DNA REPAIR

Takahiko Taguchi
Tokyo Metropolitan Institute of Gerontology, Japan

Keywords: mutator genes, environmental damage, base excision repair, nucleotide excision repair, and mismatch repair

Contents

Summary

DNA repair systems are needed by organisms as a defense against the ultraviolet light (UV) in sunshine. The ozone layer protects organisms, but as this protection is incomplete organisms still receive considerable DNA damage. For the accurate transfer of genetic information that enables organisms to survive, other systems to repair

damage and reproduce accurate genetic information are needed. These are the DNA repair systems.

There are many types of DNA damage. Therefore, various DNA repair systems must exist to provide appropriate repair and complete removal of the different kinds of DNA damage. Different systems exist that act by excising UV damaged DNA, including photoreactivation systems, base excision repair systems, nucleotide excision repair systems, and recombination repair systems. This indicates that organisms receive a great deal of damage from the UV light in sunshine. The important point for the repair of such damage is that the repair must take place before replication and transcription. DNA damage must be removed before the establishment of a mutation.

This article describes the kinds of damage and their detailed repair systems. The basics of repair systems are the release of DNA damage and gap filling by various DNA polymerases.

1. DNA Damage

The accurate transfer of genetic information to daughter cells is very important in maintaining the characteristics of cells. However, the DNA in all cells is constantly being damaged by intrinsic reactions and environmental agents. This damage influences genetic information by preventing transcription, replication, and cell division. As a result, organisms must excise the damage before transcription, replication, and cell division. Various repair systems exist to repair different kinds of damage. In the past, it was mistakenly thought possible to measure repair capacity by measuring only the size of DNA. Now repair capacity is known to involve many systems and to be reflected in enzyme activities. DNA damage related to DNA repair will be described.

1.1. Spontaneous Alterations of DNA (by Mutator Genes)

1.1.1 DNA Polymerase

As the basis of the genetic process, DNA must be synthesized with very high fidelity in living cells. Measurements of spontaneous mutation frequencies indicate that the average frequency of base-pair substitution is in the range of 10–8 to 10–11 misincorporations per base-pair replicated. It is concluded that the main source of intrinsic DNA alterations that occur during the normal metabolism of DNA synthesis is the mispairing of bases by DNA polymerases.

Most mismatch mutations that occur during *in vivo* DNA synthesis will be corrected by the mismatch repair system. The actual error rates of DNA polymerases I, III from *Escherichia coli* (*E.coli*) and the DNA polymerase from bacteriophage T4 are <10–6, while the error rates of DNA polymerases α, β, γ, δ, and ε from mammalian tissues are 10–4 to 10–5. Generally, the order of DNA polymerase fidelity is said to be δα, γ, and β. The fidelity of DNA polymerases related to following a single-strand binding protein improves the fidelity of DNA synthesis *in vitro*. Therefore, spontaneous mutation frequencies are greater than DNA polymerase fidelities. The causes of decreased DNA polymerase fidelity are partially known. DNA polymerases I, III, T4, δ, and γ possess a

3'–>5' exonuclease activity within their molecule that plays a role in proofreading during DNA synthesis. Therefore, the fidelity of DNA polymerase β may not be so high. On the other hand, the number of mismatch mutations is increased when mutant DNA polymerase is used for DNA synthesis. The fidelity of DNA synthesis falls when DNA synthesis is performed with small pools of dATP, dCTP, dGTP and dTTP, when Mn^{2+} and Co^{2+} ions are used instead of Mg^{2+}, and when error-prone DNA polymerases from cancer cells or tissues from aged animals are used. Error-prone DNA polymerases may be mutant DNA polymerases. Thus, although the fidelities of DNA polymerases are low, spontaneous mutation levels may be high because of the actions of 3'–>5' exonucleases, single-strand binding proteins, and mismatch repair.

1.1.2. Proofreading Activity

An activity that releases noncomplementary nucleotides from the 3'-end of a DNA strand is called a proofreading (editing) activity for DNA synthesis. One of the intrinsic causes of DNA alterations during DNA replication is a decrease in proofreading activity by 3'–>5'exonuclease. Most DNA polymerases possess a 3'–>5' exonuclease activity in their molecule as described above. This activity excises non-complementary nucleotides and contributes substantially to DNA replication fidelity. DNA polymerases α and β possess no 3'–>5' exonuclease activity. In rat liver, a 3'–>5' exonuclease apart from DNA polymerases α and β is present, and in the presence of both enzymes, this 3'–>5' exonuclease plays an important role in proofreading during DNA synthesis. When the 3'–>5' exonuclease activities in DNA polymerase molecules or the free form are lost, the rate of mismatch mutations increases. Thus, the decrease in proofreading activity becomes a cause for an increase in mismatch mutations.

