Review

Non-canonical hydrogen bonds in genetic information flow

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Abstract: Non-Watson-Crick-type hydrogen bonds involving minor nucleosides such as inosine in anticodon loops of transfer RNA are found in nucleic acids. Inosine contains hypoxanthine as a nucleobase and can form base pairs with various bases in the genetic decoding process. This property was applied to cloning complementary DNA by using oligodeoxyribonucleotide primers which contain deoxyinosine residues. Hypoxanthine is generated by deamination of adenine, one of the major nucleobases in DNA and RNA. Formation of unusual hydrogen bonds derived from damaged nucleobases in nucleic acids is thought to be main cause of disorders in genetic information flow. Mutagenesis and carcinogenesis of damaged bases, including hypoxanthine, 7, 8-dihydro-8-oxoguanine (8-oxoguanine) and pyrimidine photo-dimers in c-Ha-ras genes, were investigated by using synthetic genes containing modified nucleotides. The mode of recognition of unusual base pairing between a thymine photo-dimer and adenine by a repair enzyme was studied by X-ray analysis.

Key words: modified nucleobase; damaged nucleosides; synthetic gene; point mutation.

Introduction. The importance of the Watson-Crick-type hydrogen bonds[1] (Fig. 1) in molecular recognition has been shown in various steps of the genetic information flow, not only in replication and transcription of DNA but also in translation of the messenger RNA (mRNA) by transfer RNA (tRNA). The Watson-Crick type-hydrogen bonding plays an essential role in recognition between codon triplets in mRNA and anticodons in tRNA. For the confirmation of genetic codes, synthetic ribotriplets were used in a binding study for complex formation with cognate tRNA on ribosomes.[2] Inosine, a minor nucleoside containing hypoxanthine as a nucleobase, was found in the first position of the anticodon of yeast alanine tRNA and was assumed to form hydrogen bonds with all four major bases (Fig. 2). This kind of recognition process was elucidated by the wobble hypothesis.[3] We have mimicked this process in the cloning of complementary DNA deduced from amino acid sequences by using deoxyinosine-containing oligodeoxyribonucleotides.[4] The deduced nucleotide sequences include elements of ambiguity due to the degeneracy of amino acid codons. This problem was overcome by the use of this analogue because hypoxanthine can pair with any of four natural bases at the ambiguous position, with or without hydrogen bonding.

Deoxyinosine can be generated in DNA from deoxyadenosine by deamination either spontaneously or in the presence of nitrous acid.[5] Point mutations are thought to be induced in many cases by modification of a base in DNA by a mutagen and subsequent misincorporation of an incorrect deoxynucleoside triphosphate at the site opposite to the modified base catalyzed by DNA polymerase. The base pairing properties of hypoxanthine had been studied, and results had shown that hypoxanthine in oligonucleotides forms a stable base pair with an adenine or cytosine residue in the opposite strand.[6),7] Therefore, generation of hypoxanthine *in vivo* may lead to a point mutation. The mode of mutation was investigated by using synthetic oligonucleotides containing deoxyinosine at the deoxyadenosine position in the hot spot of the c-Ha-ras gene, and mutations with adenine to guanine were detected in the transformed cells.[8] Organic syntheses in combination with joining enzyme systems provide various approaches to studies of gene functions by preparation of genes that contain a modified base. Modified bases, O^6-methylguanine,[9] 7, 8-

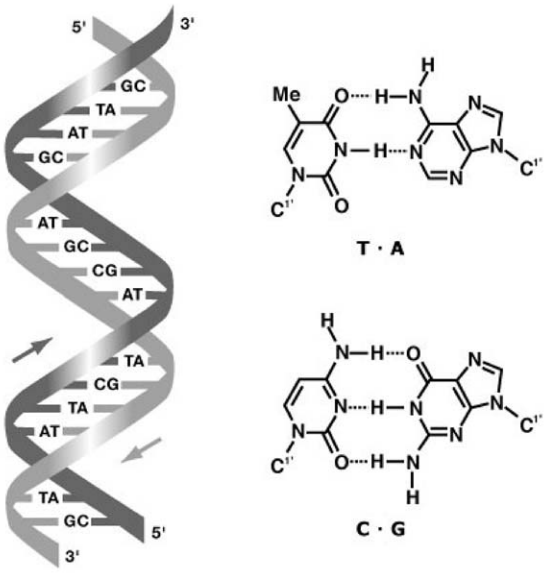

Fig. 1. Watson-Crick Type hydrogen bonds between thymine and adenine, between cytosine and guanine.

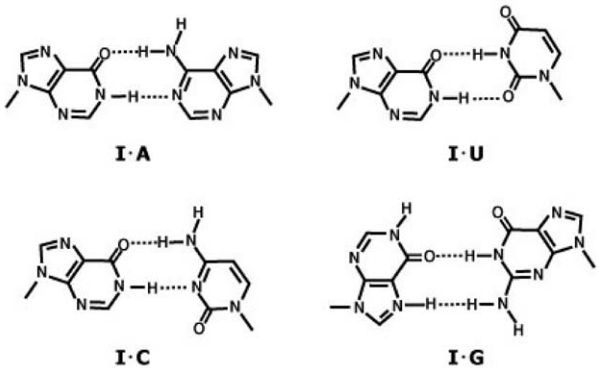

Fig. 2. Hydrogen bonds involving hypoxanthine-containing inosine.

dihydro-8-oxoguanine (8-oxoguanine),[10] pyrimidine photo-dimers[11] have been included by using chemically synthesized oligonucleotides. One of the oxidative products of guanine, 8-oxoguanine, was found to be formed by oxygen or X-ray radiation (Fig. 3). Pyrimidine photo-dimers were observed and identified in DNA after ultraviolet radiation (Fig. 4). These lesions were experimentally introduced into c-Ha-ras genes, and mutations were characterized by analysis of the genes from the transformed cells.

We also investigated the mode of interaction of this photo-lesion with a repair enzyme using synthetic DNA containing cyclobutane thymidine photo-dimers.

