In *Escherichia coli*, there are five DNA polymerases [7]. DNA pol I encoded by *polA* is the first DNA polymerase to be described and is known to be involved in DNA repair and the processing of Okazaki fragments [9,10]. The expression of DNA pol II encoded by *polB* is damage-inducible [11,12], and this polymerase is involved in the process to restart the synthesis of damaged DNA [13,14]. DNA pol III is an enzyme responsible for the chromosome replication of *E. coli*, and the *dnaE* gene encodes the catalytic subunit of this holoenzyme [15–18]. The expression of recently identified Y-family DNA polymerases, i.e. pol IV encoded by *dinB* and pol V by *umuDC*, are also damage-inducible [19–22]. Among them, DNA pol V is known to play important roles in mutagenesis induced by a variety of genotoxic agents [23,24]. DNA pol II is reported to be involved in −2 frameshift induced by 2-acetylaminofluorene (2-AAF) [25,26] and DNA pol IV plays a role in −1 frameshift induced by 4-nitroquinoline *N*-oxide (4-NQO) and benzo[*a*]pyrene (BaP) [27,28]. However, the current knowledge about the role of each DNA polymerase in mutagenesis is still limited to a small number of mutagens because oligonucleotides bearing specific DNA adducts are required for in vitro and in vivo mutation assays to address the question.

Salmonella typhimurium is a Gram negative bacterium, and the genome sequence is 70–90% homologous to that of *E. coli* at the nucleotide level [29]. In fact, there are homologues of the *polA*, *polB*, *dnaE*, *dinB* and *umuDC* genes (hereafter, they are designated as $polA_{ST}$, $polB_{ST}$, $dnaE_{ST}$, $dinB_{ST}$ and $umuDC_{ST}$). In addition, another homologue of *umuDC*, i.e. *samAB*, resides on an extra-chromosomal plasmid, pSLT [30]. The promoter regions of $polB_{ST}$, $dinB_{ST}$, $umuDC_{ST}$ and *samAB* have potential LexA binding sites, i.e. SOS box, and the damage-inducible expression of $polB_{ST}$ and $umuD_{ST}$ has been demonstrated [31,32]. Several strains of *S. typhimurium* are widely used to identify environmental mutagens and carcinogens [33]. Strain TA1538 is one of such tester strains and has a mutational hotspot of CGCGCGCG repetitive sequence in the *hisD* gene so that frameshift mutagens that induce −2 frameshifts are very sensitively detected [34,35]. In addition, the strain lacks part of lipopolysaccharide (*rfa*) of the cell wall, which increases the permeability of hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAHs) [36]. The strain also defects in an excision repair capacity, and thus DNA lesions are more likely to be bypassed rather than removed by repair enzymes [36].

To better understand the role of each DNA polymerase in chemically induced frameshift mutagenesis, we took advantage of the genetic characteristics of *S. typhimurium* TA1538. We systematically deleted the genes encoding SOS-inducible DNA polymerases of the strain and examined their mutability to 26 chemical mutagens. The mutagenicity of 13 chemicals was significantly reduced by the deletion of one of the genes encoding SOS-inducible DNA polymerases. However, remaining 13 chemicals including 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) and 4-aminobiphenyl (4-ABP) still retained the full mutagenicity even after all the genes encoding SOS-inducible DNA polymerases were deleted. We propose that the replicative DNA polymerase, i.e. DNA polymerase III (pol III) holoenzyme, in addition to SOS-inducible DNA polymerases, can skip various bulky lesions in the CG repetitive sequence, thereby inducing −2 frameshift mutations. The role of each DNA polymerase in the frameshift mutagenesis is discussed.

2. Materials and methods

2.1. Strains and plasmids

The strains and the plasmids used in this study are listed in Table 1. The plasmid pYG787 carrying *E. coli polB* was constructed by the insertion of a 2.82 kb *Cla*I-*Nde*I fragment of pTH100 carrying *polB* [11] between *Cla*I and *Sma*I sites of plasmid pWKS30 [37].

2.2. Chemicals

The names, abbreviations, CAS registry numbers and sources of the chemicals assayed in this study are listed in Table 2. The chemical structures are presented in Fig. 1.

2.3. Media

Luria-Bertani broth and agar [38] were used for bacterial cultures. Tetracycline (Tc, 10 μg/ml), spectinomycin (Sp, 50 μg/ml), kanamycin (Km, 25 μg/ml) or chloramphenicol (10 μg/ml) was added to the medium for the cultures of $polB_{ST}$, $dinB_{ST}$, $umuDC_{ST}$ or *samAB* deletion strains, respectively. Vogel-Bonner minimal agar plates and top agar were prepared as previously described [33] and used for the His^+ reversion assay with *S. typhimurium*. Nutrient broth (Difco, MI, USA) with antibiotic was used for pre-cultures of the strains for the reversion assay.

2.4. Cloning of $polB_{ST}$ and $dinB_{ST}$ genes

The DNA fragments containing the $polB_{ST}$ and $dinB_{ST}$ genes were amplified from the chromosomal DNA of *S. typhimurium* TA1538 by PCR. The sequences of the primers were as follows: for $polB_{ST}$, 5′-TCACATTGGTCTCTTCGGTATCCAGGCGGG-3′ ($polB_{ST}$-F) and 5′-TCACGGTTGGAAGGGTTAGCGCGTT-3′ ($polB_{ST}$-R); for $dinB_{ST}$, 5′-CTGCCAATTGGCGTCGGTCCATCCGGAACG-3′ ($dinB_{ST}$-F), where the underlined sequence was changed to generate the *Mfe*I recognition site (CAATTG) and 5′-CGTTTCATACCGGTCAACTTAGCCGTCGTA-3′ ($dinB_{ST}$-R), where the underlined sequence was changed to disrupt the *Hpa*I recognition site (GTTAAC). Both PCR products were blunt-ended with Klenow fragment (New England Biolabs, MA) and digested with *Mfe*I. The 4.1 kb DNA fragment

Table 1
S. typhimurium strains and plasmids

Strain or plasmid	Description	Source
Strains		
TA1538	*hisD3052*, *gal*, Δ(*chl*, *uvrB*, *bio*), *rfa*	[33]
YG5147	As TA1538, but Δ*umuDC*$_{ST}$::Km^r, Δ*samAB*::Cm^r	[35]
YG6205	As TA1538, but Δ*dinB*$_{ST}$::Sp^r	This study
YG6208	As TA1538, but Δ*polB*$_{ST}$::Tc^r	This study
YG6215	As TA1538, but Δ*umuDC*$_{ST}$::Km^r, Δ*samAB*::Cm^r, Δ*dinB*$_{ST}$::Sp^r, Δ*polB*$_{ST}$::Tc^r	This study
Plasmids		
pYG501	As pBR322 but its 1.4 kb *Eco*RI-*Msc*I fragment is replaced with a 2.2 kb fragment carrying *dinB*$_{ST}$ gene	This study
pYG503	As pBR322 but its 1.4 kb *Eco*RI-*Msc*I fragment is replaced with a 4.1 kb fragment carrying *polB*$_{ST}$ gene	This study
pYG504	As pYG503 but its 2.4 kb *Eco*RV-*Eco*RI fragment is replaced with a 1.4 kb fragment carrying a Tc^r gene	This study
pYG505	As pYG501 but its 0.6 kb *Eco*RV-*Hpa*I fragment is replaced with a 2.0 kb fragment carrying a Sp^r gene	This study
pYG768	Derivative of pWSK29 with the *E. coli dinB* gene	[37]
pYG779	As pYG768, but *dinB003* (D103N)	[19,27]
pGW2101	Derivative of pZ150 with the *E. coli umuDC* genes	[55]
pGW2020	As pGW2101, but Δ*umuC*	[55]
pYG787	Derivative of pWKS30 with the *E. coli polB* gene	This study
pYG8031	Derivative of pBR322 with the *umuDC*$_{ST}$ genes	[30]

Km^r, resistant to kanamycin; Cm^r, resistant to chloramphenicol; Sp^r, resistant to spectinomycin; Tc^r, resistant to tetracycline.

carrying *polB*$_{ST}$ or the 2.2 kb DNA fragment carrying *dinB*$_{ST}$ was inserted between *Eco*RI and *Msc*I sites of vector plasmid pBR322. The resulting plasmids were designated as pYG503 and pYG501, respectively. The sequences of the *polB*$_{ST}$ or *dinB*$_{ST}$ coding region of these plasmids were matched with the published sequences [29].

2.5. Construction of deletion strains

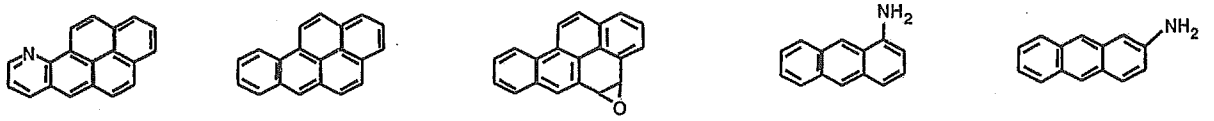
The *polB*$_{ST}$ and *dinB*$_{ST}$ genes were disrupted in strain TA1538 by the method previously described [39]. In the case of *polB*$_{ST}$ deletion strain, plasmid pYG503 was digested with *Eco*RV and *Eco*RI, and the resulting 4.6 kb DNA frag-