Cytosine, adenine, guanine, and 5-methylcytosine are converted to uracil, hypoxanthine, xanthine, and thymine, respectively, by treatment at less than pH4.0 or by high-temperature. This is called base deamination and is also a cause of mismatch mutations.

1.2. Environmental Damage to DNA

The main causes of DNA damage are free radicals, ultraviolet light, radiation, and mutagens (alkylating agents, cross-linking agents, carcinogenic agents, anticancer agents, antibiotic agents, etc.). Oxidative DNA damage is caused by free radicals, UV light, ionizing radiation, carcinogenic agents, anticancer agents, and antibiotic agents (the damage by these environmental agents is not limited to oxidative DNA damage, as shown in the following sections). More than 20 oxidized bases are known and 8-hydroxy-2-deoxyguanosine (8OHdG) is one of the most frequent products of DNA base damage induced by oxidation. The presence of 8OHdG leads to mutations during DNA replication.

Exposure to UV light at 254 nm produces pyrimidine dimers whose ring structures result from the saturation of their 5,6 double bonds. The ratio of T-T, C-T, T-C and C-C is 68 : 13 : 16 : 3. As shown in Figure 1, *cis-syn* and *trans-syn* pyrimidine dimers occur as stereoisomers. Exposure to UV light produces the thymine-cytosine 6,4 photoproduct. The thymine-cytosine 6,4 photoproduct is produced by the covalent linkage of 6

thymines and 4 cytosines. Ionizing radiation also induces strand breaks by attacking sugar residues.

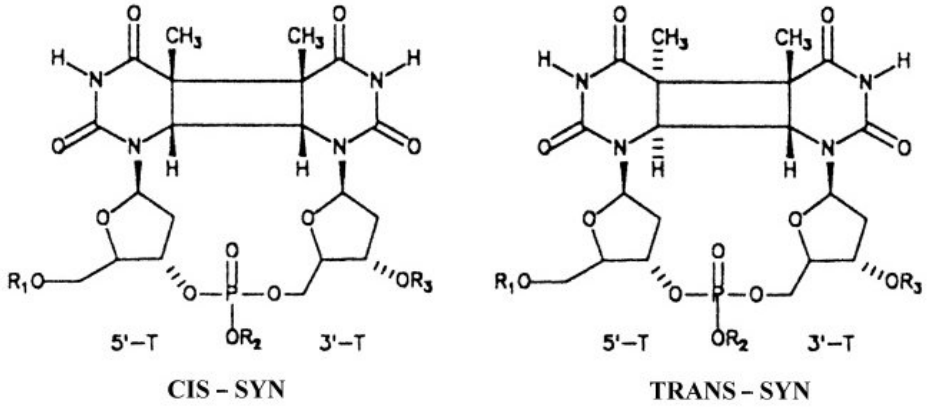

Figure 1. Two types of thymine dimers

A major group of chemical carcinogens acts as alkylating agents. These substances or their metabolites alkylate DNA at a number of sites, including the O^6-position of guanine. Many types of alkylation products are produced by various kinds of alkylating agents. The formation and persistence of O^6-alkylguanine in DNA is also linked to mutagenesis and carcinogenesis.

Interstrand DNA cross-links represent damage to DNA that completely blocks DNA replication and transcription. There are many types of cross-links caused by various cross-linking agents.

2. DNA Repair by Reversal of Damage Without Excision

2.1. Photoreactivation

It is known that the damage to organisms caused by exposure to UV light can be reversed by visible light. This phenomenon is called photoreactivation. In the case of *E.coli* , this reaction is performed by Class I cyclobutane pyrimidine dimer DNA photolyase. In higher animals and plants, Class II cyclobutane pyrimidine dimer DNA photolyase performs the reaction. On the other hand, (6,4) DNA photolyase has been reported in Drosophila and humans. These reactions involve electron transfer without any base or nucleotide excisions. These enzymes contain a chromophore.

