[CANCER RESEARCH (SUPPL.) 54, 1899s–1901s, April 1, 1994]

Enzymatic Repair of Oxidative DNA Damage[1]

Masahiko S. Satoh[2] and Tomas Lindahl

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Hertfordshire, EN6 3LD, United Kingdom

Abstract

Oxidative DNA damage, including both mutagenic and cytotoxic lesions, is implicated in aging and cancer. Studies of the processes which correct such damage in mammalian cells are, however, still in their early stages. Here we have summarized our recent work which demonstrates new features of mammalian oxidative DNA damage repair, such as (*a*) a functional role for poly(ADP-ribosyl)ation in the rejoining of DNA strand breaks and (*b*) the defective repair of oxidative DNA damage in xeroderma pigmentosum cells.

Introduction

Active oxidizing species, such as hydroxyl and superoxide radicals, arise through normal cellular metabolism as well as during inflammatory events (1, 2). Thus, mammalian cells frequently may encounter oxidative DNA damage induced by these reactive species. The lesions are heterogeneous and include single and double strand DNA breaks, AP[3] sites, and oxidized purines and pyrimidines (3, 4). Repair of these forms of damage occurs intracellularly by base excision repair, but as shown below, nucleotide excision repair also may be involved.

Defective repair of oxygen free radical-induced DNA lesions leads to a variety of biological consequences, such as mutation induction, blocking of transcription and replication, and chromosomal aberrations. In addition, DNA damage activates an abundant nuclear enzyme, PARP (5). Furthermore, recent studies indicate that persistence of DNA damage may transmit signals to other cellular components, including p53 which may be involved in a G_1-S cell cycle checkpoint (6) and trigger apoptosis (7, 8). These observations suggest that DNA damage is processed not only by DNA repair enzymes but also by other nuclear factors involved in a variety of cellular functions. The delineation of cellular responses to oxidative DNA damage in mammalian cells is still at a preliminary stage. We have investigated two specific problems in the repair of oxidative DNA damage by using gently prepared human cell-free extracts together with plasmid DNA containing oxidative lesions as substrate. Here we describe a tentative role of PARP in modulating the repair of oxygen free radical-induced DNA breaks (9, 10) and the defective repair of oxidative DNA damage in XP (11).

Functional Role of PARP in DNA Single Strand Break Repair

PARP is a M_r 113,000 nuclear enzyme that catalyzes the modification of proteins by covalent attachment of poly(ADP-ribose) polymers (for reviews, see Refs. 5, 12–16). PARP may be classified as a posttranslational protein modification enzyme, but unlike most other such enzymes, PARP mainly modifies itself (automodification). PARP adds several polymer chains containing over 200 ADP-ribose residues each onto a protein molecule which results in a general inhibition of PARP activity. Thus, poly(ADP-ribosyl)ation seems to represent an autoregulatory mechanism of PARP activity, although other proteins, including histones and topoisomerases, also are known to be acceptors for poly(ADP-ribosyl)ation.

In vitro, PARP is activated by binding to DNA breaks (17). *In vivo*, such breaks can be generated by exposure to ionizing radiation or in the process of base excision repair of damaged DNA bases. In fact, a rapid increase in the cellular content of poly(ADP-ribose) polymer has been observed after treatment of cells with DNA-damaging agents, such as ionizing radiation or alkylating agents. This activation may be explained by the binding of PARP to intracellularly generated DNA breaks, and such activation of PARP is believed to modulate the DNA break rejoining processes. In addition, inhibition of PARP by a nicotinamide analogue, 3-aminobenzamide, sensitizes cells to toxic effects of DNA-damaging agents and increases the number of unrepaired DNA breaks. These observations have been taken to indicate that PARP may stimulate the DNA break rejoining process by, for example, recruiting DNA repair enzymes to damaged sites. However, direct experimental evidence for this model has been lacking.

Using a cell-free DNA repair assay system, we found that γ-ray induced DNA single strand breaks are enzymatically rejoined, and NAD^+ strongly stimulates this activity in association with PARP activation (9). Furthermore, inhibition of PARP activity with 3-aminobenzamide suppresses NAD^+-promoted DNA break rejoining activity. Thus, consistent with *in vivo* results, poly(ADP-ribosyl)ation is apparently involved in the break rejoining reaction. Surprisingly, however, when PARP was removed from cell-free extracts by affinity chromatography, the depleted extracts could rejoin DNA breaks efficiently even without the addition of NAD^+. These results indicate that in the absence of NAD^+, or in the presence of 3-aminobenzamide with NAD^+, PARP binds tightly to γ-ray-induced DNA breaks and inhibits DNA break repair by the prevention of access of DNA repair enzymes to the damaged sites. Recently, data consistent with our conclusion were reported by Molinete *et al.* (18), who observed inhibition of DNA repair by transfection of the DNA-binding domain of PARP into cells. It may be concluded that in the process of DNA break repair, poly(ADP-ribosyl)ation serves as a release mechanism of PARP from DNA strand breaks (19, 20), which makes the breaks available to DNA repair enzymes after a short period of protection and nonaccessibility. Fig. 1 shows a summary of the process.

