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Enzymes Acting at Strand Interruptions in DNA

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Enzymes acting at strand interruptions in DNA

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SUMMARY

Endogenous and environmental DNA-damaging agents often generate single-strand interruptions in DNA. The lesions trigger a complex set of cellular reactions. In most eukaryotic cells, cellular poly(ADP-ribose) formation is the most acute response to such damage. Recently, such events have been amenable to study with soluble cell-free extracts of human cells. These investigations clarify the modulating role on DNA repair by poly (ADP-ribose), and suggest that the primary function of this unusual polymer is to act as an antirecombinant agent. Similar biochemical studies of subsequent repair events have revealed a branched pathway for the ubiquitous DNA base excision-repair process. The alternative pathway provides the cell with back-up functions for individual steps in this essential form of DNA repair.

1. INTRODUCTION

Ionizing radiation and chemicals such as bleomycin and neocarzinostatin that generate oxygen free-radicals, cause the formation of single-strand breaks in DNA. These strand interruptions have diverse and complex terminal structures, usually resulting from destruction of the deoxyribose residue at the 3′ or 5′ end of the break. Consequently, only a very small proportion of radiation-induced single-strand breaks can be rejoined directly by DNA ligase. The sensitive biophysical methods often employed to measure strand breaks in DNA have the inherent weakness of not being able to distinguish between breaks with different end groups, although various types of strand interruptions are repaired with marked differences in efficiency (Satoh *et al.* 1993). Thus one reason why rare double-strand breaks in DNA have often been implicated as the most important lethal lesions in radiological research may simply be they are easier to measure than a particular form of cytotoxic single-strand break.

Prior to their rejoining by complex excision-repair processes, oxygen radical-induced strand breaks in DNA elicit a cellular alarm response that leads to inhibition of DNA replication, apparently through unknown factors that cause elevation and stabilization of p53 protein levels (Lu & Lane 1993; Nelson & Kastan 1994). Two abundant DNA-binding nuclear enzymes are activated by strand interruptions in DNA: DNA-dependent protein kinase (Gottlieb & Jackson 1993; Anderson 1994) and poly(ADP-ribose) polymerase (de Murcia & de Murcia 1994). It is conceivable that one or both of these proteins may be involved in cellular signalling in response to DNA damage. The main role for poly(ADP-ribose) polymerase, however, appears to be to act as an antirecombinogenic factor that prevents instability of densely packed tandem-repeat sequences in chromatin. In addition, DNA-binding and activation of poly(ADP-ribose) polymerase modulates DNA repair (Satoh *et al.* 1994).

2. POLY(ADP-RIBOSE) SYNTHESIS

DNA strand interruptions that occur as reaction intermediates during lagging-strand DNA replication and nucleotide excision-repair processes are protected by multi-protein complexes and consequently are not accessible to poly(ADP-ribose) polymerase (Eki & Hurwitz 1991; Molinete *et al.* 1993; Satoh *et al.* 1993). In contrast, chain breaks generated directly by DNA-damaging agents, or appearing as intermediates in base excision-repair, elicit rapid binding and extensive automodification by poly(ADP-ribose) polymerase. This protein is abundant in cell nuclei (more than 10^5 molecules per cell, except in terminally differentiated cells) and is tightly attached to the nuclear matrix. NAD is the precursor of poly(ADP-ribose) and polymer formation is product-inhibited by nicotinamide analogues such as 3-aminobenzamide. Many investigations of poly(ADP-ribose) synthesis in response to DNA damage have been performed *in vivo* (reviewed in Cleaver & Morgan 1991) and recently the main features of the reaction have been reproduced with human cell-free extracts (Satoh & Lindahl 1992; Satoh *et al.* 1993, 1994; Smulson *et al.* 1994). DNA-free extracts of human cells (Manley *et al.* 1983) were incubated with plasmid DNA containing a single-strand break generated by ionizing radiation as well as deoxynucleoside triphosphates ATP and NAD. DNA repair was measured either by following the conversion of the nicked plasmid to a covalently closed form by agarose gel electrophoresis in the presence of ethidium bromide, or by measuring DNA repair replication by autoradiography on inclusion of a radioactively labelled deoxynucleoside triphosphate in the reaction mixture. Concurrent poly(ADP-ribose) synthesis was monitored by incorporation of ^{32}P-labelled NAD followed by gel electrophoresis and autoradiography. Within 30 seconds at 30 °C, long chains (more than 50 residues) of poly(ADP-ribose) are synthesized (Satoh *et al.* 1994). These remain attached to poly(ADP-ribose)