2.2. Repair of O^6-Alkylguanine and Alkylthymine Without DNA Strand Excision

O^6-Methylguanine-DNA methyltransferase I (O^6-MGT I), which removes methyl groups from the guanine O^6 position without DNA strand excision, was extracted from *E.coli*. This enzyme retains the ability to transfer methyl groups from O^6-methylguanine and O^4-methylthymine. O^6-Methylguanine-DNA methyltransferase I is also called the Ada protein. Another enzyme that removes methyl groups extracted from *E.coli* is DNA alkyltransferase II (O^6-MGT II). This enzyme differs from O^6-MGT I in a number of respects. For example, O^6-MGT I is inducible by treatment with low levels of alkylating

agents, whereas O^6-MGT II is not; O^6-MGT I has a larger molecular mass than O^6-MGT II; O^6-MGT I, but not O^6-MGT II, catalyzes the removal of methyl groups from methylphosphotriesters. Although O^6-MGT I and O^6-MGT II are difficult to detect in extracts of some organisms, they are widespread in nature. It is interesting that there is a tendency for O^6-MGT I expression to be higher in tumors from patients who smoke more than 20 cigarettes per day than in tumors from patients who smoke less.

3. Base Excision Repair in Non-Mammalian Cells

Excision repair that is initiated by DNA glycosylase is called base excision repair. The basis of repair by this repair system involves:

1. The removal of the damaged base by hydrolysis of the N-glycosylase bond.
2. The formation of sites of base loss called apurinic or apyrimidinic sites (deoxyribose and monophosphate), which are removed by apurinic/apyrimidinic (AP) endonucleases.
3. The arrangement of strand breaks with a 5' terminal deoxyribose-phosphate moiety by the action of a DNA deoxyribophosphodiesterase (dRpase).
4. The filling of a single nucleotide gap by DNA polymerase.
5. Completion of DNA repair by rejoining with DNA ligase.

The hydrolysis of N-glycosylase bond involved in reaction (1) differs according to the kind of base damage.

3.1. DNA Glycosylase in Non-Mammalian Cells

Methylated base damage occurs by alkylation by environmental genotoxic agents. Methylated bases not removed by O^6-MGT I or II must be removed by other enzymes. 3-Methyladenine-DNA glycosylase I excises the N-glycosilase bond and releases 3-methyladenine. The enzyme also catalyzes the excision of 3-ethyladenine and 3-methylguanine from alkylated DNA. This enzyme has a molecular mass of 21KDa and a pH optimum between 6 and 8.5, and is stimulated by Mg2+, Mn2+, and Ca2+.

3-Methyladenine-DNA glycosylase II excises N-glycosilase bonds and releases 3-methyladenine, 7-methylguanine, and 3-methylguanine. This enzyme responds adaptively to alkylation damage. A gene for this enzyme, *alkA*, maps at approximately 43 min on the *E.coli* genetic map.

Uracil bases are present in nature, but are absent from DNA. Therefore, uracil bases in DNA are recognized in animals as alien. Uracil bases are incorporated by misreading and the deamination of cytosine. Uracil is removed through the action of uracil-DNA glycosylase. This enzyme is widespread from the herpes simplex virus to humans. The homology of the genes in hepes simplex virus and human is very high. The enzyme from *E.coli* is encoded by a gene designated *ung*, which maps at 55 min on the *E.coli* circular map.

Hypoxanthine is formed by the deamination of adenine. The hypoxanthine base is also present in nature, but absent from DNA. Therefore, hypoxanthine must be removed.

Hypoxanthine is first excised at the N-glycosilase bond by hypoxanthine-DNA glycosylase.

In *E.coli*, 8-OHdG-dA mispairs can also be corrected by a DNA glycosylase called MutY. This enzyme recognizes 8-OHdG-dA pairs formed by oxidation of guanine and misincorporation caused by oxidized guanine, and releases only adenine. As a result, 8-OHdG will be released in another step by Fapy glycosylase (MutM: described below).
An endonuclease from *E.coli* that degrades UV-irradiated DNA was observed in 1976 by Radman and called endonuclease III. In addition to excising pyrimidine hydrates, the DNA glycosylase activity of endonuclease III also excises pyrimidine glycols. Pyrimidine glycols are formed by the oxidation of pyrimidine. This enzyme is one kind of thymine glycol-DNA glycosylase.

When 7-ethylguanine is treated with methyl methane sulfonate (MMS) or NH4OH, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy) forms in DNA. This blocks *in vitro* DNA replication, and is released by Fapy-DNA glycosilase. This enzyme also releases 8-hydroxy-2-deoxyguanosine (8-OHdG), which is one of the most frequent products of DNA base damage by oxidation, and is identical to MutM. The substrate specificity of MutM is rather high. Duplexes containing 8-OHdG positioned opposite dC, dG, or dT are cleaved, whereas single-stranded DNA and duplexes containing 8-OHdG-dA, or deoxy8- 8-hydroxyadenine (8-OHdA) positioned opposite any of the four DNA bases are relatively resistant.