DNA cloning using deoxyinosine-containing oligodeoxyribonucleotides. Deoxyinosine can pair with any of the four major bases as shown in Fig. 2. In analyses of genes encoding proteins, DNA sequences deduced from the amino acid sequence include ambiguities due to the degeneracy of amino acid codons. We synthesized primer oligonucleotides that contain a deoxyinosine at the position complementary to the third nucleotide of the codon in mRNA of the protein. This approach was successfully used in cloning the human cholecystokinin (CCK) gene which is thought to be of interest in studying the expression both in the gut and brain.[4] The 26 mer oligonucleotide probe (Fig. 5) was designed on the basis of the amino acid sequence of porcine CCK8, Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2, the functional octapeptide unit from the carboxyl-terminus. Because of the low concentration of the mRNA in available human tissue, rather long probes were required to isolate the gene directly from a genomic library. In the case of usual cDNA cloning in which mRNA is abundant, a mixed sequence of 14-17 nucleotides long probes was often used.[12] Lack of a CCK-producing tumor cell line, the gene was directly isolated from a genomic library.[13] To determine the amino terminal sequence, DNA fragments were subcloned and analyzed by using a mixture of 14 mer oligonucleotides representing every possible codon combination deduced from the amino terminal pentapeptide. By Southern analyses using these restriction fragments, the preprocholecystokinin gene was determined and characterized. Deoxyinosine-containing DNA was also used as a template for DNA synthesis to see how DNA polymerase reads deoxyinosine residues in templates. The 26 mer was 3'-tailed with poly(dC), annealed with poly(dG)-tailed plasmids, and incubated with DNA polymerase (Klenow fragment). The resulting DNA ligated to a plasmid vector circularized was transformed into *E. coli*. When the resulting recombinant plasmid DNA was sequenced, all of the positions for deoxyinosine were replaced by deoxyguanosine. The DNA polymerase selected deoxycytidine to pair with deoxyinosine.

Induction of mutation of a synthetic c-Ha-ras gene containing Deoxyinosine. Activated ras genes have been detected in tumor cells and are thought to be involved in tumor initiation and progression.[14),15] These activated ras genes have been found to have a point mutation at a specific site, either in codon 12, 13, or 61 or

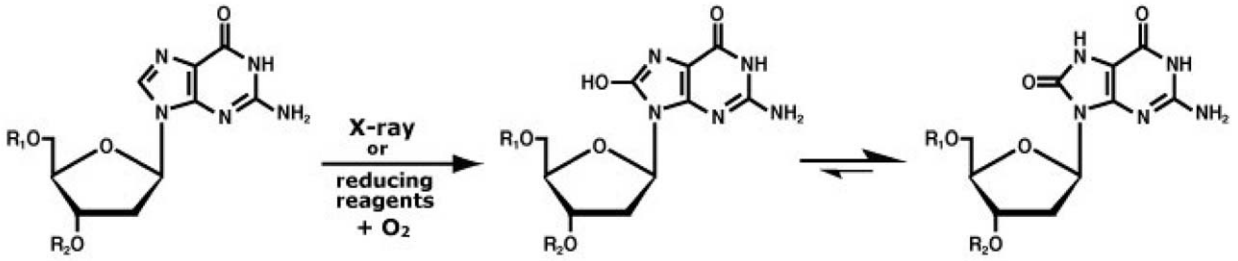

Fig. 3. Formation of 8-oxodeoxyguanosine.

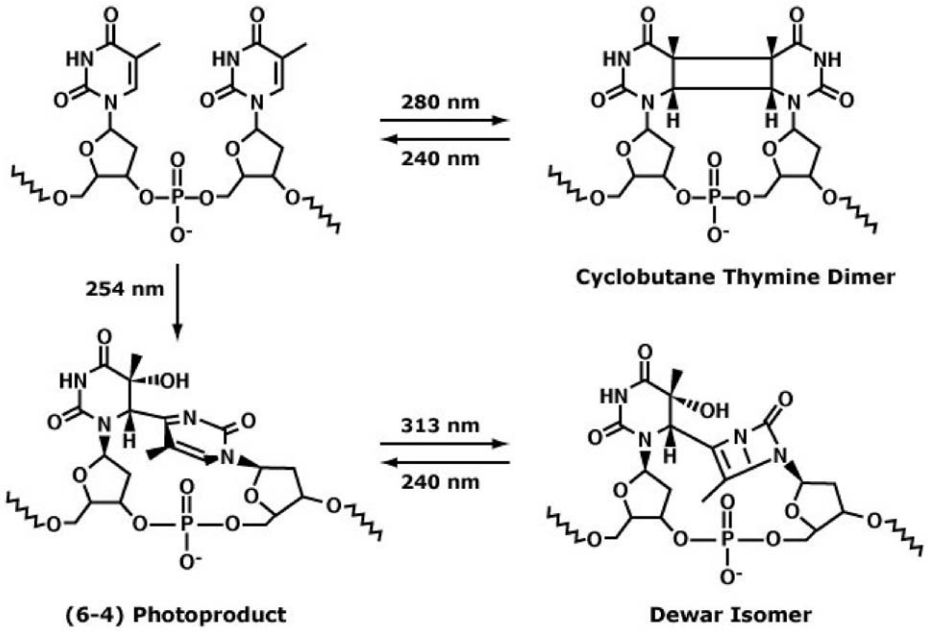

Fig. 4. Reactions of thymidines in DNA by UV irradiation.