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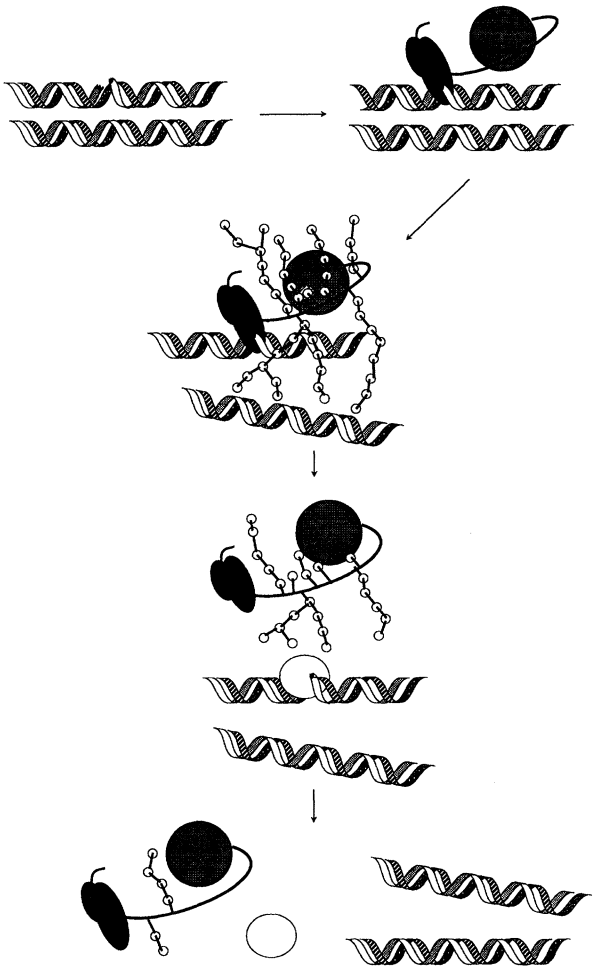

Figure 1. Poly(ADP-ribose) as an antirecombinogenic agent. Two homologous DNA sequences are shown, packed close together in the cellular chromatin. An accidental single-strand interruption generated by a DNA-damaging agent is prevented from triggering a recombination event by tight binding of poly(ADP-ribose) polymerase. Automodification of the enzyme generates negatively charged polymer chains which repel adjacent DNA sequences and prevent aligning of homologous stretches, prior to dissociation of the automodified enzyme from DNA and subsequent DNA repair. Poly(ADP-ribose) polymerase has a DNA-binding domain and a separate catalytic domain united by a short automodification region; repair enzymes are shown schematically as an open oval.

polymerase. The heavily modified enzyme, which has multiple acceptor sites, is released from DNA. Subsequently, the polymer chains are degraded by a specific poly(ADP-ribose) glycohydrolase. Very little DNA repair occurs during the first 2 min of incubation, so poly(ADP-ribose) synthesis precedes processing and rejoining of strand breaks by repair enzymes. This programme of events explains the observation of strong inhibition of DNA repair when NAD is excluded from reaction mixtures or when 3-aminobenzamide is included. The tight binding of unmodified poly(ADP-ribose) polymerase to DNA strand breaks interferes with DNA repair, but automodification of the enzyme serves as a release mechanism. If, prior to use, reaction mixtures are depleted of poly(ADP-ribose) polymerase by affinity chromatography, DNA repair proceeds efficiently even in the absence of NAD (Satoh & Lindahl 1992). These data on the *in vitro* system have been extended recently by Smulson *et al.* (1994), who employed cell extracts depleted of endogenous poly(ADP-ribose) polymerase but supplemented with mutationally altered poly(ADP-ribose) polymerase expressed in bacteria. Enzyme molecules with deletions in the N-terminal DNA-binding region were unable to suppress DNA repair. However, mutations in the C-terminal catalytic region resulted in poly(ADP-ribose) polymerase molecules that interfered with DNA repair in such a way that inhibition could not be alleviated by addition of NAD. The latter results agree well with the finding by Molinete *et al.* (1993) that expression *in vivo* of the subcloned DNA-binding domain of poly(ADP-ribose) polymerase sensitizes cells to DNA damage. Interestingly, in the experiments by Smulson's group, poly(ADP-ribose) polymerase with intact DNA-binding and catalytic domains, but a deletion in the automodification domain, behaved as a mutant with the catalytic domain deleted, *i.e.* DNA repair was inhibited and the reaction was insensitive to NAD. This mutated form of poly(ADP-ribose) polymerase had retained its capacity for low-level modification of other proteins, but such events did not affect DNA repair.

