kim, K., Cornelius, H. L., Park, M. S., and Matsumoto, Y. (1999) *J. Biol. Chem.*, **274**, 4354-4363.
46. Gomes, X. V., and Burgers, P. M. J. (2000) *EMBO J.*, **19**, 3811-3821.
47. Tom, S., Henricksen, L. A., and Bambara, R. A. (2000) *J. Biol. Chem.*, **275**, 10498-10505.
48. Levin, D. S., Bai, W., Yao, N., O'Donnell, M., and Tomkinson, A. E. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12863-12868.
49. Matsumoto, Y. (2001) *Progr. Nucleic Acids Res. Mol. Biol.*, **68**, 129-138.
50. Tom, S., Henricksen, L. A., Park, M. S., and Bambara, R. A. (2001) *J. Biol. Chem.*, **276**, 24817-24825.
51. Otterlei, M., Warbrick, E., Nagelhus, T. A., Haug, T., Slupphaug, G., Akbari, M., Aas, P. A., Steinsbekk, K., Bakke, O., and Krokan, H. E. (1999) *EMBO J.*, **18**, 3834-3844.
52. Kedar, P. S., Kim, S. J., Robertson, A., Hou, E., Prasad, R., Horton, J. K., Lewis, M., and Wilson, S. H. (2002) *J. Biol. Chem.*, **277**, 31115-31123.
53. Maga, G., Villani, G., Ramadan, K., Shevelev, I., Tanguy Le Gac, N., Blanco, L., Blanca, G., Spadari, S., and Hubscher, U. (2002) *J. Biol. Chem.*, **277**, 48434-48440.
54. Sancar, A. (1995) *J. Biol. Chem.*, **270**, 15915-15918.
55. Wood, R. (1996) *Annu. Rev. Biochem.*, **65**, 135-167.
56. Dresler, S. L., and Frattini, M. K. (1986) *Nucleic Acids Res.*, **14**, 7093-7102.
57. Nishida, C., Reinhard, P., and Linn, S. (1988) *J. Biol. Chem.*, **263**, 501-510.
58. Hunting, D. J., Gowans, B. J., and Dresler, S. L. (1991) *Biochem. Cell. Biol.*, **69**, 303-308.
59. Popanda, O., and Thielmann, H. W. (1992) *Biochim. Biophys. Acta*, **1129**, 155-160.
60. Coverley, D., Kenny, M. K., Lane, D. P., and Wood, R. D. (1992) *Nucleic Acids Res.*, **20**, 3873-3880.
61. Shivji, M. K. K., Kenny, M. K., and Wood, R. D. (1992) *Cell*, **69**, 367-374.
62. Nichols, A. F., and Sancar, A. (1992) *Nucleic Acids Res.*, **20**, 3559-3564.
63. Celis, J. E., and Madsen, P. (1986) *FEBS Lett.*, **209**, 277-283.
64. Toschi, L., and Bravo, R. (1988) *J. Cell Biol.*, **107**, 1623-1628.
65. Jackson, D. A., Hassan, A. B., Errington, R. J., and Cook, P. R. (1994) *J. Cell. Sci.*, **107**, 1753-1760.
66. Shivji, M. K., Podust, V. N., Hubscher, U., and Wood, R. D. (1995) *Biochemistry*, **34**, 5011-5017.
67. Hubscher, U., Maga, G., and Podust, V. N. (1996) in *DNA Replication in Eukaryotic Cells* (DePamphelis, M. L., ed.) Cold Spring Harbor Laboratory Press, Plainview, N. Y., pp. 525-543.
68. Tsurimoto, T., and Stillman, B. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 1023-1027.
69. Yoder, B. L., and Burgers, P. M. (1991) *J. Biol. Chem.*, **266**, 22689-22697.
70. Shiomi, Y., Usukura, J., Masamura, Y., Takeyasu, K., Nakayama, Y., Obuse, C., Yoshikawa, H., and Tsurimoto, T. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 14127-14132.
71. Gary, R., Ludwig, D. L., Cornelius, H. L., MacInnes, M. A., and Park, M. S. (1997) *J. Biol. Chem.*, **272**, 24522-24529.
72. Donahue, B. A., Yin, S., Taylor, J.-S., Reines, D., and Hanawalt, P. C. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 8502-8506.
73. Waga, S., Hannon, G. J., Beach, D., and Stillman, B. (1994) *Nature*, **369**, 574-578.

74. Sionov, R. V., and Haupt, Y. (1999) *Oncogene*, **18**, 6145-6157.
75. Podust, V. N., Podust, L. M., Goubin, F., Ducommun, B., and Hubscher, U. (1995) *Biochemistry*, **34**, 8869-8875.
76. Chen, J., Chen, S., Saha, P., and Dutta, A. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 11597-11602.
77. Warbrick, E., Lane, D. P., Glover, D. M., and Cox, L. S. (1997) *Oncogene*, **14**, 2313-2321.
78. Li, R., Waga, S., Hannon, G. J., Beach, D., and Stillman, B. (1994) *Nature*, **371**, 534-537.
79. Pan, Z.-Q., Reardon, J. T., Li, L., Flores-Rozas, H., Legerski, R., Sancar, A., and Hurwitz, J. (1995) *J. Biol. Chem.*, **270**, 22008-22016.