Table 2
Names, aberreviations, CAS registry numbers and sources of the chemicals

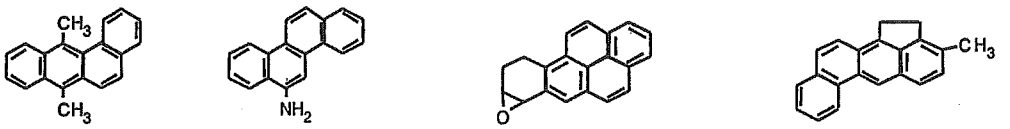
Chemical no.	Chemical	CAS registry numbers	Sources[a]
1	10-Azabenzo[*a*]pyrene (10-azaBaP)	189-92-4	1
2	Benzo[*a*]pyrene (BaP)	50-32-8	W
3	Benzo[*a*]pyrene 4, 5-dihydroepoxide (BaP4, 5-dihydroepoxide)	64437-52-1	Mi
4	1-Aminoanthracene (1-AA)	610-49-1	S
5	2-Aminoanthracene (2-AA)	613-13-8	W
6	7,12-Dimethylbenz[*a*]anthracene (DMBA)	57-97-6	W
7	6-Aminochrysene (6-AC)	2642-98-0	S
8	Benzo[*a*]pyrene 7, 8-tetrahydroepoxide (BaP7, 8-tetrahydroepoxide)	36504-67-3	Mi
9	3-Methylcholanthrene (3-MC)	56-49-5	S
10	1-Nitropyrene (1-NP)	5522-43-0	T
11	1,8-Dinitropyrene (1,8-DNP)	42397-65-9	T
12	1-Nitro-6-azabenzo[*a*]pyrene (1-*N*-6-azaBaP)	138835-35-5	4
13	3-Nitro-6-azabenzo[*a*]pyrene (3-*N*-6-azaBaP)	138835-35-6	4
14	1-Nitrobenzo[*a*]pyrene (1-NBaP)	70021-99-7	4
15	3-Nitrobenzo[*a*]pyrene (3-NBaP)	70021-98-6	4
16	4-Aminobiphenyl (4-ABP)	92-67-1	2
17	4-Nitroquinoline-*N*-oxide (4-NQO)	56-57-5	T
18	2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1)	67730-11-4	W
19	Aminophenylnorharman (APNH)	219959-86-1	3
20	Acridine orange (AO)	65-61-2	Me
21	Benz[*a*]anthracene (BA)	56-55-3	S
22	*N*-hydroxyacetylaminofluorene (*N*-OH-AAF)	53-95-2	Mi
23	2-Nitrofluorene (2-NF)	607-57-8	T
24	2-Acetylaminofluorene (2-AAF)	53-96-3	T
25	2-Aminofluorene (2-AF)	153-78-6	N
26	2-[2-(Acetylamino)-4-[bis(2-methoxyethyl)-amino]-5-amino-7-bromo-4-chloro-2H-benzotriazole (PBTA-1)	194590-84-6	3

[a] The chemicals were purchased from the following sources at the highest grade of purity: Wako Pure Chemical (W); Tokyo Kasei Kogyo (T); Sigma–Aldrich (S); Merck (Me); Midwest Research Institute (Mi); Nacalai Tesque (N). Commercially unavailable chemicals were provided by the following persons: Dr. Ken-ichi Saeki, Nagoya City University, Japan (1); Dr. Takeji Takamura-Enya, National Cancer Center Research Institute, Tokyo, Japan (2); Dr. Yukari Totsuka, National Cancer Center Research Institute, Tokyo, Japan (3); Dr. Kiyoshi Fukuhara, National Institute of Health Sciences, Tokyo, Japan (4).

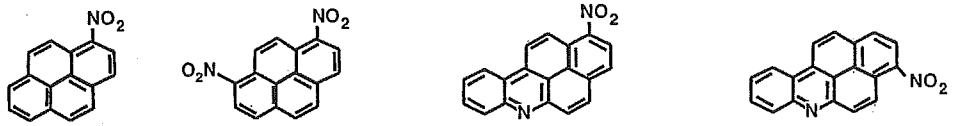
Class I



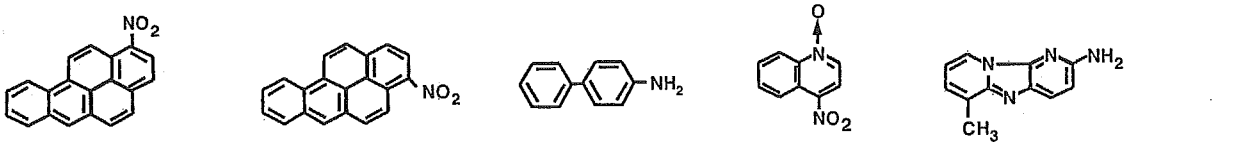
Class II

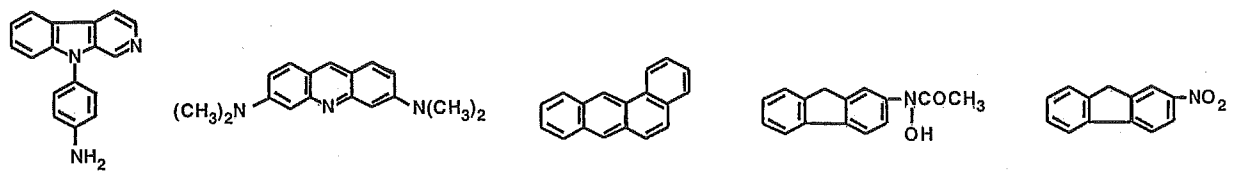


Class III



Class IV





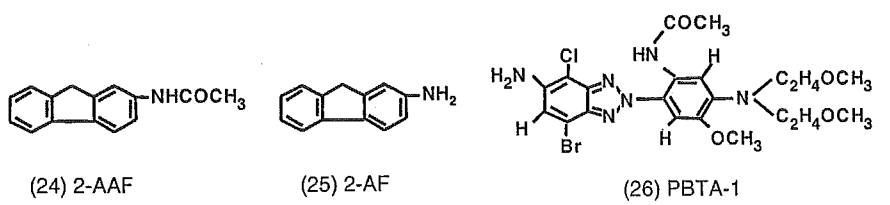


Fig. 1. Structures of the chemical mutagens used in this study.

ment was purified. A 1.4 kb *Eco*RI-*Msc*I fragment of pBR322 carrying the Tc resistance (Tc^r) gene was inserted to the purified fragment, and the resulting plasmid was designated as pYG504. The plasmid was digested with *Apa*LI to remove the replication origin of the vector. The 4.3 kb linear DNA fragment carrying the Tc^r gene between the flanking regions of $polB_{ST}$ gene was purified, treated with T4 DNA ligase, and introduced into TA1538 by electroporation. Tc resistant colonies were selected and examined for deletion of the chromosomal $polB_{ST}$ gene by PCR. The sizes of PCR products with primers $polB_{ST}$-F and $polB_{ST}$-F2 (5′-GACGTTCAA ATCTGCTCCCG-3′) were 3.7 and 2.7 kb for $polB_{ST}$ and $\Delta polB_{ST}$::Tc^r, respectively. The $\Delta polB_{ST}$ strain was designated as YG6208. In the case of $dinB_{ST}$ deletion strain, plasmid pYG501 was digested with *Eco*RV and *Hpa*I, and the resulting 4.5 kb DNA fragment was purified. A 2.0 kb *Sma*I–*Sma*I fragment of plasmid pKRP13 (National Institute of Genetics, Shizuoka, Japan) carrying the Sp resistance (Sp^r) gene was inserted to the purified fragment, and the resulting plasmid was designated as pYG505. Plasmid pYG505 was digested with *Pvu*II and *Sca*I and the 4.5 kb linear DNA fragment carrying the Sp^r gene was treated with T4 DNA ligase. The products were introduced into TA1538 by electroporation. The Sp resistant colonies were selected and examined for deletion of the chromosomal $dinB_{ST}$ gene by PCR. The sizes of PCR products with primers $dinB_{ST}$-R and $dinB_{ST}$-F2 (5′-CAAGGGTGCGCTCTTCTTTA-3′) were 1.8 and 3.2 kb for $dinB_{ST}$ and $\Delta dinB_{ST}$::Sp^r, respectively. The resulting $\Delta dinB_{ST}$ strain was designated as YG6205. In the case of four genes deletion strain, the $dinB_{ST}$ gene of strain YG5147 ($\Delta umuDC_{ST}\Delta samAB$) was disrupted as described above, and then the $polB_{ST}$ gene was disrupted. The resulting strain ($\Delta polB_{ST}$, $\Delta dinB_{ST}$, $\Delta umuDC_{ST}$ and $\Delta samAB$) was designated as YG6215.

2.6. Mutagenicity assay

The mutagenicity assay was carried out with a preincubation procedure [33]. Briefly, 0.1 ml overnight culture was incubated with the chemicals dissolved in 0.1 ml solvent and 0.5 ml of S9 mix for 20 min at 37 °C. When S9 mix is not required, 0.5 ml of 1/15 M phosphate buffer pH 7.4 was added. The mixture was then poured onto agar plates with soft agar and incubated for 2 days at 37 °C. Each chemical was assayed with four–seven doses on triplicate plates with five strains, i.e. TA1538, YG6208, YG6205, YG5147 and YG6215, in parallel. Statistical significance was analyzed with Student's *t*-test. A *P*-value < 0.01 denoted the presence of a statistically significant difference.

2.7. Identification of frameshift mutations in the CG repetitive sequence of the hisD gene of S. typhimurium TA1538 and YG6215

Total DNA was prepared from His^+ revertants of *S. typhimurium* TA1538 and YG6215. The revertants were either spontaneous or chemically induced mutants. Part of *hisD* gene including the CG repetitive sequence was amplified and the DNA was digested with *Bss*HII as described [35]. The digested DNA was analyzed by 2% agarose gel electrophoresis followed by visualization with ethidium bromide.

3. Results

3.1. Roles of multiple DNA polymerases in frameshift induced by 26 chemicals

To examine the role of each DNA polymerase in bypass of DNA lesions, we systematically deleted one or all of the genes encoding SOS-inducible DNA polymerases and examined their mutability to 26 chemical mutagens. Actual dose–response curves are presented in Fig. 2 and the numbers of induced revertants per microgram per plate of each chemical and strain are summarized in Table 3. For five mutagens, i.e. 1,8-DNP, 1-*N*-6-azaBaP, 3-*N*-6-azaBaP, 4-NQO and Glu-P-1, the numbers of revertants per nanogram instead of microgram are presented because of their potent mutagenicity. To make the comparison easier, we also calculated the relative mutability of each derivative by assigning the number of revertants per microgram (or nanogram) in strain TA1538 as 1.0. According to the pattern of the mutagenicity, we classified the 26 chemicals into four classes as follows.