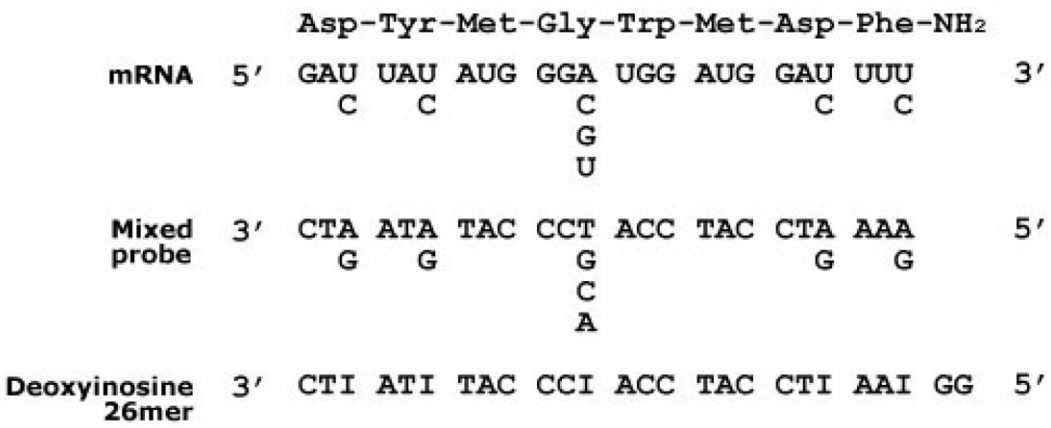

Fig. 5. Deoxyribo primer (26 mer) containing deoxyinosine at ambiguous positions.

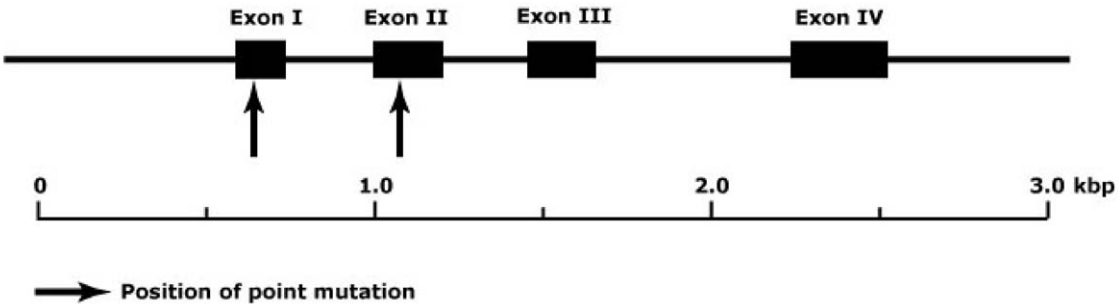

Fig. 6. c-Ha-ras gene containing four exons. Allows indicate hot spots, codon 12 and 61.

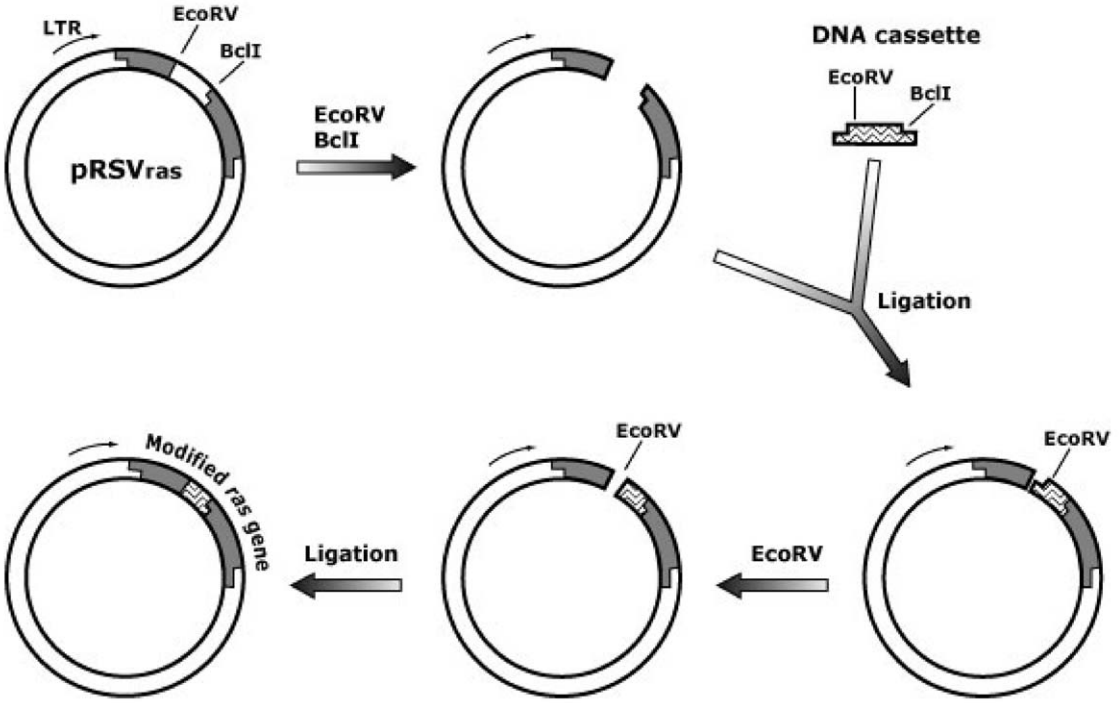

Fig. 7. Construction expression vectors by insertion of the mini duplex.

some other position that induces substitution of an amino acid in the ras-encoded protein, RAS (Fig. 6). The mutant proteins differ from normal RAS in having lower levels of GTPase activities and altered guanine nucleotide-binding properties. These mutant proteins remain as GTP complexes and disturb normal signal transduction reactions. We have synthesized c-Ha-ras genes with or without a mutation at the 12th amino acid position and expressed them in *E. coli*.[16] The tertiary structure of the RAS protein complexed with GDP or GTP analogues was investigated by X-ray structural analysis.[17),18] The regions around the 12th , 13th, and 61st amino acids were found to be in contact with a phosphate group of bound guanine nucleotides. The synthetic genes with a mutation at the 12th amino acid codon also showed transforming activity in cultured NIH3T3 cells.[19] These results showed that these cells can be used to test mutagenicity of modified bases by introduction to the oncogene at the specific position.