Although the photoreactivation system was mentioned above, another repair system that releases pyrimidine dimers by DNA glycolysis is present. Pyrimidine dimers are excised by pyrimidine dimer-DNA glycolases. These enzymes have been detected in extracts from T4 infected *E.coli*, *Micrococcus luteus*, and *Saccaromyces cerevisiae*.

4. Base Excision Repair in Mammalian Cells

Numerous DNA glycosylases have been found in mammals, and the pattern of base excision repair is the same as in non-mammalian cells.

4.1. DNA Glycosylases in Mammalian Cells

A 3-Methyladenine (3-alkyladenine, 3-methylpurine)-DNA glycosylase activity has been identified in various mammals including the Chinese hamster, mouse, rat, and human; however, it is not clear whether two distinct forms are present as in *E.coli*. The enzyme excises 3-methyladenine and 7-methylguanine, and, in the case of the human protein, its gene is located in chromosome 16.

Uracil bases are excised by uracil-DNA glycosylase. This enzyme has been observed in barley, wheat, rat mitochondria, human nuclei, and human mitochondria. All uracil-DNA glycosylases in non-mammalian cells utilize both double- and single-stranded DNA or deoxyribopolymers containing deoxyuridine as substrates. RNA-strands containing uracil are not recognized as substrate.

Hypoxanthine-DNA glycosylase has also been extracted from calf thymus and HeLa cells.

Thymine-DNA glycosylase is present in HeLa cells. This enzyme, which has not been found in procaryotes, is mismatch-specific, and prevents the mutagenic effect of G-T mispairs by the hydrolytic deamination of 5-methylcytosine with the release of thymine only. A 52.5-kDa enzyme with both 5-methylcytosine-DNA glycosylase and mismatch-specific thymine-DNA glycosylase activities has been extracted and purified from chicken embryos.

8-OHdG and thymidine glycol are formed during oxidative damage to bases in DNA and lead to mutations. Increases of 8-OHdG may be related to carcinogenesis and ageing. A glycosylase that releases 8-OHdG is also present in human, and is identical to Fapy-DNA glycosylase. Some groups have observed a human homologue (*hMMH*) to the yeast *OGG1* gene.

Thymine glycol is found in the urine of rodents and humans suggesting the presence of thymine glycol-DNA glycosylase. Actually, the mouse gene is homologous to the *E.coli* thymine glycol-DNA gene.

5-Hydroxymethyluracil-DNA glycosylase excises 5-hydroxymethyluracil. 5-Hydroxymethyluracil is produced by the base eoxidation and deamination of 5-hydroxymethylcytosine. 5-Hydroxymethyluracil-DNA glycosylase has been extracted from several mammalian cells, but the activity has not been found in procaryotic cells. The enzyme has been reported to be reduced in activity in senescent human fibloblasts.

4.2. AP Endonuclease

In the case of *E.coli*, it is generally assumed that the AP endonuclease functions by attacking sites of pyrimidine or purine loss in duplex DNA. Purified exonuclease III is 28 kDa in size, and catalyzes the hydrolysis of double AP DNA on the 5' side of sites of base loss, leaving 3'-OH and 5'-phosphate termini. The exonuclease III gene from *E.coli* has been cloned and sequenced. Its calculated molecular weight is in agreement with that of the purified protein.

Endonuclease IV is a second 5' AP endonuclease present in *E. coli*. This activity accounts for only about 10% of the total AP endonuclease activity.

Mammalian AP endonucleases have also been found in mice, calf thymus, and humans. The presence of a base excitation DNA repair system has been revealed from procaryotes to mammals.

4.3. DNA Deoxyribophosphodiesterase, DNA Polymerase, and DNA Ligase

After base-damage, gap filling and ligation by DNA polymerase and DNA ligase are necessary; these reactions require the 5'-phosphate and 3'-OH groups at the AP site. The polymerization reaction by a DNA polymerase is performed from the 3'-OH end of the primer strand arranged by a DNA deoxyribophosphodiesterase.

It is said that the DNA polymerase repair enzyme in *E.coli* is DNA polymerase I. On the other hand, the mammalian gap filling enzyme involved in base excision repair is DNA polymerase β. The requirement for DNA polymerase β has been suggested by induction and inhibition experiments.

-
-
-

Bibliography

Ahmmed, Z. and Laval, J. (1984). Enzymatic repair of O-alkylated thymidine residues in DNA: involvement of a O^4-methylthymine DNA methyltransferase and a O^2-methylthymine DNA glycosylase. *Biochemistry Biophysics Research Communication* **120**, 1–8.