Class I includes 10-azaBaP, BaP, BaP4,5-dihydroepoxide, 1-AA and 2-AA. The mutagenicity of these compounds largely depends on the presence of $dinB_{ST}$. When $dinB_{ST}$ was deleted, the mutagenicity was reduced to the level similar to that of strain YG6215, i.e. $\Delta polB_{ST}$, $\Delta dinB_{ST}$, $\Delta umuDC_{ST}$ and $\Delta samAB$ strain. Deletion of $polB_{ST}$ or $umuDC_{ST}$ plus *samAB* did not significantly reduce the mutagenicity. It should be noted, however, that 20–40% of the mutagenicity remained even after $dinB_{ST}$ was deleted.

Class II includes DMBA, 6-AC, BaP7,8-tetrahydroepoxide and 3-MC. The mutagenicity of these compounds is reduced to the level similar to that of strain YG6215 either by $dinB_{ST}$ deletion or by $umuDC_{ST}$ plus *samAB* deletion. Unlike class I or III (see below) chemicals, $dinB_{ST}$ deletion and $umuDC_{ST}$ plus *samAB* deletion reduced the mutagenicity to similar extent. For example, either $dinB_{ST}$ deletion or $umuDC_{ST}$ plus *samAB* deletion reduced the mutagenicity of DMBA by half, and the resulting mutagenicity was similar to that observed in strain YG6215. In addition to $dinB_{ST}$ and $umuDC_{ST}$ plus *samAB*, BaP7, 8-tetrahydroepoxide and 3-MC require the presence of $polB_{ST}$ for the maximum mutagenesis. When one of $polB_{ST}$, $dinB_{ST}$ or $umuDC_{ST}$ plus *samAB* was deleted, the mutagenicity of BaP7, 8-tetrahydroepoxide and 3-MC was reduced to the level similar to that of YG6215. Even after all four genes were deleted, however, substantial mutagenicity, i.e. 30–70%, of four compounds remained in strain YG6215.

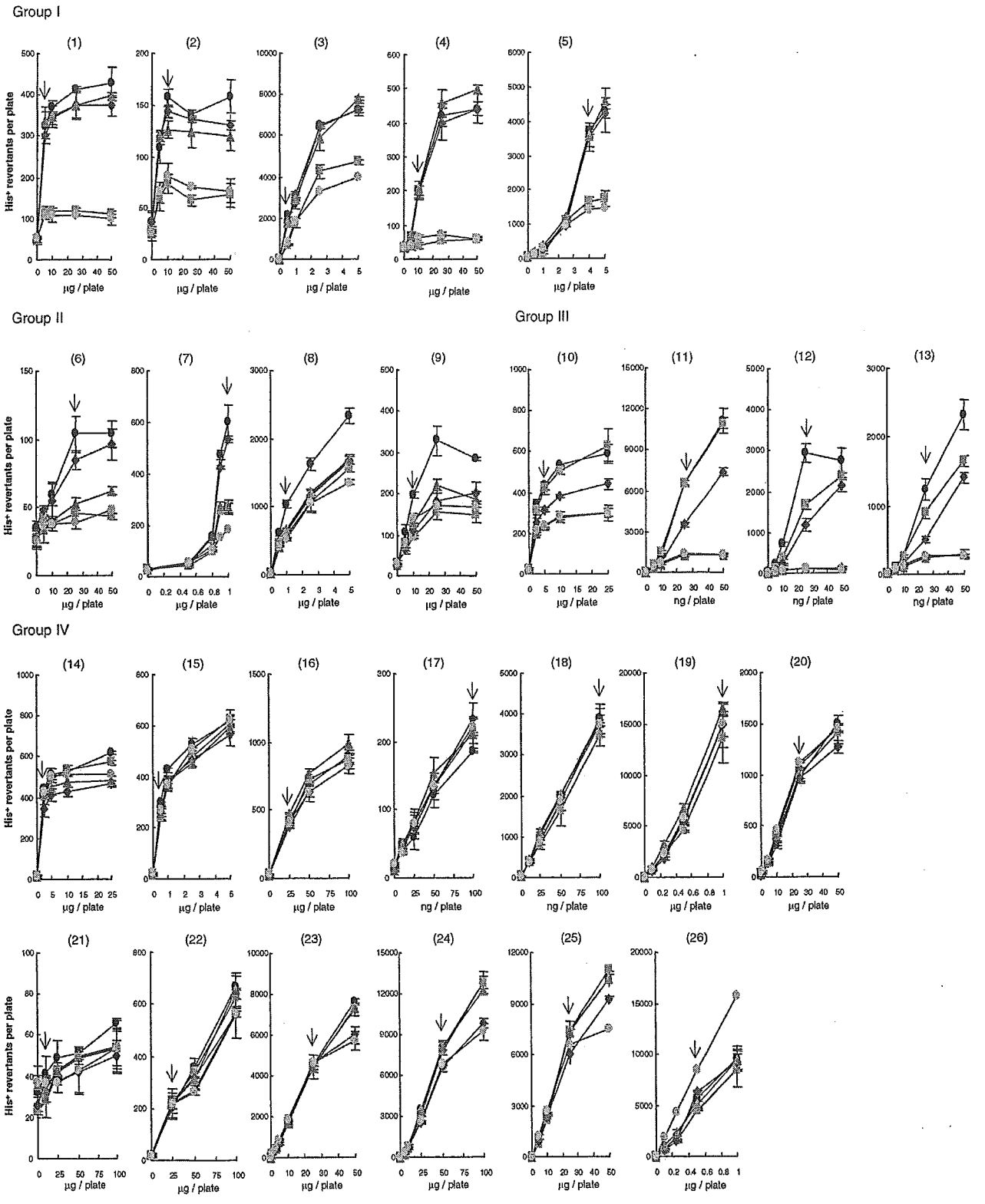


Fig. 2. Responses of *Salmonella typhimurium* tester strains to 26 chemical mutagens. The chemicals are: 10-azaBaP (1); BaP (2); BaP4,5-dihydroepoxide (3); 1-AA (4); 2-AA (5); DMBA (6); 6-AC (7); BaP7,8-tetrahydroepoxide (8); 3-MC (9); 1-NP (10); 1,8-DNP (11); 1-*N*-6-azaBaP (12); 3-*N*-6-azaBaP (13); 1-NBaP (14); 3-NBaP (15); 4-ABP (16); 4-NQO (17); Glu-P-1 (18); APNH (19); AO (20); BA (21); *N*-OH-AAF (22); 2-NF (23); 2-AAF (24); 2-AF (25) and PBTA-1 (26). The strains used are: TA1538 (black circles); YG6208 (blue diamonds); YG6205 (red squares); YG5147 (green triangles) and YG6215 (yellow circles). The arrow indicates the dose that was used for the calculation of His^+ revertants per microgram or nanogram per plate in Table 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Table 3
Mutagenicity of 26 chemicals in *S. typhimurium* strains deficient in one or all of SOS-inducible DNA polymerases

No.	Chemical	Class[a]	S9 mix[b]	His$^+$ revertants per plate per microgram[c]				
				Wild-type TA1538	Δ*polB*$_{ST}$ YG6208	Δ*dinB*$_{ST}$ YG6205	Δ*umuDC*$_{ST}$, Δ*samAB* YG5147	Δ*polB*$_{ST}$, Δ*dinB*$_{ST}$, Δ*umuDC*$_{ST}$, Δ*samAB* YG6215
1	10-AzaBap	I	+[d]	65 (1.0)	60 (0.9)	23* (0.4)	67 (1.0)	21* (0.3)
2	BaP	I	+	16 (1.0)	14 (0.9)	7* (0.4)	13 (0.8)	8* (0.5)
3	BaP4, 5-dihydroepoxide	I	–	4346 (1.0)	3702 (0.9)	1408* (0.3)	3530 (0.8)	1656* (0.4)
4	1-AA	I	+	20 (1.0)	20 (1.0)	4* (0.2)	21 (1.1)	6* (0.3)
5	2-AA	I	+	922 (1.0)	880 (1.0)	409* (0.4)	890 (1.0)	357* (0.4)
6	DMBA	II	+	4 (1.0)	3 (0.8)	2* (0.5)	2* (0.5)	2* (0.5)
7	6-AC	II	+	601 (1.0)	536 (0.9)	265* (0.4)	274* (0.5)	152* (0.3)
8	BaP7, 8-tetrahydroepoxide	II	–	1040 (1.0)	634* (0.6)	573* (0.6)	661* (0.6)	531* (0.5)
9	3-MC	II	+	20 (1.0)	11* (0.6)	10* (0.5)	13* (0.7)	14* (0.7)
10	1-NP	III	–	86 (1.0)	63* (0.7)	84 (1.0)	47* (0.5)	47* (0.5)
11	1,8-DNP	III	–	263[c] (1.0)	141[c,*] (0.5)	262[c] (1.0)	49[c,*] (0.2)	54[c,*] (0.2)
12	1-*N*-6-azaBaP	III	–	118[c] (1.0)	48[c,*] (0.4)	67[c,*] (0.6)	5[c,*] (0.04)	4[c,*] (0.03)
13	3-*N*-6-azaBaP	III	–	49[c] (1.0)	21[c,*] (0.4)	36[c,*] (0.7)	9[c,*] (0.2)	11[c,*] (0.2)
14	1-NBaP	IV	–	178 (1.0)	138 (0.8)	166 (0.9)	164 (0.9)	172 (1.0)
15	3-NBaP	IV	–	592 (1.0)	530 (0.9)	476 (0.8)	510 (0.9)	532 (0.9)
16	4-ABP	IV	+	17 (1.0)	15 (0.9)	16 (0.9)	19 (1.1)	16 (0.9)
17	4-NQO	IV	–	2[c] (1.0)	2[c] (1.0)	2[c] (1.0)	2[c] (1.0)	2[c] (1.0)
18	Glu-P-1	IV	+	39[c] (1.0)	39[c] (1.0)	35[c] (0.9)	38[c] (1.0)	37[c] (0.9)
19	APNH	IV	+	14779 (1.0)	14955 (1.0)	13525 (0.9)	16464 (1.1)	14960 (1.0)
20	AO	IV	+	39 (1.0)	39 (1.0)	40 (1.0)	43 (1.1)	44 (1.1)
21	BA	IV	+	4 (1.0)	4 (1.0)	3 (0.8)	3 (0.8)	4 (1.0)
22	*N*-OH-AAF	IV	–	8 (1.0)	9 (1.1)	9 (1.1)	9 (1.1)	9 (1.1)
23	2-NF	IV	–	182 (1.0)	186 (1.0)	177 (1.0)	176 (1.0)	184 (1.0)
24	2-AAF	IV	+	160 (1.0)	131 (0.8)	154 (1.0)	161 (1.0)	135 (0.8)
25	2-AF	IV	+	297 (1.0)	238 (0.8)	296 (1.0)	292 (1.0)	262 (0.9)
26	PBTA-1	IV	+	11446 (1.0)	12466 (1.1)	9024 (0.8)	10166 (0.9)	16928* (1.5)

Each chemical was assayed with four–seven doses on triplicate plates with five strains in parallel. The assays with chemical nos.: 1, 2, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 17, 18 and 26 were repeated to confirm the initial results. The number of His$^+$ revertants per plate per microgram or nanogram of each strain was calculated at the doses indicated with arrows in Fig. 2. The doses were chosen where the numbers of His$^+$ revertants per plate per microgram or nanogram were the highest in strain TA1538. If the doses are not appropriate to represent the different mutability of each strain (chemical nos. 2, 6, 8, 9, 10, 17 and 20), the doses where the second or third highest numbers of His$^+$ revertants per plate per microgram or nanogram were used. The numbers in parenthesis represent the relative values when the number of His$^+$ revertants per plate per microgram of TA1538 is assigned as 1.0.