80. Tom, S., Ranalli, T. A., Podust, V. N., and Bambara, R. A. (2001) *J. Biol. Chem.*, **276**, 48781-48789.
81. Chuang, L. S.-H., Ian, H.-I., Koh, T.-W., Ng, H.-H., Xu, G., and Li, B. F. L. (1997) *Science*, **277**, 1996-2000.
82. Abramova, E. B., Sharova, N. P., and Karpov, V. L. (2002) *Mol. Biol. (Moscow)*, **36**, 761-776.
83. Sweder, K., and Madura, K. (2002) *J. Biomed. Biotechnol.*, **2**, 94-105.
84. Kolodner, R. (1996) *Genes Dev.*, **10**, 1433-1442.
85. Modrich, P., and Lahue, R. (1996) *Annu. Rev. Biochem.*, **65**, 101-133.
86. Kunkel, T. A., Sabatino, R. D., and Bambara, R. A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 4865-4869.
87. Khare, V., and Eckert, K. A. (2002) *Mutat. Res.*, **510**, 45-54.
88. Fang, W. H., and Modrich, P. (1993) *J. Biol. Chem.*, **268**, 11838-11844.
89. Longley, M. J., Pierce, A. J., and Modrich, P. (1997) *J. Biol. Chem.*, **272**, 10917-10921.
90. Umar, A., Buermeyer, A. B., Simon, J. A., Thomas, D. C., Clark, A. B., Liskay, R. M., and Kunkel, T. A. (1996) *Cell*, **87**, 65-73.
91. Kleczkowska, H. E., Marra, G., Lettieri, T., and Jiricny, J. (2001) *Genes Dev.*, **15**, 724-736.
92. Johnson, R. E., Kovvali, G. K., Guzder, S. N., Amin, N. S., Holm, C., Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1996) *J. Biol. Chem.*, **271**, 27987-27990.
93. Clark, A. B., Valle, F., Drotschmann, K., Gary, R. K., and Kunkel, T. A. (2000) *J. Biol. Chem.*, **275**, 36498-36501.
94. Murray, J. M., Tavassoli, M., Al-Harithy, R., Sheldrick, K. S., Lehmann, A. R., Carr, A. M., and Watts, F. Z. (1994) *Mol. Cell. Biol.*, **14**, 4878-4888.
95. Pastink, A., Eeken, J. C., and Lohman, P. H. (2001) *Mutat. Res.*, **480/481**, 37-50.
96. Jackson, S. P. (2002) *Carcinogenesis*, **23**, 687-696.
97. Kanaar, R., Hoeijmakers, J. H., and van Gent, D. C. (1998) *Trends Cell. Biol.*, **8**, 483-489.
98. Van Dyck, E., Stasiak, A. Z., Stasiak, A., and West, S. C. (1999) *Nature*, **398**, 728-731.
99. Holmes, A. M., and Haber, J. E. (1999) *Cell*, **96**, 415-424.
100. Ferguson, D. O., and Holloman, W. K. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 5419-5424.
101. Lin, F.-L., and Sternberg, N. (1990) *Mol. Cell. Biol.*, **10**, 103-112.
102. Critchlow, S. E., and Jackson, S. P. (1998) *Trends Biochem. Sci.*, **23**, 394-398.
103. Haber, J. E. (2000) *Trends Genet.*, **16**, 259-264.
104. Gottlieb, T. M., and Jackson, S. P. (1993) *Cell*, **72**, 131-142.
105. Smith, G. C., and Jackson, S. P. (1999) *Genes Dev.*, **13**, 916-934.
106. Walker, J. R., Corpina, R. A., and Goldberg, J. (2001) *Nature*, **412**, 607-614.
107. Ramsden, D. A., and Gellert, M. (1998) *EMBO J.*, **17**, 609-614.
108. Baumann, P., and West, S. C. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 14066-14070.
109. Chen, S., Inamdar, K. V., Pfeiffer, P., Feldmann, E., Hannah, M. F., Yu, Y., Lee, J.-W., Zhou, T., Lees-Miller, S. P., and Povirk, L. F. (2001) *J. Biol. Chem.*, **276**, 24323-24330.
110. Cary, R. B., Peterson, S. R., Wang, J., Bear, D. G., Bradbury, E. M., and Chen, D. J. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 4267-4272.