Aruoma O.J., Halliwell B, and Dizdaroglu M. (1989). Iron ion-dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthin oxidase. *Journal of Biological Chemistry* **264**, 13024–13028.

Au K.G., Clark S., Miller J.H., and Modrich P. (1988). *Escherichia coli* MutY gene encodes an adenine glycosylase active on G-A mispairs. *Proceedings of the National Academy of Science United States of America* **86**, 8877–8888

Bebenek K., Joyce C.M., Fitzgerald M.P., Kunkel T.A. (1990). The fidelity of DNA synthesis catalyzed by derivatives of *Escherichia coli* DNA polymerase I. *Journal of Biological Chemistry* **265**, 13878–13887.

Bjelland S., Bjoras M., and Seeberg E. (1993). Excision of 3-methylguanine from alkylated DNA by 3-methyladenine DNA glycosylase I of *Escherichia coli*. *Nucleic Acids Research* **15**, 2045–2049.

Bohr V.A. (1993). Gene-specific DNA repair: characteristics and relations to genomic instability. *DNA Repair Mechanisms* (eds. V.A. Bohr, K. Wassemann, and K.H. Kaemer), pp. 217–227. Copenhagen: Munksgaard.

Boiteux S., Gajewski E., Laval J., and Dizdaroglu M. (1992). Substrate specificity of *Escherichia coli* Fpg protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry* **31**, 106–110.

Cathcart R., Schwiers E., Saul R.L., and Ames B.N. (1984). Thymineglycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. *Proceedings of the National Academy of Science United States of America* **81**, 5633–5637.

Chetsanga C.J., Lozon M., Makaroff C., and Savage L. (1981). Purification and characterization of *Escherichia coli* formamidopyrimidine-DNA glycosylase that excises damaged 7-methylguanine deoxyribonucleic acid. *Biochemistry* **20**, 5201–5207.

Clayton D.A., Noda J.N., and Friedberg E.C. (1974). The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proceedings of the National Academy of Science United States of America* **71**, 2777–2781.

Cleaver J.E., Green A.E., Coriell L.L., and Mulivor R.A. (1981). Xeroderma pigmentosum variant. *Cytogenetic Cellular Genetics* **31**, 188–192.

Coverey D., Kenny M.K., Lane D.P., and Wood R.D. (1992). A role for the human single-stranded DNA binding protein HSSB/RPA in an early stage of nucleotide excision repair. *Nucleic Acids Research* **20**, 3873–3880.

Domena J.D. and Mosbauugh D.W. (1985). Purification of mitochondrial uracil-DNA glycosylase from rat liver: identification of two distinct subcellular forms. *Biochemistry* **24**, 7320–7328.

Drake J.W. (1969). Comparative rates of spontaneous mutation. *Nature* **221**, 1132.

Drapkin R., Reardon J.T., Ansari A., Huang J.-C., Zawel L., Ahn K., Sancar A., and Reeinberg D. (1994). Dual role of TFIIH in DNA excision repair and in transcription by RNA polymerase II. *Nature* **368**, 769–772.

Franklin W.A., Doetsch P.W., and Haseltine W.A. (1985). Structural determination of the ultraviolet light-induced thymidine-cytosine pyrimidine-pyrimidone (6,4) photoproduct. *Nucleic Acids Research* **13**, 5317–5325.

Frey M.W., Nossal N.G., Capson T.L., and Benkovic S.J. (1993). Construction and characterization of a bacteriophage T4 DNA polymerase deficient in 3'–>5' exonuclease activity. *Proceedings of the National Academy of Science United States of America* **90**, 2579–2583.

Friedberg E.C., Walker G.C., and Siede W. (1995). *Nucleotide Excision Repair in Prokaryotes*. Washington, DC: ASM.

Ganguly T. and Duker N.J. (1990). Glycosylases that excise modified DNA pyrimidines in young and senescent human WI-38 fibroblasts. *Mutation Research* **237**, 107–115.

Goth, R. and Rajewsky M.F. (1974). Persistence of O^6-ethylguanine in rat-brain: correlation with nervous system-specific carcinogenesis by ethylnitrosourea. *Proceedings of the National Academy of Science United States of America* **71**, 639–643.

Gupta P.K. and Sirover M.A. (1981). Stimulation of the nuclear urascil DNA glycosylase in proliferating human fibroblast. *Cancer Research* **41**, 3133–3136.