A point mutation was demonstrated by introduction of hypoxanthine in the second adenine base of the hot spot at codon 61 of a synthetic c-Ha-ras gene by synthe-

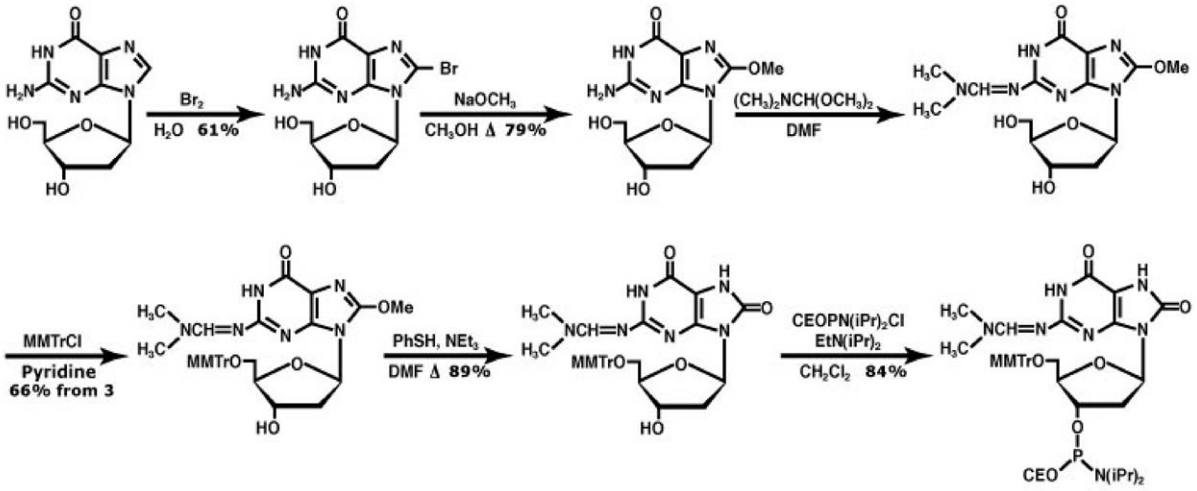

Fig. 8. Synthetic scheme for protected 8-oxodeoxyguanosine amidite unit. Abbreviations: MMTr, monomethoxytrityl; CE, cyanoethyl; iPr, isopropyl.

sizing a duplex containing deoxyinosine.[8] The gene was joined to an expression vector (Fig. 7) and transfected into NIH3T3 cells. Transfection resulted in increased focus formation. Total DNA was extracted from the transformed cells, and the sequence of the inserted c-Ha-ras DNA was analyzed by the polymerase chain reaction-single-strand conformation polymorphism method.[20] Mutations with adenine-to-guanine transition were exclusively detected, indicating that hypoxanthine paired with cytosine during replication in this case.

Possible base pairs of 8-oxoguanine during replication in the c-Ha-ras gene. One of oxidative lesions of nucleobases, 8-oxoguanine (Fig. 3), was found to be formed in DNA *in vitro* by various oxygen radical-producing agents.[21] The formation of 8-oxoguanine was also observed in cellular DNA *in vivo* by radiation or oxygen radical-forming carcinogens. This lesion is now known as a major cause of diseases related to aging. In order to investigate the mutagenicity of this compound, incorporation of nucleotides to the opposite site of 8-oxoguanine by DNA polymerase was analyzed, and various misreadings were found even in neighboring positions.[22),23] These findings suggest that one reason of mutation at guanine in a specific position of oncogenes is a result of oxidation of guanine to 8-oxoguanine.

To determine the type of mutation induced by 8-oxoguanine in an oncogene system, we constructed a synthetic c-Ha-ras gene containing 8-oxoguanine in the second position of codon 12 (GGC). For obtaining a gene fragment containing 8-oxoguanine, a properly protected deoxynucleotide unit was synthesized as shown in Fig. 8.[24] The protected amidite unit was used in the machine synthesis to obtain an oligonucleotide fragment. The fragment was ligated into the synthetic gene and joined with a c-Ha-ras-expression vector. The mutant gene was transfected into NIH3T3 cells, and focus-forming activity of the mutant gene was compared with that of the normal gene. The c-Ha-ras gene containing 8-oxoguanine induced more foci than did the normal c-Ha-ras gene but only about 1 to 2% of the number induced by the activated c-Ha-ras gene (GAC at codon 12, Asp-12). The relatively low efficiency might be due to the normal base pairing of 8-oxoguanine with cytosine. An enzyme responsible for removal of 8-oxoguanine from DNA also reduces the efficiency of transformation. The c-Ha-ras gene present in these foci was analyzed by the polymerase chain reaction-restriction enzyme method. The mutation was characterized by sequence analyses, and random mutations were revealed at the modified site (G to T, G to A, and G to C) as well as mutations of the adjacent G on the 5'-side of 8-oxoguanine (G to A and G to T). The G-to-T transversion can be explained by base pairing between 8-oxodeoxyguanosine and deoxyadenosine in the duplex DNA. The base pairing between 8-oxodeoxyguanosine and deoxycytidine and that between 8-oxodeoxyguanosine and deoxyadenosine were elucidated by nuclear magnetic resonance stud-

