Biochemical Pharmacology, Vol. 29, pp. 977–982.
Pergamon Press Ltd. 1980. Printed in Great Britain.

# COMMENTARY

## PREREPLICATIVE ERROR-FREE DNA REPAIR

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The concept of DNA repair ought to be clarified. If prereplicative excision of premutagenic or prelethal DNA damage is true repair, classification of post-replicative processes as repair mechanisms introduces confusion. For instance, SOS* decreases cell lethality but is a source of mutations. Even with regard only to the effect on lethality, the case is far from clear, since the DNA damage remains. What is repaired is not the parental DNA strand containing the lesion, which stays to cause troubles in succeeding generations, but gaps in the daughter strand.

Considering mutagenesis and carcinogenesis, SOS is on the side of the causes and not at all on the side of a repair which might help to avoid mutation or cancer. Two mechanisms leading to mutations are generally considered : (1) minor base modifications leading to mispairing; (2) major DNA damages inducing the error-prone SOS processes. In both cases, avoidance of mutation and cancer relies on prereplicative excision repair. Mutation frequency appears to depend on a competition between excision repair and DNA replication, either normal DNA replication leading to mispairing if it is a minor alteration, or induced error-prone SOS DNA replication when there is major DNA damage.

Our short review of recent developments in DNA repair will be devoted exclusively to systems involved in prereplicative excision.

### *DNA glycosylases*

DNA glycosylases were first described in bacteria. The enzyme hydrolyzes the glycosylic bond between a modified base and deoxyribose in DNA, leaving an AP site (apurinic/apyrimidinic site).

In 1974, Kirtikar and Goldthwait [1] described an enzyme activity in *Escherichia coli* which released $O^6$-methyl-guanine and 3-methyladenine from DNA treated with methylnitrosourea. The 3-methyladenine-DNA glycosylase was purified by Riazzudin and Lindahl [2], but the existence of a DNA glycosylase able to remove $O^6$-methylguanine from DNA has not been confirmed. DNA glycosylases removing uracil [3,4], hypoxanthine [5] and guanine with an opened imidazole ring [6] have also been found in *E. coli*. All these DNA glycosylases are different enzymes, coded by different genes, that do not need divalent cations for activity so that they can be assayed in the presence of EDTA.

DNA glycosylases have been characterized in other bacteria. Laval [7] has purified the 3-methyladenine-DNA glycosylase of *Micrococcus luteus*; the uracil-DNA glycosylase of the same bacterium has been found by Tomilin *et al.* [8], whereas the *Bacillus subtilis* enzyme was described by Cone *et al.* [9]. Haseltine *et al.* [10] have presented evidence showing that the UV endonucleases I and II of *M. luteus* are, at the same time, DNA glycosylases hydrolyzing the glycosylic bond of the 5′ pyrimidine of the dimer and phosphodiesterases hydrolyzing the phosphodiester bond between the two pyrimidines.

DNA glycosylases have also been described in mammals. Kühnlein *et al.* [11] found uracil–DNA glycosylase in human fibroblasts and the enzyme of calf thymus was isolated by Talpaert-Borlé *et al.* [12]. Brent [13] purified the 3-methyladenine-DNA glycosylase of human lymphoblasts.

3-Methyladenine was usually believed to have no effect by itself : it was not mutagenic, and the lethal effect was rather thought to be dependent on AP sites resulting from the rapid spontaneous loss of the alkylated purine. Studies on *E. coli tag* mutants, which are deficient in 3-methyladenine-DNA glycosylase, confirm that 3-methyladenine is not mutagenic, but the high sensitivity of *tag* mutants to methyl methanesulfonate indicates that 3-methyladenine is by itself a major lethal damage [14].