Hilbert T.P., Boorstein R.J., Kung H.C., Bolton P.H., Xing D., Cunningham R.P., and Teebor G.W. (1996). Purification of a mammalian homologue of *Escherichia coli* endonuclease III: identification of a bovine pyrimidine hydrate-thymine glycol DNA-glycosylase/ap lyase by irreversible cross linking to a thymine glycol-containing oligodeoxynucleotide. *Biochemistry* **35**, 2505–2511.

Hollstein M.C., Brooks P., Linn S., and Ames B.N. (1984). Hydroxymethylurasil DNA glycosylase in mammalian cells. *Proceedings of the National Academy of Science United States of America* **81**, 4003–4007.

Holmes J., Clark S., and Modrich P. (1990). Strand-specific mismatch correction in nuclear extracts of human and *Drosophila meranogaster* cell lines. *Proceedings of the National Academy of Science United States of America* **87**, 5837–5841.

Houmier K.L., Gerschenson M., and Low R.L. (1991). Mitochondrial enonuclease activity in the rat varies markedly among tissues in relation to the rate of tissue metabolism. *Biochimica et Biophysica Acta* **1079**, 197–202.

Jinno S., Kida K., and Taguchi T. (1995). Induction of DNA polymerase and in the lungs of age-related oxygen tolerant rats. *Mechanisms of Ageing and Development* **85**, 95–107.

Karran P. and Lindahl T. (1980). Hypoxanthine in deoxyribonucleic acid: generation by heat-induced hydrolysis of adenine residues and release in free form by a deoxyribonucleic acid glycosylase from calf thymus. *Biochemistry* **19**, 6005–6011.

Kraemer K.H., Lee M.M., and Scotto J. (1984). Xeroderma pigmentosum. cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Achimus of Dermatology* **123**, 241–250.

Kunkel T.A. and Loeb L.A. (1979). On the fidelity of DNA replication: effect of divalent metal ion activators and deoxyribonucleoside triphoshate pools on *in vitro* mutagenesis. *Journal of Biological Chemistry* **254**, 5718–5725.

Kunkel T.A. and Loeb L.A. (1981). Fidelity of mammalian DNA polymerases. *Science* **213**, 765–767.

Kunkel T.A., Meyer R.R., and Loeb L.A. (1979). Single-strand binding protein enhances fidelity of DNA synthesis *in vitro*. *Proceedings of the National Academy of Science United States of America* **76**, 6331–6335.

Leadon S.E. and Cooper P.K. (1993). Preferential repair of ionizing radiation-induced damage in the transcribed strand of an active gene is defective in Cockayne syndrome. *Proceedings of the National Academy of Science United States of America* **90**, 10499–10503.

Levis L.K., Jenkins M.E., and Mount D.W. (1992). Isolation of DNA-damage inducible promoters in *E.coli*: regulation of polB (dinA), dinG, and dinH by LexA repressor. *Journal of Bacteriology*, **174**, 3377–3385.

Lindahl T., Ljungquist S., Siegert W. Nyberg B., and Sperens B. (1977). DNA N-glycosidases. properties of uracil-DNA glycosidase from *Escherichia coli*. *Journal of Biological Chemistry* **252**, 3286–3294.

Loeb L.A. and Kunkel T.A. (1982). Fidelity of DNA synthesis. *Annual Reviews of Biochemistry* **52**, 429–457.

Mattern J., Koomagi R., and Volm M. (1998). Smoking-related increase of O^6 methylguanine-DNA methyltransferase expression in human lung carcinomas. *Carcinogenesis* **19**, 1247–1250.

Matutani C., Sugasawa K., Yanigasawa J., Soniyama T., Ui M., Enomoto K., Takio K., Tanaka K., von der Spek P.J., Bootsma D., Hoeijmakers J.H.J., and Hanaoka F. (1994). Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23. *EMBO Journal* **13**, 1831–1843.

Modrich P. (1991). Mechanisms and biological effects of mismatch repair. *Annual Review of Genetics* **25**, 229–253.

Nance M.A. and Berry S.A. (1992). Cockayne syndrome: review of 140 cases. *American Journal of Medical Genetics*, **42**, 68–84.

O'Donovan A. and Wood R.D. (1993). Identical defects in DNA repair in xeroderma pigmenentosum group G and rodent ERCC group 5. *Nature* **363**, 185–188.

Pegg A.E., Wiest L., Foote R.S., Mitra, S., and Perry W. (1983). Purification and Properties of O^6-methylguanine-DNA transmethylase from rat liver. *Journal of Biological Chemistry* **258**, 2327–2333.