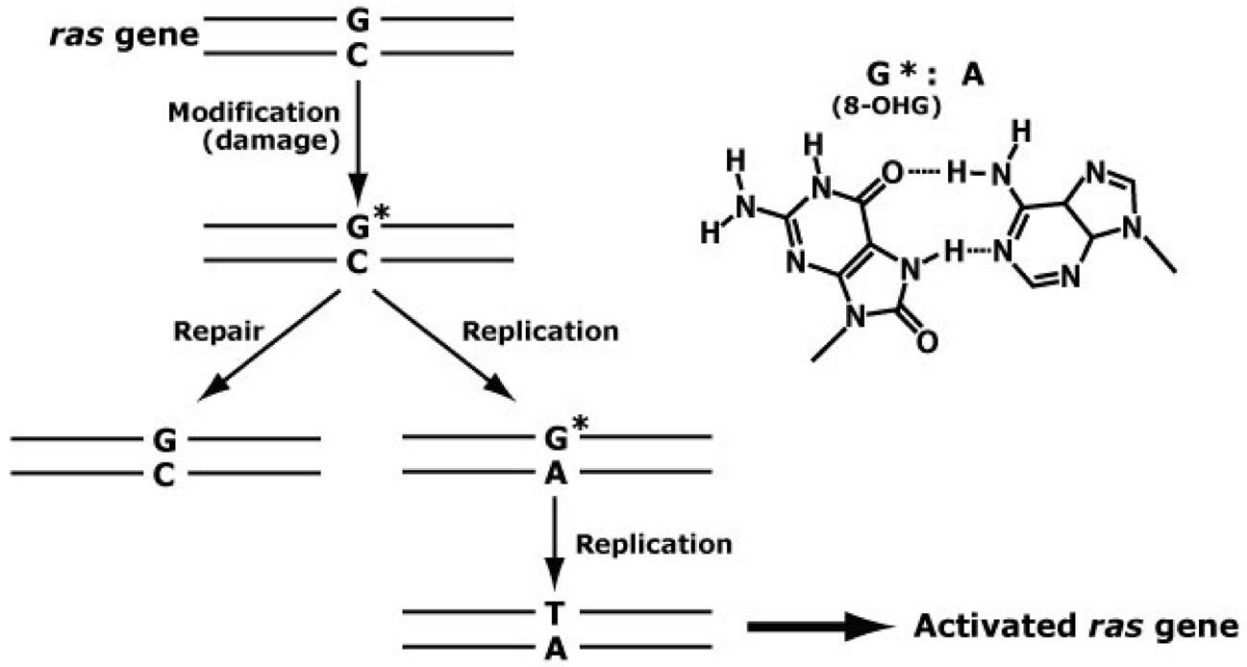

Fig. 9. Possible flow for G-to-T tranversion.

ies.[25),26)] Fig. 9 shows a possible flow of the G-to-T transversion in this system. If the repair systems of the oxidized guanine bases do not work efficiently during replication, this type of mutation can occur *in vivo*.

Mutations at thymine photo-dimers in DNA. Thymine photo-dimers (Fig. 4) are major photoproducts in duplex DNA induced by ultraviolet (UV) light and are thought to be involved in mutagenesis in prokaryotes.[27)] Patients with xeroderma pigmentosum (XP) syndrome develop skin cancer in areas exposed to sunlight, and cells derived from XP patients cannot effectively repair UV-induced DNA damage.[28)] These findings indicate that the DNA damage induced by UV light might be related to carcinogenesis at least in the case of XP patients.

To investigate the mutagenicity of these photolesions, DNA fragments containing a cyclobutane thymine photodimer (*cis-syn* or *trans-syn*) were synthesized. Fig. 10 illustrates synthetic procedures for the preparation of nucleotide units.[29)] These synthones were used to synthesize the fragments by the phosphoramidite method, and the oligonucleotides were ligated to build a cassette modified at the opposite site of codon 61. The obtained DNA cassette was joined to the synthetic Ha-ras and incorporated into expression vectors. When the vectors were transfected into mouse NIH3T3 cells, they induced focus formation. Sequence analysis of ras gene fragments derived from the transformed cells showed that the genes were activated by a point mutation. The mutations detected most frequently were 3'- T to A for the *cis-syn* isomer and 5'-T to A for the *trans-syn* isomer.

These results together with the results of mutation by modified bases are summarized in Table I.

Flipping out of an adenine base complementary to the thymine photo-dimer in the presence of a repair enzyme. *E. coli* cells become relatively resistant to UV radiation when infected with bacteriophage T4. T4 endonuclease V is an enzyme that repairs DNA by cleaving the 5'-side of the thymine base in thymine photo-dimers in a DNA duplex as a pyrimidine dimer glycosylase.[30)] This small enzyme having 168 amino acids also catalyzes cleavage of the DNA chain as an apurinic-apyrimidinic endonuclease. This latter reaction proceeds through β-elimination of the 3'-phosphate of an abasic site rather than through actual hydrolysis of the phosphodiester bond.[31)] The enzyme nonspecifically binds by electrostatic force before binding to a pyrimidine dimer. Once the enzyme has specifically bound to a pyrimidine dimer, the 5'-glycosyl bond is incised in the dimer, and scission of the phosphodiester