Without repair, deamination of cytosine to uracil is mutagenic since the complementary base of uracil is adenine while the complementary base of cytosine is guanine. Deamination of cytosine can be spontaneous [14,15] or caused by bisulfite, nitrous acid or ultraviolet irradiation. The uracil is very efficiently removed from DNA by the uracil-DNA glycosylase so that deamination of cytosine has a very low mutagenicity. A mutation in the *ung* gene, which codes for the uracil–DNA glycosylase in *E. coli*, leads to an increased mutation rate either spontaneously or under the influence of deaminating agents [14]. The importance of uracil-DNA glycosylase in keeping a low mutation frequency is indirectly supported by the work of Coulondre *et al.* [16] showing

* Abbreviations used: dUTP, deoxyuridine triphosphate; dTTP, deoxythymidine triphosphate; dCTP, deoxycytidine triphosphate; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate; EDTA, ethylenediaminetetraacetate; dUTPase, deoxyuridine triphosphatase; *tag*, *Escherichia coli* gene coding for the 3-methyladenine-DNA glycosylase; *ung*, *E. coli* gene coding for the uracil-DNA glycosylase; *dut*, *E. coli* gene coding for the dUTPase; *thy*, *E. coli* gene coding for the enzyme involved in thymine biosynthesis; *ada*, *E. coli* gene coding for the factor responsible for the disappearance of $O^6$-methylguanine from DNA; *recA*, *E. coli* gene coding for a protein needed for SOS processes and recombination; SOS, processes induced when DNA synthesis is blocked by a major DNA lesion.

that the hot spots of the *lacI* gene in *E. coli* correspond to 5-methylcytosine residues; the spontaneous deamination of 5-methylcytosine yields thymine which the uracil-DNA glycosylase does not eliminate even if this pyrimidine stands in front of a guanine.

Lindahl *et al.* [3] formulated the hypothesis that DNA contains thymine instead of uracil as found in RNA to resist the genetic drift resulting from the deamination of cytosine. Deamination of cytosine gives a uracil sitting in front of a guanine. The mispairing might be the signal for repair, but it is not recognized by the uracil-DNA glycosylase (enzymes correcting mispairing in nascent DNA are happily unable to act because both strands are methylated; they would not know which of the mispaired bases, uracil or guanine, must be replaced). Uracil-DNA glycosylase removes uracil from single-stranded or double-stranded DNA; when double-stranded DNA is the substrate, the uracil is released whatever the purine, adenine or guanine in the complementary strand. Since it is the cytosine deamination product which is recognized and removed by the enzyme, this product cannot be a normal DNA base; thymine, which is left untouched by the uracil-DNA glycosylase, is thus used to build DNA instead of uracil.

dUTP is normally present in cells along with dTTP so that uracil is incorporated into nascent DNA where it is hydrogen-linked to adenine of the template strand. But this uracil is quickly replaced by thymine : the uracil is removed by the DNA glycosylase and the resulting AP site is repaired as explained in another section; the first step of this repair is catalyzed by an AP endodeoxyribonuclease which cuts the DNA strand near the AP site. The fragmentation of nascent DNA observed by Okazaki *et al.* [17] thus derives from two causes : discontinuous synthesis but also excision of uracil.

The cell contains an enzyme, dUTPase, which hydrolyzes dUTP into PPi and dUMP, which is the precursor of dTTP, so as to keep a low dUTP/dTTP ratio. Mutations in gene *dut*, coding for this enzyme in *E. coli*, result in a higher intracellular concentration of dUTP, the production of much shorter Okazaki pieces and a higher recombination frequency [18]; the mature DNA, however, does not contain uracil [19]. Introduction of an additional *ung* mutation increases the length of the Okazaki pieces [20] and the DNA is found to contain up to 1% of uracil without any obvious effect on cell survival [21].

The importance of uracil excision in the fragmentation of nascent DNA has been explored *in vitro* by Olivera [22] who studied the replication of DNA on cellophane disks using *E. coli* lysates and the four usual precursors (dATP, dGTP, dCTP, dTTP) in the presence or absence of dUTP. In absence of dUTP, the leading strand is formed continuously and only the lagging strand is synthesized by pieces. The addition of dUTP results in the appearance of breaks in the leading strand and a greater fragmentation of the lagging strand. With the ratio dUTP/dTTP found in wild-type cells, the pieces of the two newly-synthesized strands cannot be distinguished on sedimentation analysis. The effect of dUTP addition is much lower if a lysate from an *ung* mutant is used [23].