The amazingly elaborate polymer synthesis and automodification of poly(ADP-ribose) polymerase triggered by DNA strand breaks is unlikely to be required to induce a conformational change in the protein itself, which is often the function of more discrete modification events such as phosphorylation or mono(ADP-ribosyl)ation. Instead, the interaction between nuclear macromolecules could be modulated in this fashion. Short chains of poly(ADP-ribose) at multiple acceptor sites in the automodified enzyme are sufficient for its release from strand interruptions in DNA (Satoh *et al.* 1994). Such relatively persistent short chains, which normally result from partial degradation of initially-formed long poly(ADP-ribose) chains by poly(ADP-ribose) glycohydrolase, apparently serve to prohibit rapid reattachment of poly(ADP-ribose) polymerase to DNA strand breaks, allowing time for DNA repair to proceed. Thus, the overall effect of poly(ADP-ribose) polymerase on repair of DNA strand interruptions, resulting in a slight delay of DNA rejoining, does not appear to be the primary reason for the synthesis of long chains of poly(ADP-ribose), but a secondary consequence. A number of scenarios have been proposed to explain the occurrence of polymer synthesis, such as wilful depletion of cellular NAD pools (Berger 1985), inhibition of DNA topoisomerase I (Ferro *et al.* 1984), or histone rearrangements in chromatin (Panzeter *et al.* 1993), but none of these models seems particularly convincing. Interestingly, in lower eukaryotes, there seems to be a marked correlation between the occurrence of poly(ADP-ribose) polymerase and its accompanying poly(ADP-ribose) glycohydrolase with the abundance of repeated DNA sequences in the cellular genome (Satoh *et al.* 1994). For example, dinoflagellates, which belong to the protozoa, have almost one-half of their DNA in the form of interspersed repeated DNA sequences and have

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a poly(ADP-ribose) polymerase (but no histones), whereas *Saccharomyces cerevisiae* has very little repeated DNA of this type and apparently lacks poly(ADP-ribose) polymerase. Large numbers of complex sequence repeats must be a major challenge to mechanisms that serve to retain genomic stability, especially if a DNA strand interruption accidentally occurs in such a region of the chromatin to generate a potential hot spot for homologous recombination. The biochemical properties of poly(ADP-ribose) polymerase seem admirably suited to prevent this problem. The protein binds very rapidly and tightly to nicks in DNA and protects them from repair and recombination enzymes such as exonucleases. The subsequent slight delay in DNA repair seems a small price to pay for this efficient shielding. The automodification of the bound enzyme with multiple long branched chains of poly(ADP-ribose) results in a zone of high negative charge around the strand break and will effectively repel any immediately adjacent DNA sequences in tightly packed chromatin and prevent them from aligning as potential partners in homologous recombination events. A model is shown in figure 1. In apparent agreement with this scheme of events, several reports have appeared on the stimulation of homologous recombination, gene amplification and sister chromatid exchange by inhibition of poly(ADP-ribose) synthesis (Oikawa *et al.* 1980; Natarajan *et al.* 1981; Ferro *et al.* 1984; Waldman & Waldman 1991; Smulson *et al.* 1994). More definite experimental tests of the model in cell-free systems (Kawasaki *et al.* 1994) should now be possible however, cell lines or transgenic mice lacking poly (ADP-ribose) polymerase might be required to confirm finally the physiological roles of this enzyme.

3. DNA BASE EXCISION-REPAIR

The most common fate of a DNA lesion in *E. coli*, or in human cells, is its removal by 'excision-repair'. This general term covers two entirely different pathways employing different enzymes and DNA reaction intermediates. These pathways have been called base excision-repair versus nucleotide excision-repair, the semantic problems with this nomenclature have been discussed by Friedberg (1985). In addition, DNA mismatch correction occurs by a third route of excision-repair. Bulky DNA lesions that cause major helix distortion, e.g. the major lesions induced by ultraviolet light, are handled by nucleotide excision-repair, which is discussed elsewhere in this Volume. Base excision-repair is largely employed to correct potentially mutagenic or toxic lesions that only cause minor distortion of the DNA double helix; these include many lesions (including strand interruptions) generated by spontaneous DNA hydrolysis, oxygen free-radicals and methylating agents. Thymine residues in G·T mismatches in the DNA of mammalian cells are also removed by this pathway (Neddermann & Jiricny 1993).