Involvement of PARP in Base Excision Repair but not in Nucleotide Excision Repair

In the repair process of DNA single strand breaks induced by radiomimetic agents such as bleomycin and neocarzinostatin, NAD^+-dependent repair associated with PARP automodification occurs (10). In particular, repair of bleomycin-induced DNA breaks seems totally dependent on PARP activation. Repair of modified bases generated by alkylating agents also is promoted by PARP activation. Such altered bases are known to be corrected by base excision repair (see Fig. 2), which is initiated by the elimination of damaged bases by DNA glycosylases. Breaks are induced at the AP sites by AP endonuclease, followed by excision of deoxyribosephosphate, DNA polymerization, and ligation. PARP apparently is involved in the process of DNA break rejoining initiated by AP endonuclease, while the preceding process of removal of modified bases is unaffected by PARP.

In contrast to DNA base excision repair, major UV-induced DNA damage, *i.e.*, cyclobutane pyrimidine dimers and pyrimidine-pyrimidone 6-4 products, is repaired by a poly(ADP-ribosyl)ation-independent process. During the repair of the damage, PARP is not activated, nor does NAD^+ stimulate the repair. These photoproducts are elim-

[1] Presented at the 4th International Conference on Anticarcinogenesis & Radiation Protection, April 18–23, 1993, Baltimore, MD.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: AP, abasic; PARP, poly(ADP-ribose)polymerase; XP, xeroderma pigmentosum.

inated by nucleotide excision repair, which requires formation of multiprotein repair complexes before DNA incision (21); such complexes may prevent the binding of PARP to DNA breaks. These results are summarized in Fig. 2. PARP is involved only in base excision repair and in repair of DNA breaks induced by ionizing radiation [poly(ADP-ribosyl)ation-dependent repair] but not in nucleotide excision repair [poly(ADP-ribosyl)ation-independent repair]. What is the main physiological function of PARP? Considering the abundance of this protein in cell nuclei, PARP presumably serves important functions, perhaps having a structural role in chromatin and/or acting as a temporary protection mechanism for DNA breaks during the early stages of recombination and repair.

Defective Repair of Oxygen Free Radical-induced DNA Lesions in XP

XP is a human genetic disorder with an autosomal recessive mode of inheritance (for reviews, see Refs. 22 and 23). There are seven genetic complementation groups from A through G, and the defective gene products in these groups are involved in nucleotide excision repair, particularly in damage recognition and incision processes. Typical clinical features of XP are skin lesions, including frequent carcinomas and melanomas, and hypersensitivity to sunlight. In addition, severe cases of XP exhibit neurological abnormalities as well as increased frequency of endogenous tumor formation. The origins of the latter symptoms have been unclear, although formation of DNA damage by endogenous agents has been proposed as a possible explanation (24).

By using a cell-free assay system, we have observed DNA repair of previously unrecognized lesions in plasmids treated with γ-rays or H_2O_2/Cu^{2+} (11). The oxidatively damaged plasmid substrate used in the assay was pretreated with *Escherichia coli* endonuclease III and formamidopyrimidine-DNA glycosylase to cleave DNA molecules containing oxidized pyrimidines and purines with fragmented or oxidized imidazole rings and then subjected to ethidium bromide/CsCl centrifugation to purify the remaining closed circular DNA. The resulting DNA plasmids essentially were freed from known types of oxidative damage, including DNA single strand breaks, and formamidopyrimidine-DNA glycosylase- and *E. coli* endonuclease III-sensitive sites. When this plasmid substrate was used in the assay, DNA repair replication occurred in the reaction with cell-free extracts from normal cells. Such repair synthesis was not observed with untreated control DNA. Furthermore, the repair reaction showed several characteristics of nucleotide excision repair, such as slow kinetics and lack of PARP activation (10).