The thymineless death of *thy* mutants apparently results from an excessive fragmentation of nascent DNA resulting from a high dUTP/dTTP ratio. *Ung, thy* double mutants have a greater resistance to thymine deficiency [14].

Repair of DNA containing 5-bromouracil seems to proceed via dehalogenation followed by excision of the resulting uracil [24].

*AP endodeoxyribonucleases and repair of DNA containing AP sites*

AP sites appear spontaneously in DNA. Lindahl and Nyberg [25] have calculated that the probability, at 37°, for a purine to be lost is $10^{-7}hr^{-1}$; the probability is 20 times lower for pyrimidines [26]. Modification of DNA bases can accelerate their loss : alkylation of adenine on N-3 or of guanine on N-7 increases the rate of depurination by a factor of $10^5$ to $10^6$. AP sites may also result from the activity of DNA glycosylases.

An AP endodeoxyribonuclease was first discovered in *E. coli* [27]; the enzyme was completely purified [28] and, to avoid a wide-spread confusion with endonuclease II, it was named endonuclease VI by Gossard and Verly [29]. The endonucleolytic activity of endonuclease VI is specific for AP sites in double-stranded DNA [28–30]; endonuclease VI is the same protein as exonuclease III : it is a 3′-phosphatase and a 3′–5′ exonuclease working from single-strand breaks in double-stranded DNA [29,31,32]. DNA containing AP sites has been repaired *in vitro* on incubation with endonuclease VI, DNA polymerase I of *E. coli* and phage T4 ligase [33]. The details of the repair have been worked out [29] : the AP endodeoxyribonuclease hydrolyzes the phosphodiester bond which is neighbouring the AP site on its 5′ side, leaving a 3′-hydroxyl and a 5′-phosphate; the exonuclease III activity of endonuclease VI degrades the knicked strand in the 3′-5′ direction leaving the AP site in DNA; DNA polymerase I, using the intact strand as template, fills the gap, excises the AP site in a di- or tri-nucleotide by its 5′-3′ exonuclease activity, then catalyzes the translation of a break in the 5′-3′ direction by the conjugated action of the polymerase and exonuclease activities; the sliding break, which is limited by a 3′-hydroxyl and a 5′-phosphate, is sealed by ligase.

*E. coli* possesses a second endodeoxyribonuclease specific for AP sites named endonuclease IV [34]; this enzyme has no associated exonuclease activity. AP endodeoxyribonucleases have been isolated from other bacteria : *Micrococcus luteus* [7,8,35], *Bacillus stearothermophilus* [36], *Hemophilus influenzae* [37] and *Bacillus subtilis* [38].

AP endodeoxyribonucleases have been found in fungi, algae and the roots and leaves of higher plants [39]. The *Phaseolus multiflorus* embryo enzyme has been isolated [40]; the AP endodeoxyribonucleases of *Saccharomyces cerevisiae* [41] and of barley leaves [42] have also been purified. Endodeoxyribonucleases specific for AP sites have been prepared from rat liver [43], calf thymus [44], human fibroblasts [45], lymphoblasts [46] and placenta [47].

It soon appeared that eukaryotic cells contain several AP endodeoxyribonucleases [39,47,48]. On the

other hand, Thibodeau and Verly [39] showed that, in *Phaseolus multiflorus* embryo cells, most of the AP endodeoxyribonuclease activity is located in the non-histone proteins of chromatin.

In more recent work, Thibodeau, in our laboratory, found that, in rat liver, AP endodeoxyribonuclease activities are present in nuclei, mitochondria, membranes and cytosol. The nucleus activity is shared between chromatin, nuclear sap and nuclear membranes. Thibodeau, moreover, demonstrated that the nuclear membrane and cytoplasmic membrane enzymes are probably identical and different from the enzymes of other cell compartments : they have the same half-life at 45° and they are activated by Triton, whereas the other enzymes are inhibited. The nuclear sap enzyme has a higher molecular weight than the chromatin enzyme; the latter enzyme, which has 12,500 daltons, has been isolated [49,50]. The conclusion seems to be that different enzymes are located in different cell compartments. The following hypotheses can be formulated : (1) the only biologically significant enzyme for nuclear DNA repair is the chromatin AP endodeoxyribonuclease; (2) the enzymes found in other cell compartments are precursors of the chromatin enzyme. The protein might first be bound to the membrane and transported into the nucleus; a first maturation step would release the enzyme in the nuclear sap and a second maturation step would lead to the formation of a species which is taken up by the chromatin.