Following a similar strategy to that employed for our studies on nucleotide excision-repair (Wood *et al.* 1988) and poly(ADP-ribose) formation during DNA repair (Satoh & Lindahl 1992), base excision-repair has been investigated in DNA-free soluble cell extracts supplemented with a defined DNA substrate (Dianov *et al.* 1992). In this case, we have used double-stranded oligonucleotides containing a single, centrally located dUMP residue opposite a dGMP residue. Such oligonucleotides are repaired very efficiently by cell extracts of *E. coli* or human cells, with close to 100% replacement of the dUMP with a dCMP residue after 10–20 min incubation at 37 °C. The repair process apparently comprises five steps. A schematic outline of the process, with candidate human enzymes involved, is shown in figure 2. The first two steps are well-known and supported by a large body of biochemical and genetic data: the dUMP residue is cleaved by a DNA glycosylase that catalyses the release of free uracil, an apurinic/apyrimidinic (AP) endonuclease then hydrolyses the phosphodiester bond on the 5′ side of the base-free site. Several other distinct DNA lesions are dealt with in an analogous way, employing different DNA glycosylases. After removal of an altered base, the AP site remains well accommodated in the double-helix and causes surprisingly little distortion (Withka *et al.* 1991), although such an informationless residue in the DNA template acts as a partial block to replication. After incision next to the AP site, however, the hydrophilic deoxyribose-phosphate residue, which does not form hydrogen bonds with the base in the complementary strand, would be expected to swing out into an extra-helical position. The one-nucleotide gap in the double helix is then filled in by a DNA polymerase. In 80–90% of the repair events, no further polymerization takes place, so that DNA repair replication is confined to the replacement of a single nucleotide (Dianov *et al.* 1992). Occasionally, however, repair replication covers several additional nucleotides. The factors that regulate such additional synthesis are unclear at present, but in mammalian cells could simply depend on which DNA polymerase first encounters the gap. The longer repair patches may reflect a minor, back-up pathway for this particular step in the repair process, employing enzymes also used for late steps in lagging-strand DNA replication and nucleotide excision-repair. Several observations on a heterogenous mode of gap-filling during base excision-repair have been made *in vivo* (Snyder & Regan 1982; DiGiuseppe & Dresler 1989) and *in vitro* (Price & Lindahl 1991; Matsumoto & Bogenhagen 1991; Satoh *et al.* 1993). In the single nucleotide replacement route, the displaced deoxyribose-phosphate residue is excised as such, and candidate enzymes catalysing this step have been described in human cells (Price & Lindahl 1991) and in *E. coli* (Graves *et al.* 1992; Dianov *et al.* 1994). In the bacterial system, the RecJ protein (Lovett & Kolodner 1989) is the only enzyme found, so far, with the ability to release a 5′ terminal deoxyribose-phosphate residue in DNA by hydrolysis of the phosphodiester bond on its 3′ side. However, the Fpg protein, as well as basic proteins and polyamines, can promote a β-elimination event to liberate the sugar-phosphate and similarly generate a one-nucleotide