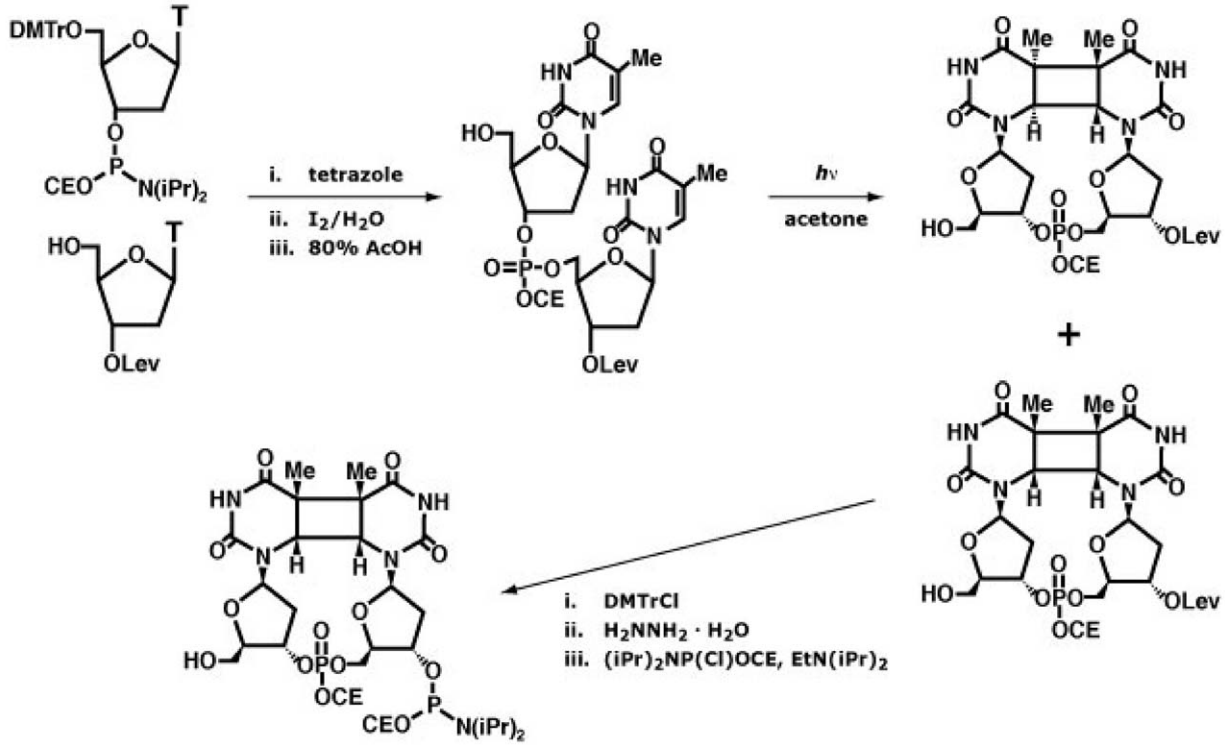

Fig. 10. Synthetic scheme for *cis-syn* thymidine dimer amidite units. Lev refers to levulynyl. Other abbreviations are described in Fig. 8.

Table I. Major mutations by modified bases at hot spots in the *ras* gene

Nucleoside containing modified base	Introduced position	Major mutation
O^6-Methyldeoxyguanosine	12th GGC	GAC
8-Oxodeoxyguanosine	12th GGC	GTC, GAC
Deoxyinosine	61st CAA	CGA
cis-syn Thymine dimer	61st GTT	GAT
trans-syn Thymine dimer	61st GTT	GTA

bond subsequently occurs at the backbone. The three-dimensional X-ray structure of the enzyme was determined by using the gene product of the synthetic gene,[32] and site-directed mutagenesis was performed to identify residues participating in substrate binding and the catalytic reaction.[33] From the amino acid sequence, a characteristic aromatic cluster was found in the C-terminal region and was thought to be involved in the elimination reaction. However, the three-dimensional structure showed an all α-structure, and the C-terminal segment forms a rigid loop near the active site to stabilize the structure by polar interactions between the loop and the two helical ends.[34] The crystal structure of this enzyme complexed with a duplex DNA substrate containing a thymine dimer revealed the unique conformation of the DNA duplex, which exhibits a sharp kink with a 60 degree inclination at the central thymine dimer.[35] The adenine base complementary to the 5'-side of the thymine dimer is completely flipped out of the DNA duplex and trapped in a cavity on the protein surface as shown in Fig. 11. The hydrogen bonds between the 5'-thymine and the opposite adenine are broken by interaction with the enzyme, and this event stimulates cleavage reaction of the 5'-glycosyl bond by facilitating