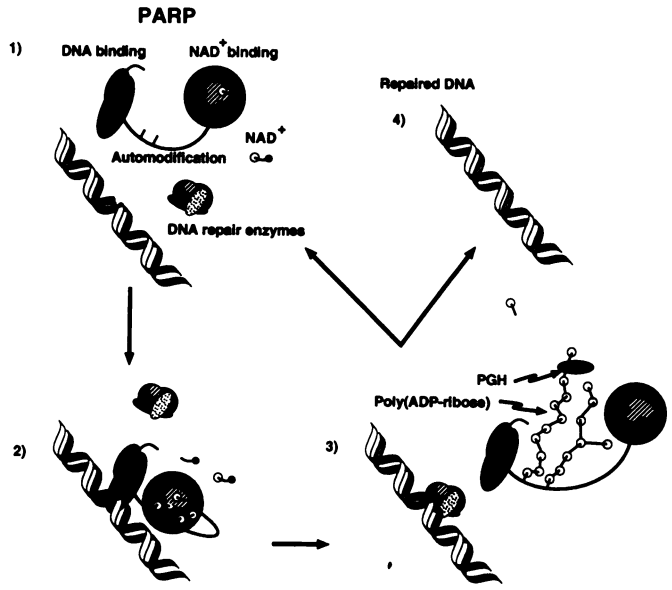

Fig. 1. Model for the involvement of poly(ADP-ribosyl)ation in DNA break rejoining. (1) PARP competes with DNA repair enzymes in binding to DNA breaks. (2) Binding of PARP inhibits DNA repair but initiates automodification which reduces the DNA binding affinity of PARP. (3) As a consequence of automodification, PARP dissociates from DNA breaks. (4) Digestion of the poly(ADP-ribose) polymers by poly(ADP-ribose)glycohydrolase (*PGH*) returns PARP to its original form, while DNA repair enzymes complete DNA strand break rejoining.

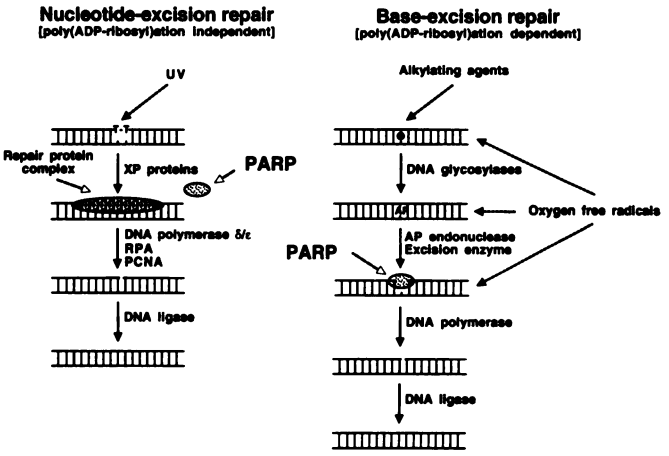

Fig. 2. DNA binding of PARP during base excision repair but not in nucleotide excision repair. Nucleotide excision repair is initiated by enzymatic recognition of pyrimidine dimers (*T-T*), followed by removal of an oligonucleotide and gap filling. This process requires the formation of repair protein complexes which may inhibit binding of PARP to the DNA breaks. On the other hand, base excision repair and oxygen free radicals generate DNA breaks to which PARP binds.

When XP cell-free extracts were used in the assay with the oxidized and enzyme-treated plasmid DNA, negligible amounts of repair replication were observed. Furthermore, the strongly reduced repair replication, compared with that seen with normal cell extracts, was restored by mixing two XP cell extracts derived from different XP complementation groups and by supplementation of purified XPA protein to XPA cell-free extracts, indicating that the reduced repair activity of oxidative damage was due to the known deficiency of nucleotide excision repair in XP.

Since reactive oxidizing species are generated through normal cell metabolism, cellular DNA may undergo continuous oxidation at a low level (25). Hence, any oxidative DNA damage requiring nucleotide excision repair for correction would accumulate in XP cells, and such a buildup of nonrepaired lesions may be a cause of the neurological deterioration and increased frequency of endogenous tumor formation in XP patients.

In cell survival assays, most XP cells show close to normal resistance to ionizing radiation. Thus, XP cells, in general, appear to have normal repair activity for oxidative DNA damage. However, cell survival assays tend to show the effects of the most lethal and abundant DNA lesions, which mask the effects of other forms of DNA damage. Thus, the cell-free DNA repair assay used here has the advantage of disclosing the nature of lesions requiring nucleotide excision repair among other forms of more abundant damage. Currently, we are attempting to identify the nature of the lesion that cannot be repaired by XP cell extracts. Preliminary data indicate that this lesion may be identical to covalent intrastrand purine dimers, AA and AG, recently found by Carmichael *et al.* (26) in H_2O_2/Cu^{2+}-treated DNA.

Acknowledgments

We thank Dr. B. Sedgwick for critical reading of the manuscript.