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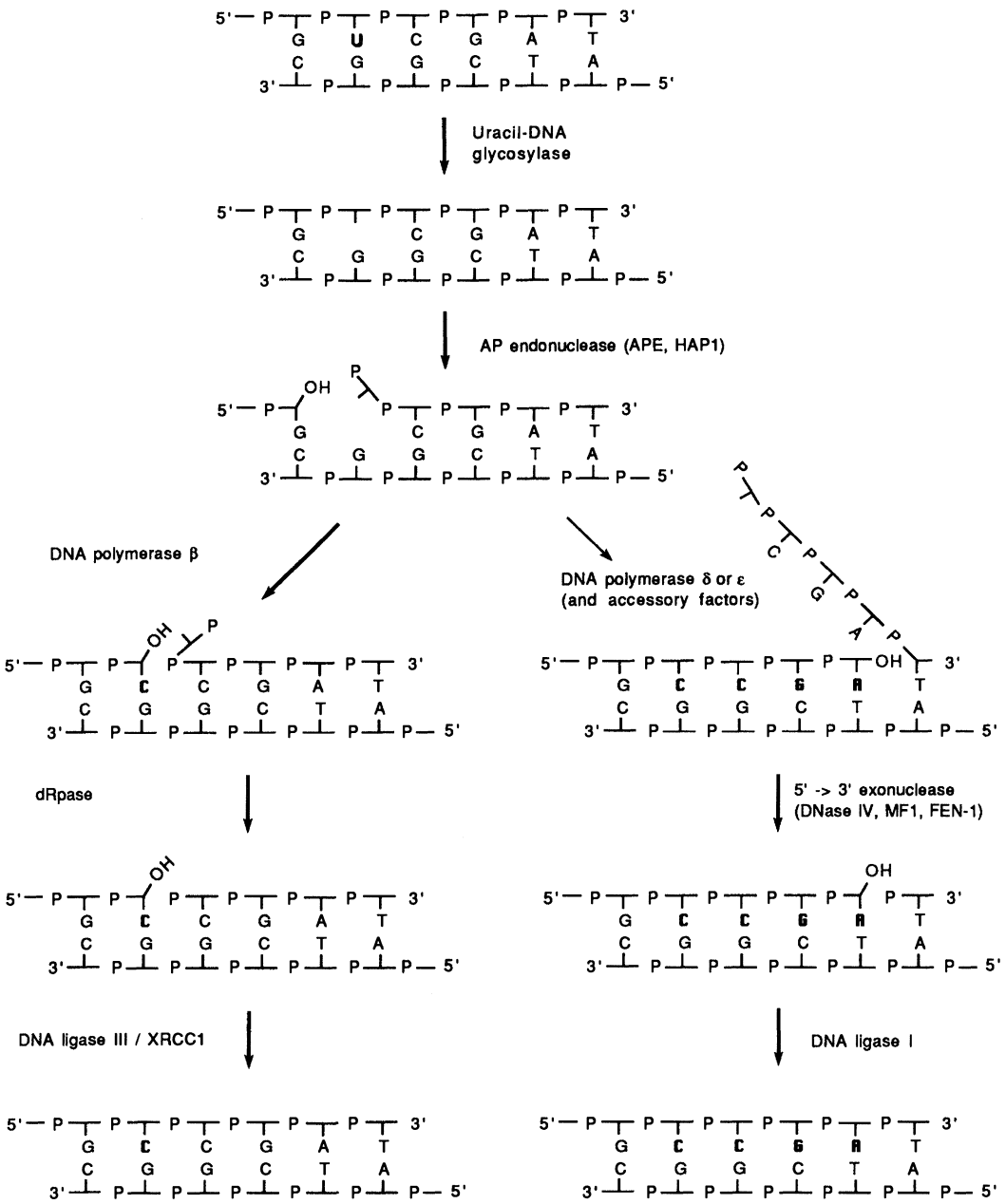

Figure 2. Branched pathway of DNA base excision-repair, resulting in heterogeneity of repair patch sizes. The human enzymes that tentatively account for each separate step are indicated.

gap. The 5′→3′ exonuclease function of *E. coli* DNA polymerase I is totally unable to catalyse this particular event, but it can slowly release a 5′-terminal deoxyribose-phosphate residue as part of a small oligonucleotide (Price 1992). A mammalian exonuclease with biochemical properties and apparent physiological roles very similar to those of the 5′→3′ exonuclease function of *E. coli* DNA polymerase I acts in an identical fashion in this regard. This mammalian 5′→3′ exonuclease, which is not covalently bound to a DNA polymerase, was initially called DNase IV (Lindahl 1971*a*, *b*; Price & Lindahl 1991) but has been renamed several times by various authors on rediscovery. DNase IV is the only known 5′→3′ exonuclease acting on double-stranded DNA in mammalian cell nuclei; the other major exonuclease, DNase III, acts in the 3′→5′ direction and preferentially degrades single-stranded DNA (Lindahl 1971*b*). Thus, DNase IV appears to be identical to the 5′→3′ exonuclease that is an essential component of lagging-strand DNA replication (Ishimi *et al.* 1988; Kenny *et al.* 1988), as well as the related cca/exo activity (Goulian *et al.* 1990), the replication factor MF1 (Waga *et al.* 1994), the FEN-1 endonuclease (Harrington & Lieber 1994), and the 5′→3′ exonuclease associated with DNA polymerase ϵ (Murante *et al.* 1994).