Radman M. (1976). An endonuclease from *Escherichia coli* that introduces single polynucleotide chain scissions in ultraviolet-irradiated DNA. *Journal of Biological Chemistry* **251**, 1438–1445.

Randahl H., Eliott G.C., and Linn S. (1988). DNA repair reactions by purified HeLa DNA polymerases and exonucleases. *Journal of Biological Chemistry* **263**, 12228–12234.

Reardon J.T., Nichols A.F., Keeney C.A., Smith C.A., Taylor J.-S., Linn S., and Sancar A. (1993). Comparative analysis of binding of human damaged DNA-binding protein (XPE) and *Escherichia coli* damage recognition protein (UvrA) to the major ultraviolet photoproducts: T[c, s]T, T[t, s]T, T[6-4]T, and T[Dewar]T. *Journal of Biological Chemistry* **268**, 21301–21308.

Reha-Krantz L.J. and Bessman M.J. (1977). Studies on the biochemical basis of mutation IV: effect of amino acid substitution on the enzymatic and biological properties of bacteriophage T4 DNA polymerase. *Journal of Molecular Biology* **116**, 99–113.

Richter C., Park J.-W., and Ames B.N. (1988). Normal oxidative damage to mitochondrial and nuclearDNA is extensive. *Proceedings of the National Academy of Science United States of America* **71**, 6465–6467.

Saki S., Akiyama K., Watanabe S., Hatsushika M., Ikeda S., and Tsutsui K. (1991). cDNA and deduced amino acid sequence of a mouse DNA repair enzyme (APEX nuclease) with significant homology to *Escherichia coli* exonuclease III. *Journal of Biological Chemistry* **266**, 20797–20802.

Samson L., Derfler B., Boosalis M., and Call K. (1991). Cloning and characterization of a 3-methyladenine DNA glycosylase cDNA from human cells whose gene maps to chromosome 16. *Proceedings of the National Academy of Science United States of America* **88**, 9127–9131.

Sancar, G.B., Smith, F.W. Lorence M.C., Rupert C.S., and Sancar A. (1984). Sequence of the *Escherichia coli* photolyase gene and protein. *Journal of Biological Chemistry 2***59**, 6033–6038.

Saparbaev M. and Laval J. (1994). Excision of hypoxanthine from DNA containing dIMP residues by the *Escherichia coli*, yeast, rat, and human alkylpurine DNA glycosylases. *Proceedings of the National Academy of Science United States of America* **91**, 5873–5877.

Sarker A.H., Ikeda S., Nakano H., Terato H., Ide H., Imai K., Akiyama K., Tsutsui K., Bo Z., Kubo K., Yamamoto K., Yasui A., Yoshida M.C., and Seki S. (1998). Cloning and characterization of a mouse homologue (mNth1) of *Escherichia coli* endonuclease III. *Journal of Molecular Biology* **282**, 761–774.

Seal G. and Sirover M.A. (1986). Physical association of the human base-excision repair enzyme uracil DNA glycosylase with the 70,000-dalton catalytic subunit of DNA polymerase α. *Proceedings of the National Academy of Science United States of America* **83**, 7608–7612.

Setlow R.B. (1966). Cyclobutane-type pyrimidine dimers in polynucleotides. *Science* **153**, 379–386.

Smerdon M.J.R., Gupta R., and Murad A.O. (1992). DNA repair in transcriptionally active chromatin. *DNA Repair Mechanisms* (eds. V.A. Bohr, K. Wassemann, and K.H. Kraemer), pp. 258–270. Copenhagen: Munksgaard.

Sung P., Bailly V., Weber C., Thompson L.H., Prakash L., and Prakash S. (1993). Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature* **365**, 852–855.

Taguchi T. and Ohashi M. (1996). Age-associated changes in the template-reading fidelity of DNA polymerase α from regenerating rat liver. *Mechanisms of Ageing and Development* **92**, 143–157.

Taguchi T. and Ohashi M. (1997). Changes in fidelity levels of DNA polymerases α-1, α-2, and β during ageing in rats. *Mechanisms of Ageing and Development* **99**, 33–47.

Taguchi T., Toda T., Fukuda M., and Ohashi M. (1998). Effect of a 3'–>5' exonuclease with a proofreading function on the fidelity of error-prone DNA polymerase α from regenerating liver of aged rats. *Mechanisms of Ageing and Development* **100**, 1–16.