References

1. Klebanoff, S. J. Oxygen metabolism and the toxic properties of phagocytes. Ann. Intern. Med., *93:* 480–489, 1980.
2. Fridovich, I. Superoxide radical: an endogenous toxicant. Annu. Rev. Pharmacol. Toxicol., *23:* 239–257, 1983.
3. von Sonntag, C. The Chemical Basis of Radiation Biology. London: Taylor & Francis, Ltd., 1987.
4. Wallace, S. S. AP endonucleases and DNA glycosylases that recognize oxidative DNA damage. Environ. Mol. Mutagen., *12:* 431–477, 1988.
5. Althaus, F. R., and Richter, C. ADP-ribosylation of Proteins: Enzymology and Biological Significance. Berlin: Springer-Verlag, 1987.
6. Prives, C., and Manfredi, J. J. The p53 tumor suppressor protein: meeting review. Genes Dev., *7:* 529–534, 1993.
7. Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, M. L., and Wyllie, A. H. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature (Lond.), *362:* 849–852, 1993.
8. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature (Lond.), *362:* 847–849, 1993.
9. Satoh, M. S., and Lindahl, T. Role of poly(ADP-ribose) formation in DNA repair. Nature (Lond.), *356:* 356–358, 1992.
10. Satoh, M. S., Poirier, G. G., and Lindahl, T. NAD^+-dependent repair of damaged DNA by human cell extracts. J. Biol. Chem., *268:* 5480–5487, 1993.
11. Satoh, M. S., Jones, C. J., Wood, R. D., and Lindahl, T. DNA excision-repair defect of xerodermapigmentosum prevents removal of a class of oxygen free radical-induced base lesions. Proc. Natl. Acad. Sci. USA, *90:* 6335–6339, 1993.
12. Sugimura, T. Poly(adenosine diphosphate ribose). Prog. Nucleic Acid Res. Mol. Biol., *13:* 127–151, 1973.
13. Shall, S. ADP-ribose in DNA repair: a new component of DNA excision repair. Adv. Radiat. Biol., *11:* 1–69, 1984.
14. Ueda, K., and Hayaishi, O. ADP-ribosylation. Annu. Rev. Biochem., *54:* 73–100, 1985.
15. Cleaver, J. E., and Morgan, W. F. Poly(ADP-ribose)polymerase: a perplexing participant in cellular responses to DNA breakage. Mutat. Res., *257:* 1–18, 1991.
16. de Murcia, G., Ménissier-de Murcia, J., and Schreiber, V. Poly(ADP-ribose) polymerase: molecular biological aspects. Bioessays, *13:* 455–462, 1991.
17. Benjamin, R. C., and Gill, D. M. Poly(ADP-ribose) synthesis *in vitro* programmed by damaged DNA. J. Biol. Chem., *255:* 10502–10508, 1980.
18. Molinete, M., Vermeulen W., Bürkle A., Ménissier-de Murcia, J., Küpper, J. H., Hoeijmakers, J. H. J., and de Murcia, G. Overproduction of the poly(ADP-ribose) polymerase DNA-binding domain blocks alkylation-induced DNA repair synthesis in mammalian cells. EMBO J., *12:* 2109–2118, 1993.
19. Ferro, A. M., and Olivera, B. M. Poly(ADP-ribosyl)ation *in vitro*. J. Biol. Chem., *257:* 7808–7813, 1982.
20. Zahradka, P., and Ebisuzaki, K. A shuttle mechanism for DNA-protein interactions. Eur. J. Biochem., *127:* 579–585, 1982.
21. Bootsma, D., and Hoeijmakers, J. H. J. Engagement with transcription. Nature (Lond.), *363:* 114–115, 1993.
22. Cleaver, J. E., and Kraemer, K. H. Xeroderma pigmentosum. *In:* C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (eds.), The Metabolic Basis of Inherited Disease, pp. 2949–2971. New York: McGraw-Hill Book Co., 1986.
23. Kraemer, K. H., Lee, M. M., and Scotto, J. Xeroderma pigmentosum. Arch. Dermatol., *123:* 241–250, 1987.
24. Robbins, J. H. J. A childhood neurodegeneration due to defective DNA repair: a novel concept of disease based on studies xeroderma pigmentosum. J. Child. Neurol., *4:* 143–146, 1989.
25. Lindahl, T. Instability and decay of the primary structure of DNA. Nature (Lond.), *362:* 709–715, 1993.
26. Carmichael, P. L., Ni Shé, M., and Phillips, D. H. Detection and chractarization by ^{32}P-postlabelling of DNA adducts induced by a Fenton-type oxygen radical-generating system. Carcinogenesis (Lond.), *13:* 1127–1135, 1992.