The occurrence of a branched pathway of base excision-repair (see figure 2) may provide increased versatility after inhibition of replication as a consequence of DNA damage, when the alternative pathway in figure 2 involving replication factors such as DNA polymerase δ (or ε) and associated components in replisomes may not be operative. The main pathway involving DNA polymerase β allows for effective excision-repair of DNA damage caused by ionizing radiation or alkylating agents even in the absence of

replication factors. Moreover, the forked pathway offers a possible explanation for the unexpected finding that two different DNA ligases in mammalian cells (reviewed by Lindahl & Barnes 1992) are involved in base excision-repair. Simple S_N2 alkylating agents such as methyl methanesulphonate (MMS) and dimethyl sulphate induce the formation of 7-methylguanine and 3-methyladenine in DNA; the former lesion is not miscoding and remains in DNA for long periods. In contrast, the cytotoxic and mutagenic 3-methyladenine lesion is excised very rapidly by a DNA glycosylase, which acts in a fashion analogous to that of uracil-DNA glycosylase. DNA repair of MMS-induced damage consequently occurs by base excision-repair (Figure 2). There is now strong genetic evidence for a role of both DNA ligase I and DNA ligase III in this process. DNA ligase I is required for the efficient joining of Okazaki fragments during DNA replication (Li *et al.* 1994; Prigent *et al.* 1994; Waga *et al.* 1994.) A human cell line derived from an immunodeficient patient of stunted growth, 46BR, encodes a malfunctioning DNA ligase I with an amino acid substitution within a conserved region. This cell line is anomalously hypersensitive to MMS and exhibits altered repair with excessive DNA repair synthesis at repair patches (Prigent *et al.* 1994). DNA ligase III occurs as a heterodimer composed of the ligase catalytic subunit and the regulatory XRCC-1 protein (Caldecott *et al.* 1994; Ljungquist *et al.* 1994). In the absence of the XRCC-1 protein, the DNA ligase III catalytic subunit exhibits strongly reduced activity. Mammalian cell mutants deficient in the XRCC-1 protein are tenfold more sensitive to MMS than normal cells, strongly implying a defect in base excision-repair in these cells. It now seems possible that the major branch of base excision-repair, with replacement of only a single nucleotide, might depend on the DNA ligase III/XRCC-1 protein complex for efficient rejoining, whereas repair involving longer patches would require the same DNA ligase as lagging-strand DNA replication and nucleotide excision-repair, i.e. DNA ligase I.

DNA base excision-repair is likely to emerge as the most common DNA repair process in all cells (Lindahl 1993). Whereas loss of the nucleotide excision-repair system results in an ultraviolet radiation-sensitive but viable cell, and loss of mismatch repair leads to a mutator phenotype, genetic experiments on micro-organisms strongly indicate that loss of repair capacity for AP sites in DNA leads to rapid cell death. Because of the critical importance of the pathway, back-up activities occur at every catalytic step. Thus, a deaminated cytosine residue is not only recognized and cleaved by the abundant uracil-DNA glycosylase, but also is a substrate for a mismatch correction function in mammalian cells, the mismatch-specific thymine-DNA glycosylate (Neddermann & Jiricny, 1994). Similarly, minor AP endonuclease activities occur in addition to the major one in mammalian cells, and *E. coli* has two distinct AP endonuclease with very similar modes of action (Demple & Harrison 1994). The branched pathway shown in figure 2 may be similarly advantageous to cells in that the right-hand part of the scheme provides a back-up pathway for completion of base excision-repair. This situation is in contrast to nucleotide excision-repair, where no back-up system has been detected when any of the components of the multi-subunit complex for damage recognition/DNA incision/damage excision is defective. Consequently, DNA repair-deficient inherited human diseases similar to xeroderma pigmentosum, but with defects in base excision-repair components, have not been detected except for a single patient (Prigent *et al.* 1994). They would be expected to be very rare, because defects in both a main pathway and a back-up pathway might be required. Nevertheless, further studies on base excision-repair is likely to reveal this pathway as a key strategy of cells for retaining genomic stability.

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