Taylor J.-S., Garrett D.S., Brockie I.R., Svoboda D.L., and Telser J. (1990). 1H NMR assignment and melting temperature study of cis-syn and trans-syn thymine dimer containing duplexes of d(CGTATTATGC)-d(GCATAATAACG). *Biochemistry* **29**, 8855–8866.

Tchou J., Kasai H., Shibutani S., Chung M.-H., Laval J., Grollman A.P., and Nishimura S. (1991). 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proceedings of the National Academy of Science United States of America* **88**, 4690–4694.

Todo T., Takamori H., Ryo H., Ihara M., Matsunaga T., Nikaido O., Sato K., and Nomura T. (1993). A new photoreactivating enzyme that specifically ultraviolet light-induced (6,4) photoproducts *Nature* **361**, 371–374.

Tomkinson A.E., Bonk R.T., Kin N. Bartfeld N., and Linn S. (1990). Mammalian mitochondrial endonuclease activities specific for ultraviolet-irradiated DNA. *Nucleic Acids Research* **18**, 929–935.

Tomkinson A.E., Bonk R.T., and Linn S. (1988). Mitochondrial endonuclease activity specific for apurinic/apyrimidinic site in DNA from mouse cells. *Journal of Biological Chemistry* **263**, 12532–12537.

Troelstra C., van Gool A., de Wit J., Vermeulen W., Bootsma D., and Hoeijmakerrs J.H.J. (1992). ERCC, a member of a subfamily of putative helicases, is involved in Cockayne syndrome and preferential repair of active genes. *Cell* **71**, 939–953.

Varlet I., Radman M., and Brooks P. (1990). DNA mismatch repair in Xenopus egg extracts: repair efficiency and DNA repair synthesis for all single base-pair mismatches. *Proceedings of the National Academy of Science United States of America* **87**, 7883–7887.

Vermeulen R.A., van Vunren A.J., Chipoulet M., Scheffer L., Appeldoorm E., Weeda G., Jaspers N.G.J., Priestly A., Priestly A., Arlett C.F., Lehmann A.R., Stefanini M., Mezzina M., Sarasin A., Bootsma D., Egly J.-M., and Hoeijmakers J.H. (1994). Three unusual repair deficiencies associated with transcription factor BTF2 (TFIIH). evidence for the existence for the transcription syndrome. *Cold Spring Harber Symposia Quantative Biology* **59**, 317–329.

Wang T.S.-F. (1991). Eukaryotic DNA polymerases. *Annual Reviews of Biochemistry* **60**, 513–552.

White R.L. and Fox M.S. (1975). Genetic consequences of transfection with heteroduplex bacteriophages lambda DNA. *Molecular Gene Genetics* **141**, 163–171.

Wiebauer K. and Jiricny J. (1989). *In vitro* correction of G-T mispairs to G-C pairs in nuclear extracts from human cells. *Nature* **339**, 234–236.

Wiebauer K. and Jiricny J. (1990). Mismatch-specific thymine DNA glycosylase and DNA polymerase β mediate the correction of G-T mispairs in nuclear extracts from human cells. *Proceedings of the National Academy of Science United States of America* **87**, 5842–5845.

Yasuda S. and Sekiguchi M. (1970). T4 endonuclease involved in repair of DNA. *Proceedings of the National Academy of Science United States of America* **67**, 1839–1845.

Yu C.-E., Oshima J., Fu Y.-H., Wijsman E.M., Hisama F., Alisch R., Matthews S., Nakura J., Miki T., Ouais S., Martin G.M., Mulligan J., and Schellenberg G.D. (1996). Positional cloning of the Werner's syndrome gene. *Science* **272**, 258–262.

Biographical Sketch

Takahiko Taguchi was born in 1941. From 1962 to 1966 he studied at the Department of Zoology, Faculty of Science, Tokyo University of Education, receiving his B.Sc. in 1966. He then undertook post-graduate studies at the Master Course, Department of Zoology, Division for Research of Sciences, Graduate School of Tokyo University of Education, receiving his M.Sc. in 1968 and his Ph.D. in 1971.

From 1965 to 1972 he worked as a student fellow under Dr. Tetsuo Ono in the Laboratory of Biochemistry, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo. He served at the Department of Biochemistry, Tokyo Metropolitan Institute of Gerontology, as a Research Scientist from 1972 to 1980, and since as Senior Research Scientist (Department of Molecular Biology, and Gene Regulation and Protein Function).

He specializes in biochemistry in the field of ageing research, and is an active member of the Japanese Biochemical Society, the Japanese CancerAssociation, and the Japan Society for Biomedical Gerontology.