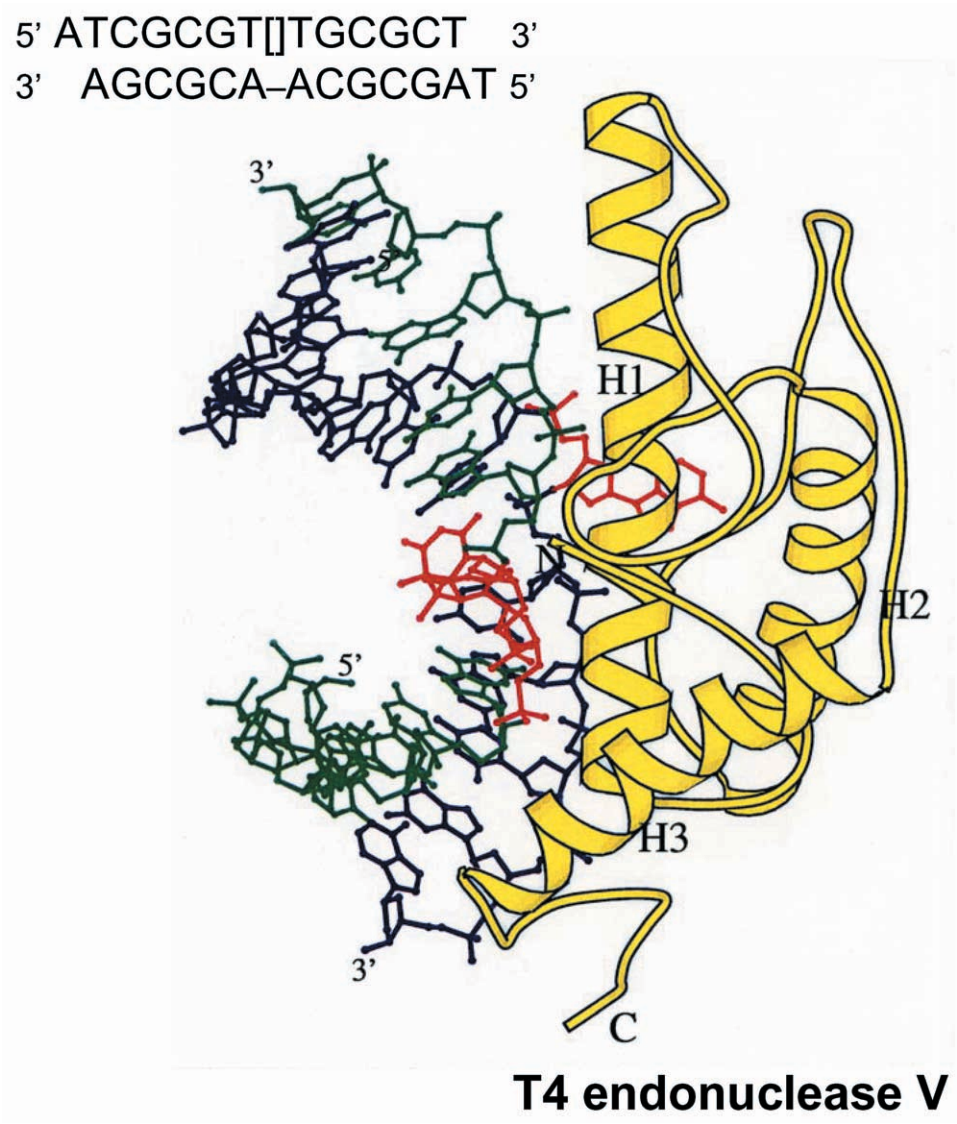

Fig. 11. Structure of T4 endonuclease V-DNA complex. The flipped-out nucleotide and the 5'-side adenylate are colored red.

contact to the active site. Recognition of unusual hydrogen bonds between the thymine dimer and the complementary adenine by the enzyme is the initiation for the repair reaction. These structural features suggest the catalytic mechanism of how repair enzymes recognize damaged DNA duplexes.

Future direction of structure-function relationship studies of nucleic acids. Recent findings on the function of double stranded RNA in a range of sequence-specific gene silencing phenomena in many organisms suggest the importance of hydrogen bonds in RNA.[36] Depending on the nature of the target sequence and the participating proteins, different type of silencing can occur. RNA interference (RNAi) is initiated by small interfering RNA (siRNA) duplexes, 21-26 nucleotides in length. Another class of small RNA, microRNAs (miRNAs), can induce silencing by targeting mRNA. Depending on their degree of complementarity to the target mRNA, they elicit either translational repression or mRNA cleavage. Post-transcriptional gene silencing that is induced by siRNA and miRNA achieves specificity through RNA-RNA sequence recognition

and base pairing. The RNAi process also affects gene function at the level of genomic DNA. Studies on properties of hydrogen bonds in DNA and RNA will become important in functional genomics.

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References

1) Watson, J. D., and Crick, F. H. (1953) Genetical implications of the structure of deoxyribonucleic acid. Nature **171**, 964-967.
2) Söll, D., Ohtsuka, E., Jones, D. S., Lohrmann, R., Hayatsu, H., Nishimura, S., and Khorana, H. G. (1965) Studies on polynucleotides, XLIX. Stimulation of the binding of aminoacyl-sRNA's to ribosomes by ribotrinucleotides and a survey of codon assignments for 20 amino acids. Proc. Natl. Acad. Sci. USA **54**, 1378-1385.
3) Crick, F. H. (1966) Codon—anticodon pairing: the wobble hypothesis. J. Mol. Biol. **19**, 548-555.
4) Ohtsuka, E., Matsuki, S., Ikehara, M., Takahashi, Y., and Matsubara, K. (1985) An alternative approach to deoxyoligonucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions. J. Biol. Chem. **260**, 2605-2608.
5) Shapiro, H. S., and Chargaff, E. (1966) Studies on the nucleotide arrangement in deoxyribonucleic acids. XI. Selective removal of cytosine as a tool for the study of the nucleotide arrangement in deoxyribonucleic acid. Biochemistry **5**, 3012-3018.
6) Uesugi, S., Oda, Y., Ikehara, M., Kawase, Y., and Ohtsuka, E. (1987) Identification of I:A mismatch base-pairing structure in DNA. J. Biol. Chem. **262**, 6965-6968.
7) Kawase, R., Iwai, S., and Ohtsuka, E. (1989) Synthesis and thermal stability. Chem. Pharm. Bull. (Tokyo) **37**, 2313-2317.
8) Kamiya, H., Miura, H., Kato, H., Nishimura, S., and Ohtsuka, E. (1992) Induction of mutation of a synthetic c-Ha-ras gene containing hypoxanthine. Cancer Res. **52**, 1836-1839.
9) Kamiya, H., Miura, K., Ohtomo, N., Koda, T., Kakinuma, M., Nishimura, S., and Ohtsuka, E. (1989) Transformation of NIH3T3 cells with synthetic c-Ha-*ras* genes. Jpn. J. Cancer Res. **80**, 200-203.
10) Kamiya, H., Miura, K., Ishikawa, H., Inoue, H., Nishimura, S., and Ohtsuka, E. (1992) c-Ha-ras containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. Cancer Res. **52**, 3483-3485.
11) Kamiya, H., Murata, N., Murata, T., Iwai, S., Matsukage, A., Masutani, C., Hanaoka, F., and Ohtsuka, E. (1993) Cyclobutane thymine dimers in a *ras* proto-oncogene hot spot activate the gene by point mutation. Nucleic Acids Res. **21**, 2355-2361.
12) Ito, H., Ike, Y., Ikuta, S., and Itakura, K. (1982) Solid phase synthesis of polynucleotides. VI. Further studies on polystyrene copolymers for the solid support. Nucleic Acids Res. **10**, 1755-1769.
13) Takahashi, Y., Kato, K., Hayashizaki, Y., Wakabayashi, T., Ohtsuka, E., Matsuki, S., Ikehara, M., and Matsubara, K. (1985) Molecular cloning of the human cholecystokinin gene by use of a synthetic probe containing deoxyinosine. Proc. Natl. Acad. Sci. USA **82**, 1931-1935.
14) Barbacid, M. (1987) ras Genes. Annu. Rev. Biochem. **56**, 779-827.
15) Nishimura, S., and Sekiya, T. (1987) Human cancer and cellular oncogenes. Biochem. J. **243**, 313-327.
16) Miura, K., Kamiya, H., Tominaga, M., Inoue, Y., Ikehara, M., Noguchi, S., Nishimura, S., and Ohtsuka, E. (1987) Overproduction of cellular and activated Ha-ras proteins by mutating a synthetic gene. Chem. Pharm. Bull. (Tokyo) **35**, 4878-4882.
17) Brünger, A. T., Milburn, M. V., Tong, L., deVos, A. M., Jancarik, J., Yamaizumi, Z., Nishimura, S., Ohtsuka, E., and Kim, S. H. (1990) Crystal structure of an active form of RAS protein, a complex of a GTP analog and the HRAS p21 catalytic domain. Proc. Natl. Acad. Sci. USA **87**, 4849-4853.
18) Pai, E. F., Kabsch, W., Krengel, U., Holmes, K. C., John, J., and Wittinghofer, A. (1989) Structure of the guanine-nucleotide-binding domain of the Ha-ras oncogene product p21 in the triphosphate conformation. Nature **341**, 209-214.
19) Kamiya, H., Miura, K., Ohtomo, N., Nishimura, S., and Ohtsuka, E. (1991) Transforming activity of a synthetic c-Ha-ras gene containing O^6-methylguanine in codon 12. Jpn. J. Cancer Res. **82**, 997-1002.
20) Iwahana, H., Orita, M., Kanazawa, H., Hayashi, K., and Sekiya, T. (1990) A new RFLP in intron 1 of the human c-Ha-ras1 gene and its close relationship with the variable tandem repeats in the region 3' to the gene. Oncogene **5**, 1049-1053.
21) Kasai, H., and Nishimura, S. (1984) Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res. **12**, 2137-2145.
22) Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E., and Nishimura, S. (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. Nature **327**, 77-79.
23) Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G → T and A → C substitutions. J. Biol. Chem. **267**, 166-172.
24) Koizume, S., Kamiya, H., Inoue, H., and Ohtsuka, E. (1994) Synthesis and thermodynamic stabilities of damaged DNA involving 8-hydroxyguanine(7,8-dihydro-8-oxoguanine) in a ras-gene fragment. Nucleosides & Nucleotides **13**, 1517-1534.