[a] The chemicals were classified into four classes based on their mutagenicity to the strains.

[b] + and – mean that the assay was performed in the presence or the absence of metabolic activation with S9 mix, respectively.

[c] The number of His$^+$ revertants per plate per nanogram instead of microgram is indicated.

[d] The amount of S9 used is 50 μl but in the case of 10-azaBaP, 5 μl.

* Significantly different ($p < 0.01$) from value for TA1538.

Class III includes 1-NP, 1,8-DNP, 1-*N*-6-azaBaP and 3-*N*-6-azaBaP. The mutagenicity of these compounds largely depends on the presence of *umuDC*$_{ST}$ plus *samAB*. When the genes were deleted, the mutagenicity was reduced to the level observed in strain YG6215. In particular, the mutagenicity of 1-*N*-6-azaBaP was reduced by more than 95% by the deletion of *umuDC*$_{ST}$ plus *samAB*. Elimination of other DNA polymerases displayed less pronounced effects on the mutagenicity of this class of chemicals: deletion of *polB*$_{ST}$ reduced the mutagenicity of all four compounds by 30–60% and deletion of *dinB*$_{ST}$ reduced the mutagenicity of 1-*N*-6-azaBaP and 3-*N*-6-azaBaP by 30–40%. Strain YG6215 exhibited 20–50% mutability to 1-NP, 1,8-DNP and 3-*N*-6-azaBaP compared with strain TA1538.

Class IV includes 1-NBaP, 3-NBaP, 4-ABP, 4-NQO, Glu-P-1, APNH, AO, BA, *N*-OH-AAF, 2-NF, 2-AAF, 2-AF and PBTA-1. The mutagenicity of these compounds is not significantly reduced by deletion of any of the genes encoding SOS-inducible DNA polymerases. The mutagenicity of PBTA-1 is exceptional in that the mutagenicity was enhanced when *polB*$_{ST}$, *dinB*$_{ST}$ and *umuDC*$_{ST}$ plus *samAB* were all deleted.

3.2. Induction of –2 frameshift in CGCGCGCG sequence in the hisD gene

To examine whether the mutations induced by the chemicals indeed occurred in the CG repetitive sequence in the *hisD* gene, DNA containing the repetitive sequence was amplified from His$^+$ revertants by PCR and the amplified DNA was digested with restriction enzyme *Bss*HII, whose target sequence is GCGCGC. If CG deletions occur in the CGCGCGCG sequence, this enzyme will digest the DNA at

Table 4
Effect of the introduction of various plasmids on the frequency of His^+ reversion induced by 6-AC or 3-MC

	Strain	Number of His^+ revertants per plate per microgram									
		No plasmid	pBR322 vector	pYG787 *polB*$^+$	pYG503 *polB*$_{ST}^+$	pYG768 *dinB*$^+$	pYG779 *dinB*$^-$	pYG501 *dinB*$_{ST}^+$	pGW2101 *umuDC*$^+$	pGW2020 *umuD*$^+$	pYG8031 *umuDC*$_{ST}^+$
6-AC	TA1538	704 (1.0)	699 (1.0)								
	YG6215	214 (0.3)	154 (0.2)	147 (0.2)	172 (0.2)	3712 (5.3)	227 (0.3)	4624 (6.6)	687 (1.0)	159 (0.2)	2952 (4.2)
3-MC	TA1538	13 (1.0)	15 (1.2)								
	YG6215	6 (0.5)	4 (0.3)	12 (0.9)	10 (0.8)	51 (3.9)	8 (0.6)	58 (4.5)	25 (1.9)	5 (0.4)	26 (2.0)

The mutagenicity assays of 6-AC (1 μg per plate) or 3-MC (25 μg per plate) were performed twice with duplicate plates. The numbers of His^+ revertants per plate per microgram were calculated as mean numbers of four plates for two experiments. The numbers in parenthesis represent the relative values when the number of His^+ revertants per plate per microgram of TA1538 is assigned as 1.0. Strain YG6215 is the same as TA1538 but is Δ*polB*$_{ST}$, Δ*dinB*$_{ST}$, Δ*umuDC*$_{ST}$ and Δ*samAB*.

only one site outside of the CG repetitive sequence, resulting in 296 and 63 bp DNA fragments [35]. If no deletions occur in the repetitive sequence, it will digest the DNA at two sites, resulting in 173, 125 and 63 bp. In strain TA1538, frameshift mutations occurred in the repetitive sequences in almost all the His^+ revertants induced by chemicals. The numbers of mutants having −2 frameshift in the CG repetitive sequence are as follows: 15 out of 15 His^+ revertants analyzed (15/15) for 10-azaBaP; 9/10 for 3-MC; 9/9 for 1,8-DNP; 10/10 for PBTA-1 and 15/15 for 2-AAF. Similar results were obtained in strain YG6215: 13/15 for 10-azaBaP; 10/10 for 3-MC; 8/8 for 1,8-DNP; 10/10 for PBTA-1 and 15/15 for 2-AAF. In contrast, spontaneous frameshift mutations occurred less frequently in the CG repetitive sequence, i.e. 11/14 (78%) in strain TA1538 and 10/15 (67%) in strain YG6215.

3.3. Effect of the introduction of plasmids carrying E. coli polB, dinB and umuDC genes on the mutagenicity of 6-AC and 3-MC in strain YG6215

The mutagenicity of class II compounds, e.g. 6-AC and 3-MC, was reduced to the level similar to that in YG6215 either by *dinB*$_{ST}$ deletion or by *umuDC*$_{ST}$ plus *samAB* deletion (Table 3). To examine whether the catalytic activities of both DNA polymerases are required for the maximum mutagenicity, the complementation assay was conducted (Table 4). Introduction of plasmid pYG768 carrying *E. coli dinB* into strain 6215 enhanced the mutagenicity of 6-AC and 3-MC substantially. Similar results were observed with plasmid pYG501 carrying *dinB*$_{ST}$. In contrast, introduction of pYG779 carrying *dinB003* encoding inactive DNA pol IV by the change of 103rd amino acid from Asp to Asn had virtually no effects, indicating the necessity of the polymerase activity of DNA pol IV for the enhancement of mutagenesis. The mutagenicity of 6-AC and 3-MC was also enhanced by the introduction of plasmid pGW2101 carrying *E. coli umuDC*, but not by the introduction of plasmid pGW2020 carrying *E. coli umuD* without *umuC*. The *umuC* gene encodes the catalytic subunit of DNA pol V. It indicates the essential role of polymerase activity of DNA pol V for the enhancement of mutagenesis. The mutagenicity was also enhanced with plasmid pYG8031 carrying *umuDC*$_{ST}$. The presence of *polB*$_{ST}$ was required for the maximum mutagenesis by 3-MC but not by 6-AC (Table 3). In agreement with the results, introduction of plasmid pYG787 carrying *E. coli polB* enhanced the mutagenicity of 3-MC but not the mutagenicity of 6-AC. Similar results were observed with plasmid pYG503 carrying *polB*$_{ST}$.

4. Discussion

DNA pol III holoenzyme in *E. coli* is a high fidelity polymerase that is occasionally blocked by DNA lesions, and DNA polymerase V encoded by *umuDC* plays an important role in bypass reactions that mostly result in base substitution mutations [10,24]. Less is known, however, about the mechanisms of translesion bypass resulting in frameshift [40–42]. In this study, we have engineered a set of isogenic derivatives of *S. typhimurium* strain TA1538 to examine the role of specialized and replicative DNA polymerases in translesion bypass leading to frameshift. The strain possesses a hot spot sequence of CGCGCGCG for −2 frameshift [34]. The derivatives are deficient in one or all of the genes encoding SOS-inducible DNA polymerase, i.e. *polB*$_{ST}$, *dinB*$_{ST}$, *umuDC*$_{ST}$ and *samAB* (Table 1). The strain YG6215 deficient in all the genes encoding SOS-inducible DNA polymerases exhibited normal phenotypes regarding the growth rate and the morphology, and the spontaneous His^+ reversion frequency was indistinguishable from that of the parent strain TA1538 (data not shown). Among the SOS polymerase genes, *samAB* seems functionally inactive because *samAB* is only active when it resides on multi-copy-number plasmids, such as pBR322 [30,43]. Previous studies indicate that deletion of *umuDC*$_{ST}$, but not *samAB*, reduces the mutagenesis induced by UV, 1-NP and 1,8-DNP [35,43]. Nevertheless, we have constructed the strains deficient in both *samAB* and *umuDC*$_{ST}$, i.e. YG5147 and YG6215, and used them for the mutation assays with 26 chemicals (Fig. 1).