111. Burgers, P. M. J., Koonin, E. V., Bruford, E., Blanco, L., Burtis, K. C., Christman, M. F., Copeland, W. C., Friedberg, E. C., Hanaoka, F., Hinkle, D. C., Lawrence, C. W., Nakanishi, M., Ohmori, H., Prakash, L., Prakash, S., Reynaud, C.-A., Sugino, A., Todo, T., Wang, Z., Weill, J.-C., and Woodgate, R. (2001) *J. Biol. Chem.*, **276**, 43487-43490.
112. Zwelling, L. A., Michaels, S., Schwartz, S., Dobson, P. P., and Kohn, K. W. (1981) *Cancer Res.*, **41**, 640-649.
113. Cole, R. S. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 1064-1068.
114. Van Houten, B., Gamper, H., Holbrook, S. R., Hearst, J. E., and Sancar, A. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8077-8081.
115. Cheng, S., van Houten, B., Gamper, H. B., Sancar, A., and Hearst, J. E. (1988) *J. Biol. Chem.*, **263**, 15110-15117.
116. Sladek, F. M., Munn, M. M., Rupp, W. D., and Howard-Flanders, P. (1989) *J. Biol. Chem.*, **264**, 6755-6765.
117. Cheng, S., Sancar, A., and Hearst, J. E. (1991) *Nucleic Acids Res.*, **19**, 657-663.
118. Jachymczyk, W. J., von Borstel, R. C., Mowat, M. R., and Hastings, P. J. (1981) *Mol. Gen. Genet.*, **182**, 196-205.
119. Magana-Schwencke, N., Henriques, J. A., Chanet, R., and Moustacchi, E. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1722-1726.
120. Saffran, W. A., Greenberg, R. B., Thaler-Scheer, M. S., and Jones, M. M. (1994) *Nucleic Acids Res.*, **22**, 2823-2829.
121. Dardalhon, M., and Averbeck, D. (1995) *Mutat. Res.*, **336**, 49-60.
122. Besshno, T., Mu, D., and Sancar, A. (1997) *Mol. Cell. Biol.*, **17**, 6822-6830.
123. Nelson, J. R., Lawrence, C. W., and Hinkle, D. C. (1996) *Science*, **272**, 1646-1649.
124. Johnson, R. E., Prakash, S., and Prakash, L. (1999) *Science*, **283**, 1001-1104.
125. Yuan, F., Zhang, Y., Rajpal, D. K., Wu, X., Guo, D., Wang, M., Taylor, J.-S., and Wang, Z. (2000) *J. Biol. Chem.*, **275**, 8233-8239.
126. Johnson, R. E., Prakash, S., and Prakash, L. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 3838-3843.
127. Zhang, Y., Yuan, F., Wu, X., Wang, M., Rechkoblit, O., Taylor, J.-S., Geacintov, N. E., and Wang, Z. (2000) *Nucleic Acids Res.*, **28**, 4138-4146.
128. Vaisman, A., and Woodgate, R. (2001) *EMBO J.*, **20**, 6520-6529.

129. Haracska, L., Johnson, R. E., Unk, I., Phillips, B. B., Hurwitz, J., Prakash, L., and Prakash, S. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 14256-14261.
130. Zhang, Y., Wu, X., Yuan, F., Xie, Z., and Wang, Z. (2001) *Mol. Cell. Biol.*, **21**, 7995-8006.
131. Nelson, J. R., Lawrence, C. W., and Hinkle, D. C. (1996) *Nature*, **382**, 729-731.
132. Haracska, L., Prakash, S., and Prakash, L. (2002) *J. Biol. Chem.*, **277**, 15546-15551.
133. Zhang, Y., Wu, X., Guo, D., Rechkoblit, O., Taylor, J. S., Geacintov, N. E., and Wang, Z. (2002) *J. Biol. Chem.*, **277**, 44582-44587.
134. Kannouche, P., and Stary, A. (2003) *Biochimie*, **85**, 1123-1132.
135. Goodsell, D. S. (2004) *Oncologist*, **9**, 108-109.
136. McCulloch, S. D., Kokoska, R. J., Masutani, C., Iwai, S., Hanaoka, F., and Kunkel, T. A. (2004) *Nature*, **428**, 97-100.
137. Watanabe, K., Tateishi, S., Kawasuji, M., Tsurimoto, T., Inoue, H., and Yamaizumi, M. (2004) *EMBO J.*, **23**, 3886-3896.
138. Kannouche, P. L., and Lehmann, A. R. (2004) *Cell Cycle*, **3**, 1011-1013.
139. Kannouche, P. L., Wing, J., and Lehmann, A. R. (2004) *Mol. Cell*, **14**, 491-500.
140. Trincao, J., Johnson, R. E., Escalante, C. R., Prakash, S., Prakash, L., and Aggarwal, A. K. (2001) *Mol. Cell*, **8**, 417-426.