It is remarkable that native chromatin has only a very low activity on an added DNA containing AP sites, but that a high activity is found in the non-histones prepared from chromatin. This result suggests that the enzyme is not free to move in chromatin, but oriented in such a way that it can work on chromatin DNA but not on a foreign DNA.

It is tempting to speculate that the necessity of maturation steps is not particular to AP endodeoxyribonucleases, but might be general for DNA repair enzymes in eukaryotic cells. Repair deficiencies might depend not only on mutations in the gene coding for the repair enzyme, but also in genes coding for enzymes involved in their processing or for proteins needed to give to the chromatin enzyme a correct orientation to work on nuclear DNA. It could be the explanation for the numerous complementation groups found in a DNA repair disease such as *xeroderma pigmentosum*. Several observations reported in the literature could be explained by the necessity of maturation steps before the DNA repair enzyme can do its work. Kühnlein *et al.* [48] observed that the AP endodeoxyribonuclease with the lowest $K_m$ was absent from the fibroblasts of *xeroderma pigmentosum* patients, group D, with severe nervous defects; the absence of this enzyme might well be due to an incomplete maturation of the precursor protein. A lack of processing might also explain the observation of Mortelmans *et al.* [51] of the presence of an UV endodeoxyribonuclease activity in *xeroderma pigmentosum*, group A, fibroblasts, although the enzyme was not capable to act on the chromatin DNA containing pyrimidine dimers.

Maturation of deoxyribonucleases has been observed by Kwong and Fraser [52] in *Neurospora crassa* : the cell extract contains an inactive precursor which proteases transform first into an endoexonuclease active on single-stranded and double-stranded DNA, then into an endonuclease specific for single-stranded DNA.

Coming back to the repair of apurinic sites in DNA, it has been suggested recently that the missing purine could be replaced by an enzyme called insertase without cutting the DNA strand. Two insertases have been described : one that uses free purines [53] and one that uses deoxynucleoside triphosphates [54]. The insertase using free purines is probably not a repair enzyme : the concentration of free purines necessary to drive the reaction in the right direction does not exist in cells. The insertase which uses deoxynucleoside triphosphates raises a more complex problem; the substrate contains energy-rich bonds and the reaction is a transfer of purine between two deoxyriboses. There seems to be no thermodynamical difficulty, but the substrate used so far looks artificial : it contains intact apurinic sites, whereas *in vivo*, due to the high AP endodeoxyribonuclease activity, most apurinic sites are associated with breaks. Nobody has shown that insertase can utilize such a substrate; if this was so, insertase alone would not be sufficient and ligase would be needed to complete the repair.

### *Repair of DNA containing* $O^6$*-alkylguanine*

$O^6$-Alkylguanine in DNA is believed to be a premutagenic and a precarcinogenic lesion [55]. The complementary base of $O^6$-alkylguanine can be uracil or thymine instead of cytosine [56–58].

$O^6$-Methylguanine is not lost spontaneously from DNA, but *in vivo* it disappears from the chromosome of *E. coli* [59]. As seen previously, the disappearance is not due to the release of the methylated base by a DNA glycosylase. A preliminary low dose of methylnitronitrosoguanidine (MNNG) decreases the mutagenic effect of a second higher dose [60]; adapted bacteria have less $O^6$-methylguanine in their DNA after the high dose of MNNG than non-adapted bacteria [61]. The adapted bacteria, after the challenge dose of MNNG, show a rapid disappearance of $O^6$-methylguanine which occurs even at 0°, followed by a slow disappearance which is temperature-dependent; the non-adapted bacteria exhibit only the slow disappearance. Both fast and slow disappearances of $O^6$-methylguanine depend on the same *ada* gene [62]. The adaptation factor seems to be a protein but it is not an enzyme; it reacts stoichiometrically with $O^6$-methylguanine even at 0° and disappears during the reaction. During the adaptation (i.e. in the presence of a low concentration of MNNG), in the *E. coli* strain studied by Robins and Cairns [62], molecules of the adaptation factor accumulate at a rate of 100 per min per cell (it can be 5 times higher in other strains). After the challenging dose of MNNG, a maximum number of $O^6$-methylguanine residues equal to the number of the accumulated adaptation factor molecules can disappear immediately from the cell DNA; excess of $O^6$-methylguanine disappears at a rate of 100 residues per min per cell at 37°, which is the rate observed