Interestingly, the results suggest that not only specialized DNA polymerases but also the replicative DNA polymerase, i.e. DNA pol III holoenzyme, is deeply involved in chemically induced frameshift. As most typically shown in class IV chemicals, such as Glu-P-1, 4-ABP or *N*-OH-AAF, the

mutagenicity was not reduced by deletion of any of the genes encoding SOS-inducible DNA polymerases (Table 3, Fig. 2). In addition, most of the chemicals in classes I–II exhibited substantial remaining mutagenicity even after all the genes encoding SOS-inducible DNA polymerases were deleted. Only exception was 1-*N*-6-azaBaP in that the mutagenicity was reduced by more than 95% by the deletion of *umuDC*$_{ST}$ plus *samAB*. We suggest that *S. typhimurium* DNA pol III holoenzyme has an ability to bypass various DNA lesions when adducts are formed in the CGCGCGCG sequence. Since the involvement of DNA pol I encoded by *polA*$_{ST}$ is an obvious possibility, we at first attempted to disrupt the *polA*$_{ST}$ gene (ΔKlenow). Unfortunately, we could not successfully isolate a *polA*$_{ST}$ derivative of strain TA1538. We then introduced a plasmid carrying the *E. coli polA* gene into strain TA1538 and YG6215 and examined the mutability to some of class IV chemicals (Glu-P-1, APNH and 2-NF). We expected that enhancement or suppression of the mutability would be induced by the introduction of the plasmid if DNA pol I were involved in the translesion. However, we observed no enhancement or suppression of the mutability of the strains to the compounds (Nohmi et al., unpublished). Thus, we prefer the possibility that DNA pol III, rather than DNA pol I, plays a major role in the TLS in the CG repetitive sequence. If DNA pol I were involved, it should induce sequence changes during the processing of Okazaki fragments in the lagging strands because excision repair function is inactivated in the strains. The frameshift may result either from incorporation of cytosine opposite the adducted guanine by DNA pol III and perhaps also by DNA pol I, followed by a 2 bp slippage, or from correct incorporation of cytosine opposite 2 bp ahead guanine following formation of a bulge containing the adducted guanine [44].

In *E. coli*, DNA pol III holoenzyme is shown to bypass a synthetic abasic site in a gapped plasmid in vitro [45]. The efficiency of bypass by DNA pol III holoenzyme is much higher than that of bypass by DNA pol I, pol II or pol III core. *E. coli* DNA pol III holoenzyme is also able to bypass 2-AAF-guanine adduct in 5′-GGCG*CC-3′ sequence in a plasmid in vitro, thereby generating −2 frameshift (G* represents the adducted guanine) [46]. Thus, we suggest that *S. typhimurium* and perhaps *E. coli* DNA pol III holoenzymes are able to skip bulky lesions in the CGCGCGCG sequence, which results in −2 frameshift. DNA pol III holoenzymes would halt replication, however, if they encountered bulky DNA lesions in other sequence contests. In fact, some of the class IV chemicals, i.e. 4-ABP, 4-NQO, Glu-P-1, AO, BA, 2-NF and 2-AAF, are shown to be strong inducers of SOS response in *S. typhimurium*, suggesting that the chemicals are able to block DNA replication [47,48]. The efficient skip over bulky adducts by DNA pol III holoenzymes may occur only in certain sequence contexts, such as CGCGCGCG, which makes this sequence a hot spot for frameshift mutagenesis.

It was a bit surprise that *polB*$_{ST}$ deletion did not significantly affect the frameshift induced by 2-AAF or *N*-OH-AAF while the deletion reduced the mutagenicity of other six chemicals, such as 3-MC and 1,8-DNP (Table 3, Fig. 2). It is reported that bypass of 2-AAF guanine adducts located in the *Nar*I site (GGCGCC sequence) requires DNA pol II for −2 frameshift [25,26]. The discrepancy may be due to either the difference of local sequence contexts, i.e. four times CG repeat in the *hisD* gene versus two times GC repeat in the *Nar*I site, or the difference of the position of target sequences, i.e. chromosome versus plasmid. It was also unexpected that the mutagenicity of PBTA-1 was enhanced instead of reduced by the deletion of all the genes encoding SOS-inducible polymerases (Table 3, Fig. 2). PBTA-1 is a potent aromatic amine mutagen in a polluted river [49]. We speculate that deletion of SOS-inducible DNA polymerases might affect the fidelity of DNA pol III holoenzyme, thereby enhancing the mutations induced by PBTA-1. We also observed that deletion of all the genes encoding SOS-inducible DNA polymerases enhanced the mutagenicity of the nucleoside analogue, i.e. deoxyribosyl dihydropyrimido[4,5-*c*][1,2]oxazin-7-one (dP) [50] in *S. typhimurium* TA1535 (Kokubo et al., unpublished results).

In addition to the replicative DNA polymerase, specialized DNA polymerases apparently play important roles in −2 frameshift induced by chemicals. Deletion of *dinB*$_{ST}$ significantly reduced the mutagenicity of classes I and II chemicals, most of which are PAHs (Table 3, Fig. 2). *E. coli* DNA pol IV (DinB) bypasses N^2-guanine adducts of BaP-7,8-diol 9,10-epoxide (BPDE-G) in vitro with higher efficiency and fidelity compared to DNA pol II and DNA pol V [51]. Both *E. coli* DNA pol IV and pol V are required for error-free and error-prone (−1 frameshift) bypass reactions across BPDE-G adducts in plasmid DNA [25]. Thus, it seems likely that *S. typhimurium* DNA pol IV encoded by *dinB*$_{ST}$ efficiently bypasses across guanine modified with PAHs, which leads to frameshift. It was unexpected, however, that the mutagenicity of aromatic amines, i.e. 1-AA, 2-AA and 6-AC (Fig. 1) was severely reduced by the deletion of *dinB*$_{ST}$ (Table 3, Fig. 2). In fact, 1-AA was the most severely affected compound among the class I chemicals. The mutagenicity of other aromatic amines, such as Glu-P-1 and 2-AF (class IV) was not affected by *dinB*$_{ST}$ deletion. Although DNA adducts by 1-AA and 2-AA are not identified yet, 6-nitrochrysene is reported to form the N^2-guanine adduct, i.e. 5-(deoxyguanosin-N^2-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene [52]. Thus, unlike Glu-P-1 and 2-AF, which form C8-guanine adducts through *N*-oxidation, 1-AA, 2-AA and 6-AC may form N^2-guanine adducts through ring oxidation leading to the formation of the epoxides, as in the case of PAHs.

Class II chemicals are intriguing since they appear to require multiple SOS-inducible DNA polymerases for the maximum mutagenesis. DMBA and 6-AC require both *dinB*$_{ST}$ and *umuDC*$_{ST}$ plus *samAB*. BaP7,8-tetrahydroepoxide and 3-MC need *polB*$_{ST}$ additionally (Table 3, Fig. 2). The necessity of *polB*$_{ST}$ for the maximum mutagenesis by 3-MC was supported by the results that introduction of plasmid carrying either *E. coli polB* or *polB*$_{ST}$ into strain YG6215 enhanced the mutagenicity of 3-MC but not 6-AC (Table 4). The results of complementation assay also confirm

the involvement of both *dinB*$_{ST}$ and *umuDC*$_{ST}$ in the mutagenesis by 6-AC and 3-MC. Thus, two or three SOS-inducible DNA polymerases appear to be involved in frameshift by the class II chemicals. A possible explanation for the phenomenon is that the polymerases are involved in distinct biochemical steps such as restart of DNA replication (see below), incorporation and extension of bypass reactions, although the exact mechanisms remain to be elucidated. The results of complementation assay also caution that over-expression of single DNA polymerase may mask the necessity of other DNA polymerases that are important in mutagenesis when they are present at the physiological levels (Tables 3 and 4).

S. typhimurium DNA pol V encoded by *umuDC*$_{ST}$ plays important roles in the mutagenicity of classes II and III chemicals. In particular, the mutagenicity of 1-*N*-6-azaBaP was severely diminished by the deletion of *umuDC*$_{ST}$ plus *samAB*. Intriguingly, its analogue, i.e. 1-NBaP, does not require any SOS-inducible DNA polymerases for the mutagenesis. Similarly, the mutagenicity of 3-*N*-6azaBaP is significantly reduced by the deletion of *umuDC*$_{ST}$ plus *samAB*, but the mutagenicity of 3-NBaP is not. 1-NBaP and 3-NBaP are environmental contaminants and form the N^2-guanine adducts via the C6 of aromatic rings, such as 6-(deoxyguanosin-N^2-yl)-3-aminobenzo[*a*]pyrene, as a major adduct [53]. Thus, the N^6 of 1-*N*-6-azaBaP and 3-*N*-6-azaBaP may inhibit the formation of the N^2-guanine adducts. 1-NP and 1,8-DNP, which need the presence of *umuDC*$_{ST}$ for the maximum mutagenesis (class III), induce C8 guanine adducts [54]. By analogy to the adduct formation by 1-NP and 1,8-DNP, we speculate that 1-*N*-6azaBaP and 3-*N*-6azaBaP might induce C8 guanine adducts via the formation of nitrenium ions. The presence of C8 guanine adducts of class III compounds may block replication by DNA pol III holoenzyme, which requires the presence of DNA pol V to bypass the lesions.

S. typhimurium DNA pol II encoded by *polB*$_{ST}$ was involved in the frameshift induced by six chemicals that belong to classes II and III. However, the effects of *polB*$_{ST}$ deletion were moderate compared to *dinB*$_{ST}$ deletion or *umuDC*$_{ST}$ plus *samAB* deletion. There was no chemical whose mutagenicity was largely depended upon the presence of *polB*$_{ST}$. *S. typhimurium* DNA pol II appears to collaborate with DNA pol IV or pol V to achieve mutagenic bypass across the lesion. *E. coli* DNA pol II is known to be involved in the immediate recovery of DNA synthesis after UV irradiation [13]. Thus, it is tempting to speculate that *S. typhimurium* DNA pol II might be required to re-synthesize the primer strand to reach the lesion when the primer strand was degraded. This degradation might occur when DNA pol III holoenzyme encountered the lesion and stopped the replication. If DNA synthesis by DNA pol II as well as DNA pol III holoenzyme was blocked by the lesion, other SOS-inducible DNA polymerases, such as DNA pol IV and pol V would be recruited to accomplish the lesion bypass, thereby inducing frameshift.