25) Oda, Y., Uesugi, S., Ikehara, M., Nishimura, S., Kawase, Y., Ishikawa, H., Inoue, H., and Ohtsuka, E. (1991) NMR studies of a DNA containing 8-hydroxydeoxyguanosine. Nucleic Acids Res. **19**, 1407-1412.
26) Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P., and Patel, D. J. (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-Oxo-7H-dG(syn). dA(anti) alignment at lesion site. Biochemistry **30**, 1403-1412.
27) LeClerc, J. E., and Istock, N. L. (1982) Specificity of UV mutagenesis in the lac promoter of M13lac hybrid phage DNA. Nature **297**, 596-598.
28) Cleaver, J. E. (1968) Defective repair replication of DNA in xeroderma pigmentosum. Nature **218**, 652-656.
29) Murata, T., Iwai, S., and Ohtsuka, E. (1990) Synthesis and characterization of a substrate for T4 endonuclease V containing a phosphorodithioate linkage at the thymine dimer site. Nucleic Acids Res. **18**, 7279-7286.
30) Yasuda, S., and Sekiguchi, M. (1970) T4 endonuclease involved in repair of DNA. Proc. Natl. Acad. Sci. USA **67**, 1839-1845.
31) Mazumder, A., Gerlt, J. A., Rabow, L., Absalon, M. J., Stubbe, J., and Philip H. Bolton, P. H. (1989) UV endonuclease V from bacteriophage T4 catalyzes DNA strand cleavage at aldehydic abasic sites by a syn. beta.-elimination reaction. J. Am. Chem. Soc. **111**, 8029-8030.
32) Inaoka, T., Ishida, M., and Ohtsuka, E. (1989) Affinity of single- or double-stranded oligodeoxyribonucleotides containing a thymine photodimer for T4 endonuclease V. J. Biol. Chem. **264**, 2609-2614.
33) Hori, N., Doi, T., Karaki, Y., Kikuchi, M., Ikehara, M., and Ohtsuka, E. (1992) Participation of glutamic acid 23 of T4 endonuclease V in the beta-elimination reaction of an abasic site in a synthetic duplex DNA. Nucleic Acids Res. **20**, 4761-4764.
34) Morikawa, K., Matsumoto, O., Tsujimoto, M., Katayanagi, K., Ariyoshi, M., Doi, T., Ikehara, M., Inaoka, T., and Ohtsuka, E. (1992) X-ray structure of T4 endonuclease V: an excision repair enzyme specific for a pyrimidine dimer. Science **256**, 523-526.
35) Vassylyev, D. G., Kashiwagi, T., Mikami, Y., Ariyoshi, M., Iwai, S., Ohtsuka, E., and Morikawa, K. (1995) Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition. Cell **83**, 773-782.
36) Matzke, M. A., and Birchler, J. A. (2005) RNAi-mediated pathways in the nucleus. Nature Rev. Genetics **6**, 24-35.

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Profile

Eiko Ohtsuka was born in 1936 and received her Ph. D. under Yoshihisa Mizuno at Hokkaido University, where she collaborated with Yuji Tonomura and Morio Ikehara in studies of ATP analogs in muscle contraction. She then joined Khorana's group as a postdoctoral fellow at the University of Wisconsin to work on synthesis of nucleic acids for the genetic code. She participated in the work on the total synthesis of formylmethionine tRNA in Osaka University in Ikehara's group and performed the gene synthesis of human growth hormone. She was promoted to Professor in Hokkaido University in 1984 and started synthetic approaches of structure-function relationship of catalytic RNA, and protein engineering for molecular recognition in nucleic acids including synthesis of damaged DNA as substrates of cognate enzymes or as a part of synthetic genes. She has received an award from the pharmaceutical Society of Japan, the twentieth anniversary award of the Princess Takamatsu Cancer Research Fund, and the Japan Academy Prize in 1996. She is an honorary member of the Japanese Biochemical Society, and the Pharmaceutical Society of Japan. Since 2004, she is Auditor of Hokkaido University.