in unadapted bacteria. Karran *et al.* [63] show that extracts of adapted *E. coli* cells contain a factor responsible for the disappearance of $O^6$-methylguanine from an added methylated DNA : $O^6$-methylguanine is not released as a free base; it is modified into something else, not yet identified, which stays in DNA. Extracts of non-adapted bacteria or of *ada* mutants pretreated with a low dose of MNNG do not contain the adaptation factor.

It must be emphasized that the adaptative response is a pre-replicative error-free repair mechanism which is different from the SOS error-prone processes; it is present in *recA* mutants [64].

$O^6$-Alkylguanine also disappears from DNA in eukaryotic cells, although the rate of the reaction in rat liver (where it is particularly high) is much lower than in *E. coli*. The capacity of tissues to remove $O^6$-alkylguanine from DNA varies widely. It has been suggested that the very slow removal of the modified purine from DNA in some tissues is responsible for the localization of cancers induced by alkylating agents in these tissues [65,66]. It is not, of course, the only parameter which fixes the localization of a cancer; level of persisting alkylation and probability of cell division are also important [67].

Kleihues and Margison [68] reported that a first treatment with non-labeled methylnitrosourea decreases the rate of disappearance of labeled $O^6$-methylguanine from rat liver DNA resulting from a second treatment with radioactive methylnitrosourea. Pegg [69] made a similar observation using dimethylnitrosamine (20 mg/kg). These results can be explained as deriving from a competition, the first high dose of alkylating agent forming unlabeled $O^6$-methylguanine which competes with the labeled $O^6$-methylguanine introduced by a second dose. But the result is completely different if the animal is first treated with a low dose of alkylating agent. Montesano *et al.* [70] found that a chronic administration of 2 mg/kg of dimethylnitrosamine for 9 weeks to rats increases the excision, from liver DNA, of labeled $O^6$-methylguanine produced by a challenging dose of radioactive dimethylnitrosamine.

Pegg and Hui [71] obtained, by precipitation with ammonium sulfate of 15,000 *g* supernatants from rat liver homogenates, proteins which lead to the disappearance of $O^6$-methylguanine from an added alkylated DNA. The $O^6$-methylguanine is not released as a free base. Treatment of the rat with a high dose of dimethylnitrosamine gives a liver extract with a lower efficiency [69], but, if the animal is chronically treated with a low dose according to the procedure of Montesano *et al.* [70], the liver extract has a higher efficiency than if prepared from an unadapted animal (Pegg, personal communication).

There is some likeness, but there are also differences between rat liver and *E. coli* : although there seems to be a possibility of adaptation in rat liver cell, the repair factor exists constitutively, which is not the case in *E. coli*. We do not know yet if the rat liver factor is consumed by the reaction, although the decrease in activity when the extract is prepared from an animal which has received a high dose of alkylating agent could be explained in this way. We no longer know if the reaction responsible for the disappearance of $O^6$-alkylguanine from DNA is the same in mammalian cells as in *E. coli* cells.

Purified rat liver nuclei treated with ethylnitrosourea lose $O^6$-ethylguanine from their DNA on incubation [72]. Renard, in our laboratory, found that the proteins of rat liver chromatin contain a factor removing $O^6$-ethylguanine from an added ethylated DNA without releasing the alkylated residue as a free base. We do not know whether the factor observed by Pegg and his collaborators in 15,000 *g* supernatants and the chromatin protein are identical. It may be that, as for the AP endodeoxyribonucleases, there is a particular chromatin factor which is probably the only one of biological importance and that proteins with the same function found in other cell compartments are only precursors of the true repair agent.

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