In summary, our results suggest that different sets of DNA polymerases are engaged in lesion bypass in the CGCGCGCG sequence depending upon the environmental threats by chemicals. The replicative DNA polymerase, i.e. DNA pol III holoenzyme, appears to play important roles in −2 frameshift by skipping various lesions in the CG repetitive sequence. As for specialized DNA polymerases, DNA pol IV appears to bypass guanine adducts with PAHs efficiently, and DNA pol V can bypass lesions with various structures. DNA pol II contributes to frameshift in a collaborative manner with DNA pol V and DNA pol IV. Lesion structures, types of mutation (frameshift versus base substitution) and sequence contexts could strongly affect the roles of DNA polymerases in mutagenesis.

Acknowledgements

We wish to thank Drs. Ken-ichi Saeki (Nagoya City University, Nagoya, Japan), Takeji Takamura-Enya (National Cancer Center Research Institute, Tokyo, Japan), Yukari Totsuka (National Cancer Center Research Institute, Tokyo, Japan) and Kiyoshi Fukuhara (National Institute of Health Sciences, Tokyo, Japan) for providing us the chemicals used. We acknowledge the helpful discussion by Dr. Kiyoshi Fukuhara for the adduct formation of 1-*N*-6-azaBaP and 3-*N*-6-azaBaP. We also appreciate Dr. Kazuo Yamamoto, Tohoku University, for providing the plasmid carrying *E. coli pol A*. Part of this study was financially supported by the Budget for Nuclear Research of the Ministry of Education, Culture, Sports, Science and Technology Japan, based on the screening and counseling by the Atomic Energy Commission. This work was also supported by grants-in-aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan, and for Basic Research from the Japan Health Science Foundation.

References

[1] E.C. Friedberg, G.C. Walker, W. Siede, DNA Repair and Mutagenesis, ASM Press, Washington, DC, 1995, pp. 1–697.
[2] S.C. Kowalczykowski, D.A. Dixon, A.K. Eggleston, S.D. Lauder, W.M. Rehrauer, Biochemistry of homologous recombination in *Escherichia coli*, Microbiol. Rev. 58 (1994) 401–465.
[3] H. Echols, M.F. Goodman, Fidelity mechanisms in DNA replication, Annu. Rev. Biochem. 60 (1991) 477–511.
[4] G.C. Walker, SOS-regulated proteins in translesion DNA synthesis and mutagenesis, Trends Biochem. Sci. 20 (1995) 416–420.
[5] B. Bridges, DNA polymerases and SOS mutagenesis: can one reconcile the biochemical and genetic data? Bioessays 22 (2000) 933–937.
[6] Z. Livneh, DNA damage control by novel DNA polymerases: translesion replication and mutagenesis, J. Biol. Chem. 276 (2001) 25639–25642.
[7] A.J. Rattray, J.N. Strathern, Error-prone DNA polymerases: when making a mistake is the only way to get ahead, Annu. Rev. Genet. 37 (2003) 31–66.
[8] Z. Wang, Translesion synthesis by the UmuC family of DNA polymerases, Mutat. Res. 486 (2001) 59–70.
[9] C.M. Joyce, W.S. Kelley, N.D. Grindley, Nucleotide sequence of the *Escherichia coli polA* gene and primary structure of DNA polymerase I, J. Biol. Chem. 257 (1982) 1958–1964.

[10] A. Kornberg, T.A. Baker, DNA Replication, W.H. Freeman and Co., New York, 1992, pp. 165–181.
[11] H. Iwasaki, A. Nakata, G.C. Walker, H. Shinagawa, The *Escherichia coli polB* gene, which encodes DNA polymerase II, is regulated by the SOS system, J. Bacteriol. 172 (1990) 6268–6273.
[12] C.A. Bonner, S. Hays, K. McEntee, M.F. Goodman, DNA polymerase II is encoded by the DNA damage-inducible *dinA* gene of *Escherichia coli*, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 7663–7667.
[13] S. Rangarajan, R. Woodgate, M.F. Goodman, Replication restart in UV-irradiated *Escherichia coli* involving pols II, III, V, PriA, RecA and RecFOR proteins, Mol. Microbiol. 43 (2002) 617–628.
[14] S. Rangarajan, G. Gudmundsson, Z. Qiu, P.L. Foster, M.F. Goodman, *Escherichia coli* DNA polymerase II catalyzes chromosomal and episomal DNA synthesis in vivo, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 946–951.
[15] H. Maki, A. Kornberg, The polymerase subunit of DNA polymerase III of *Escherichia coli*. II. Purification of the alpha subunit, devoid of nuclease activities, J. Biol. Chem. 260 (1985) 12987–12992.
[16] H. Maki, T. Horiuchi, A. Kornberg, The polymerase subunit of DNA polymerase III of *Escherichia coli*. I. Amplification of the *dnaE* gene product and polymerase activity of the alpha subunit, J. Biol. Chem. 260 (1985) 12982–12986.
[17] M.M. Welch, C.S. McHenry, Cloning and identification of the product of the *dnaE* gene of *Escherichia coli*, J. Bacteriol. 152 (1982) 351–356.
[18] C.S. McHenry, DNA polymerase III holoenzyme of *Escherichia coli*: components and function of a true replicative complex, Mol. Cell Biochem. 66 (1985) 71–85.
[19] J. Wagner, P. Gruz, S.R. Kim, M. Yamada, K. Matsui, R.P. Fuchs, T. Nohmi, The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis, Mol. Cell 4 (1999) 281–286.
[20] M. Tang, X. Shen, E.G. Frank, M. O'Donnell, R. Woodgate, M.F. Goodman, UmuD'(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 8919–8924.
[21] N.B. Reuven, G. Arad, A. Maor-Shoshani, Z. Livneh, The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA, and SSB and is specialized for translesion replication, J. Biol. Chem. 274 (1999) 31763–31766.
[22] C.J. Kenyon, G.C. Walker, DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 2819–2823.
[23] G.C. Walker, Bryn Bridges and mutagenesis: exploring the intellectual space, Mutat. Res. 485 (2001) 69–81.
[24] M.F. Goodman, R. Woodgate, The biochemical basis and in vivo regulation of SOS-induced mutagenesis promoted by *Escherichia coli* DNA polymerase V (UmuD'2C), Cold Spring Harb. Symp. Quant. Biol. 65 (2000) 31–40.
[25] R. Napolitano, R. Janel-Bintz, J. Wagner, R.P. Fuchs, All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis, EMBO J. 19 (2000) 6259–6265.
[26] O.J. Becherel, R.P. Fuchs, Mechanism of DNA polymerase II-mediated frameshift mutagenesis, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8566–8571.
[27] S.R. Kim, K. Matsui, M. Yamada, P. Gruz, T. Nohmi, Roles of chromosomal and episomal *dinB* genes encoding DNA pol IV in targeted and untargeted mutagenesis in *Escherichia coli*, Mol. Genet. Genomics 266 (2001) 207–215.
[28] N. Lenne-Samuel, R. Janel-Bintz, A. Kolbanovskiy, N.E. Geacintov, R.P. Fuchs, The processing of a Benzo(*a*)pyrene adduct into a frameshift or a base substitution mutation requires a different set of genes in *Escherichia coli*, Mol. Microbiol. 38 (2000) 299–307.
[29] M. McClelland, K.E. Sanderson, J. Spieth, S.W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, R.K. Wilson, Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2, Nature 413 (2001) 852–856.
[30] T. Nohmi, A. Hakura, Y. Nakai, M. Watanabe, S.Y. Murayama, T. Sofuni, *Salmonella typhimurium* has two homologous but different *umuDC* operons: cloning of a new *umuDC*-like operon (*samAB*) present in a 60-megadalton cryptic plasmid of *S. typhimurium*, J. Bacteriol. 173 (1991) 1051–1063.
[31] M. Escarceller, J. Hicks, G. Gudmundsson, G. Trump, D. Touati, S. Lovett, P.L. Foster, K. McEntee, M.F. Goodman, Involvement of *Escherichia coli* DNA polymerase II in response to oxidative damage and adaptive mutation, J. Bacteriol. 176 (1994) 6221–6228.
[32] R. Woodgate, A.S. Levine, W.H. Koch, T.A. Cebula, E. Eisenstadt, Induction and cleavage of *Salmonella typhimurium* UmuD protein, Mol. Gen. Genet. 229 (1991) 81–85.
[33] D.M. Maron, B.N. Ames, Revised methods for the Salmonella mutagenicity test, Mutat. Res. 113 (1983) 173–215.
[34] K. Isono, J. Yourno, Chemical carcinogens as frameshift mutagens: Salmonella DNA sequence sensitive to mutagenesis by polycyclic carcinogens, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 1612–1617.
[35] T. Nohmi, M. Yamada, M. Matsui, K. Matsui, M. Watanabe, T. Sofuni, Involvement of $umuDC_{ST}$ genes in nitropyrene-induced -CG frameshift mutagenesis at the repetitive CG sequence in the *hisD3052* allele of *Salmonella typhimurium*, Mol. Gen. Genet. 247 (1995) 7–16.
[36] B.N. Ames, F.D. Lee, W.E. Durston, An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 782–786.
[37] S.R. Kim, G. Maenhaut-Michel, M. Yamada, Y. Yamamoto, K. Matsui, T. Sofuni, T. Nohmi, H. Ohmori, Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an overexpression of *dinB/dinP* results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 13792–13797.
[38] J.H. Miller, A Short Course in Bacterial Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1992.
[39] M. Yamada, A. Hakura, T. Sofuni, T. Nohmi, New method for gene disruption in *Salmonella typhimurium*: construction and characterization of an ada-deletion derivative of *Salmonella typhimurium* TA1535, J. Bacteriol. 175 (1993) 5539–5547.
[40] G. Streisinger, Y. Okada, J. Emrich, J. Newton, A. Tsugita, E. Terzaghi, M. Inouye, Frameshift mutations and the genetic code, Cold Spring Harb. Symp. Quant. Biol. 31 (1966) 77–84.
[41] K. Bebenek, T.A. Kunkel, Frameshift errors initiated by nucleotide misincorporation, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 4946–4950.
[42] L.B. Bloom, X. Chen, D.K. Fygenson, J. Turner, M. O'Donnell, M.F. Goodman, Fidelity of *Escherichia coli* DNA polymerase III holoenzyme. The effects of beta, gamma complex processivity proteins and epsilon proofreading exonuclease on nucleotide misincorporation efficiencies, J. Biol. Chem. 272 (1997) 27919–27930.
[43] T. Nohmi, M. Yamada, M. Watanabe, S.Y. Murayama, T. Sofuni, Roles of *Salmonella typhimurium umuDC* and *samAB* in UV mutagenesis and UV sensitivity, J. Bacteriol. 174 (1992) 6948–6955.
[44] M.L. Shelton, D.M. DeMarini, Mutagenicity and mutation spectra of 2-acetylaminofluorene at frameshift and base-substitution alleles in four DNA repair backgrounds of Salmonella, Mutat. Res. 327 (1995) 75–86.
[45] G. Tomer, Z. Livneh, Analysis of unassisted translesion replication by the DNA polymerase III holoenzyme, Biochemistry 38 (1999) 5948–5958.
[46] S. Fujii, R.P. Fuchs, Defining the position of the switches between replicative and bypass DNA polymerases, EMBO J. 23 (2004) 4342–4352.
[47] S.I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto, SOS-inducing activity of chemical carcinogens and mutagens in

Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals, Mutat. Res. 192 (1987) 239–246.

[48] G. Reifferscheid, J. Heil, Validation of the SOS/umu test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data, Mutat. Res. 369 (1996) 129–145.

[49] T. Ohe, D.T. Shaughnessy, S. Landi, Y. Terao, H. Sawanishi, H. Nukaya, K. Wakabayashi, D.M. DeMarini, Mutation spectra in Salmonella TA98, TA100, and TA104 of two phenylbenzotriazole mutagens (PBTA-1 and PBTA-2) detected in the Nishitakase River in Kyoto, Japan, Mutat. Res. 429 (1999) 189–198.

[50] K. Negishi, D.M. Williams, Y. Inoue, K. Moriyama, D.M. Brown, H. Hayatsu, The mechanism of mutation induction by a hydrogen bond ambivalent, bicyclic N4-oxy-2′-deoxycytidine in *Escherichia coli*, Nucl. Acids Res. 25 (1997) 1548–1552.

[51] X. Shen, J.M. Sayer, H. Kroth, I. Ponten, M. O'Donnell, R. Woodgate, D.M. Jerina, M.F. Goodman, Efficiency and accuracy of SOS-induced DNA polymerases replicating benzo[*a*]pyrene-7,8-diol 9,10-epoxide A and G adducts, J. Biol. Chem. 277 (2002) 5265–5274.

[52] K. El Bayoumy, A.K. Sharma, J.M. Lin, J. Krzeminski, T. Boyiri, L.C. King, G. Lambert, W. Padgett, S. Nesnow, S. Amin, Identification of 5-(deoxyguanosin-N^2-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene as the major DNA lesion in the mammary gland of rats treated with the environmental pollutant 6-nitrochrysene, Chem. Res. Toxicol. 17 (2004) 1591–1599.

[53] P.P. Fu, D. Herreno-Saenz, L.S. Von Tungeln, J.O. Lay, Y.S. Wu, J.S. Lai, F.E. Evans, DNA adducts and carcinogenicity of nitro-polycyclic aromatic hydrocarbons, Environ. Health Perspect. 102 (Suppl. 6) (1994) 177–183.

[54] P. Hilario, S. Yan, B.E. Hingerty, S. Broyde, A.K. Basu, Comparative mutagenesis of the C8-guanine adducts of 1-nitropyrene and 1,6- and 1,8-dinitropyrene in a CpG repeat sequence. A slipped frameshift intermediate model for dinucleotide deletion, J. Biol. Chem. 277 (2002) 45068–45074.

[55] T. Nohmi, J.R. Battista, L.A. Dodson, G.C. Walker, RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 1816–1820.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Mutation Research 591 (2005) 60–73
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Modulation of oxidative mutagenesis and carcinogenesis by polymorphic forms of human DNA repair enzymes

Takehiko Nohmi *, Su-Ryang Kim [1], Masami Yamada

Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

Received 4 February 2005; received in revised form 24 March 2005; accepted 25 March 2005
Available online 2 August 2005

Abstract

Chromosome DNA is continuously exposed to various endogenous and exogenous mutagens. Among them, oxidation is one of the most common threats to genetic stability, and multiple DNA repair enzymes protect chromosome DNA from the oxidative damage. In *Escherichia coli*, three repair enzymes synergistically reduce the mutagenicity of oxidized base 8-hydroxy-guanine (8-OH-G). MutM DNA glycosylase excises 8-OH-G from 8-OH-G:C pairs in DNA and MutY DNA glycosylase removes adenine incorporated opposite template 8-OH-G during DNA replication. MutT hydrolyzes 8-OH-dGTP to 8-OH-dGMP in dNTP pool, thereby reducing the chance of misincorporation of 8-OH-dGTP by DNA polymerases. Simultaneous inactivation of MutM and MutY dramatically increases the frequency of spontaneous G:C to T:A mutations, and the deficiency of MutT leads to the enhancement of T:A to G:C transversions more than 1000-fold over the control level. In humans, the functional homologues of MutM, MutY and MutT, i.e., OGG1, MUTYH (MYH) and MTH1, contribute to the protection of genomic DNA from oxidative stress. Interestingly, several polymorphic forms of these proteins exist in human populations, and some of them are suggested to be associated with cancer susceptibility. Here, we review the polymorphic forms of OGG1, MUTYH and MTH1 involved in repair of 8-OH-G and 8-OH-dGTP, and discuss the significance of the polymorphisms in the maintenance of genomic integrity. We also summarize the polymorphic forms of human DNA polymerase η, which may be involved in damage tolerance and mutagenesis induced by oxidative stress.

Keywords: 8-Hydroxyguanine; OGG1; MUTYH; MTH1; DNA polymerase η; Polymorphism; Cancer susceptibility

1. Introduction

Each human cell metabolizes approximately 10^{12} molecules of oxygen per day [1,2]. Although oxygen is normally metabolized to water to generate ATP without the accumulation of reactive intermediates, about 1% of oxygen metabolism results in the production

* Corresponding author. Tel.: +81 3 3700 9873; fax: +81 3 3707 6950.
E-mail address: nohmi@nihs.go.jp (T. Nohmi).
[1] Present address: Project Team for Pharmacogenetics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan.

0027-5107/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrfmmm.2005.03.033

of reactive oxygen species (ROS) by a sequence of one election reduction [1,2]. These reactive molecules include superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen, and hydroxyl radicals are thought to be the most predominant reactive species. ROS is also generated in cells by exposure to radiation and chemical carcinogens. Because ROS damages nearby cellular components such as DNA, proteins and lipids in membrane, cells must have evolved multiple defense mechanisms to combat the oxidative stress. Enzymes such as catalase or superoxide dismutase detoxify ROS, and low-molecular-weight scavengers such as glutathione alleviate the toxicity of ROS. Nevertheless, some ROS molecules escape from the defense systems and inevitably damage the bio-molecules. Thus, ROS has been implicated in the etiology of human degenerative diseases, aging and cancer [3,4].

Although ROS generates a variety of modified bases in DNA, 8-OH-G is the best characterized oxidized base in chemistry and biology [5,6]. 8-OH-G has been used as a biomaker for oxidative stress because it can be detected sensitively by a high performance liquid chromatography system equipped with an electrochemical detector [7]. Approximately 10^3 8-OH-G is estimated to be generated in normal human cells per day [8]. Structural studies indicate that 8-OH-G pairs with cytosine in the *anti* conformation but assumes the *syn* conformation when pairing with adenine [9–11]. In fact, both dATP and dCTP are inserted opposite template 8-OH-G during in vitro DNA synthesis. In general, the repair polymerases, such as *Escherichia coli* DNA polymerase I and DNA polymerase β, incorporate dCTP preferentially over dATP and the replicative polymerases, DNA polymerase α and *E. coli* DNA polymerase III, preferentially incorporate dATP [12]. The incorporation of dATP opposite template 8-OH-G during DNA replication can induce G:C to T:A transversions. In agreement of this, the *mutM mutY* mutants of *E. coli* deficient in the ability to remove 8-OH-G in DNA exhibit extremely high spontaneous G:C to T:A transversions [13]. In addition to base damage in DNA, the nucleotide pool, i.e., dNTPs, is oxidized by ROS. When an oxidized dGTP, i.e., 8-OH-dGTP, is incorporated opposite template adenine, it can cause T:A to G:C transversions [14]. The *E. coli mutT* mutants, which are reduced in the activity of hydrolyzing 8-OH-dGTP, exhibit more than 1000 times higher spontaneous T:A to G:C mutations over the control level [15].

To counteract the mutagenic threats by the lesion, eukaryotes including humans possess several repair enzymes that remove 8-OH-G in DNA and degrade 8-OH-dGTP in the nucleotide pool (Fig. 1). OGG1, a functional counterpart of *E. coli* MutM, has both DNA glycosylase and apurinic/apyrimidinic (AP) lyase activities to excise 8-OH-G opposite cytosine [16–18]. The glycosylase also excises another oxidative DNA lesion, i.e., 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy) [19–21]. The resulting 3′-blocking end is removed by AP endonuclease, and the gap is filled in by DNA polymerase β, thereby removing 8-OH-G and Fapy from DNA in an accurate manner [22,23]. The mice deficient in the *OGG1* gene exhibit an increased accumulation of 8-OH-G in DNA and the spontaneous mutation frequency is higher than that of the wild-type mice [24,25]. Eukaryotic cells also possess MUTYH (MYH), a homologue of *E. coli* MutY, which excises adenine or 2-hydroxy-adenine (2-OH-A) misincorporated opposite template 8-OH-G or guanine [26,27]. 2-OH-A is an oxidized base that can pair with thymine, guanine or cytosine during DNA replication, thereby inducing base substitutions and frameshifts [28,29]. Despite the homology between *E. coli* MutY and human MUTYH, the bacterial protein removes 2-OH-A from the substrate containing 2-OH-A:G pairs very poorly [30]. The importance of MUTYH in human genome integrity is highlighted by the fact that biallelic germ-line variants of the *MUTYH* gene is associated with multiple colorectal adenomas that display somatic G:C to T:A transversions in the *APC* gene [31]. Inactivation of both *Mutyh* and *Ogg1* causes an age-associated accumulation of DNA 8-OH-G in lung and small intestine in mice, and increases an incidence of lung and small intestine cancer [32]. G:C to T:A mutations are identified in 75% of the lung tumors at codon 12 of the *K-ras* oncogene in $Mutyh^{-/-}Ogg1^{-/-}$ mice [33].

For the prevention of mutagenesis induced by oxidized dNTPs, MTH1, a homologue of *E. coli* MutT, hydrolyzes 8-OH-dGTP, 2-OH-dATP and 8-OH-dATP to the mono-phosphate forms [34,35]. The mice deficient in MTH1 display an enhanced tumor formation in the lung, liver and stomach, suggesting the importance of oxidized dNTP pool as a source of carcinogenesis [36]. Although the mutation frequency is not

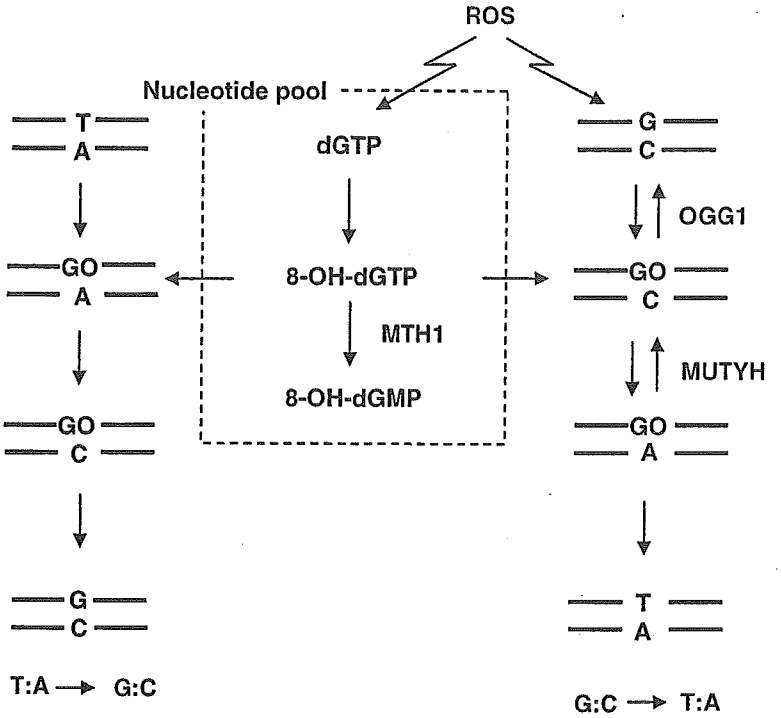


Fig. 1. Possible roles of OGG1, MUTYH (MYH) and MTH1 to prevent the genotoxicity of oxidative stress. Reactive oxygen species (ROS) generates 8-OH-G (GO) in DNA and 8-OH-dGTP in the nucleotide pool. The GO lesion can be removed by OGG1, and the subsequent process can restore the original G:C base pair. If it is not removed, GO can be a template for translesion DNA synthesis, which may result in GO:A base pair. The misincorporated A opposite template GO can be removed by MUTYH, and the following repair synthesis can restore the GO:A base pair. If the incorporated A is not removed, however, it pairs with T in the next round of DNA replication, leading to G:C to T:A transversions. 8-OH-dGTP in the nucleotide pool is hydrolyzed to the mono-phosphate form by MTH1. When 8-OH-dGTP is misincorporated opposite template A by DNA polymerase, GO will direct the insertion of C in the next DNA replication. The incorporated C pairs with G in the next round of DNA replication, which induces T:A to G:C transversions.

enhanced, the mutation spectrum in *Mutyh*$1^{-/-}$ cells is significantly shifted to single base deletions in mononucleotide runs [37]. In addition to MTH1, mouse possesses MTH2, which hydrolyzes 8-OH-dGTP to 8-OH-dGMP [38], and human Nudix type 5 (NUDT5) protein hydrolyses 8-OH-dGDP to monophosphate [39].

In human populations, there are numerous inter-individual sequence variations in coding and non-coding regions of the genome [40]. In fact, inter-individual differences in metabolic and DNA repair capacities have been shown to affect the susceptibility to various cancers [41,42]. Here, we review the variant forms of OGG1, MUTYH and MTH1, and discuss the significance of the polymorphism in the protection of genome integrity from endogenous and exogenous oxidative damaging agents (Table 1). We also discuss the polymorphism of one of specialized DNA polymerases, DNA polymerases η, which may be involved in translesion bypass across 8-OH-G in DNA and incorporation of 8-OH-dGTP into nascent DNA strands [43–45].

2. OGG1

Although OGG1 is a functional counterpart of *E. coli* MutM, there is no particular homology between them at the amino acid sequence level [16,18]. The human *OGG1* gene consists of seven exons and is mapped in 3p26.2, which is frequently deleted in a variety of human cancers. Alternative splicing of the C-terminal region of *OGG1* produces α-OGG1 (345

Table 1
Major polymorphic forms of OGG1, MUTYH and MTH1

Allele	Characteristic	Reference
OGG1		
Ser326Cys	The most common polymorphism of *OGG1*. The change of Ser326 to Cys reduces the activity for excision of 8-OH-G. It may be a risk factor for various cancers	[49–51,53–60]
Arg46Gln	OGG1-Gln46 has a reduced activity for excision of 8-OH-G	[49,55,61–63]
Arg154His	OGG1-His154 is less effective than OGG1-Ser326 and OGG1-Gln46 for excision of 8-OH-G and Fapy	[62–65]
MUTYH		
Gln324His (type 1)	Type 1 MUTYH is transferred to mitochondria. The amino acid change does not reduce the repair activity significantly	[71]
Gln310His (type 2)	Type 2 MUTYH is transferred to nucleus. The change does not affect the repair activity significantly	[71]
G/C intron 1	It induces an alternative splicing and reduces the translation efficiency	[72]
A/G intron 10	It induces the production of truncated protein, which is not localized in the nucleus	[73]
Gly382Asp	The change of Gly382 to Asp diminishes the glycosylase activity towards 8-OH-G:A. A risk factor for colorectal tumors	[31,74–85]
Tyr165Cys	The change of Tyr165 to Cys diminishes the glycosylase activity towards 8-OH-G:A. A risk factor for colorectal tumors	[31,74–85]
Tyr90 to stop	A possible risk factor for colorectal tumors	[83]
Glu466 to stop	A possible risk factor for colorectal tumors	[83]
MTH1		
Val83Met	MTH1-Met83 is more thermolabile than that of MTH1-Val83	[94–97]

amino acids) and β-OGG1 (424 amino acids) depending on the last exon of the sequence. Both proteins share the N-terminal region, which contains a mitochondria targeting signal (MTS), and α-OGG1 and β-OGG1 have exons 7 and 8, respectively [46]. β-OGG1 is transferred to the mitochondria while α-OGG1 is localized in the nucleus because of a nuclear localization signal (NLS) in the exon 7 [27,47,48].

Several single nucleotide polymorphisms (SNPs) of *OGG1* with amino acid substitutions have been registered in NCBI database (http://www.ncbi.nlm.nih.gov/SNP). Ser326Cys is the most common polymorphism of *OGG1* and the allele frequency of Cys326 is markedly different among ethnic groups [49–52]. In Japanese population, about 25% of the people are homozygous at codon 326 possessing Cys326/Cys326, but the frequency of Cys326 allele seems lower in Caucasian population [53]. The k_{cat}/K_m value of GST-OGG1-Cys326 for excision of 8-OH-G is about half of that of GST-OGG1-Ser326 [54]. GST-OGG1-Cys326 suppresses the spontaneous G:C to T:A transversions in *E. coli mutM mutY* mutants less effectively than does GST-OGG1-Ser326 (Fig. 2), suggesting that OGG1-Cys326 may have a lower ability to remove 8-OH-G in DNA than does OGG1-Ser326 [49]. However, both GST-OGG1-Ser326 and GST-OGG1-Cys326 suppress oxidative mutagenesis induced by 4-nitroquinoline N-oxide, methylene blue plus visible light and benzo[*a*]pyrene plus visible light

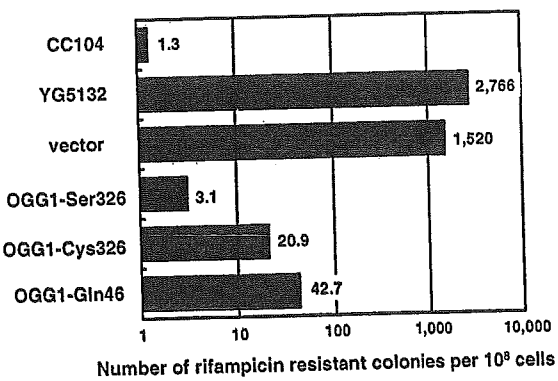


Fig. 2. Different abilities of polymorphic forms of OGG1 to suppress high spontaneous mutations of *E. coli mutM mutY* strain (YG5132). CC104 is the same as YG5132 but is *mutM*$^+$ *mutY*$^+$. GST-OGG1-Ser326, -Cys326, -Gln46 or vector plasmid (pGEX-1λT) is introduced to strain YG5132, and the resulting spontaneous rifampicin resistant mutations are assayed [49]. *p*-Values for the differences between GST-OGG1-Ser326 and GST-OGG1-Cys326 and between GST-OGG1-Ser326 and GST-OGG1-Gln46 are below 0.02.

in *Salmonella typhimurium* at similar efficiencies [55]. They also equally suppress spontaneous G:C to T:A mutations in *mutM mutY* mutants of *S. typhimurium*. In human cells, mutations by 8-OH-G in the plasmid are more efficiently suppressed by OGG1-Ser326 transduced cells than OGG1-Cys326 transduced cells [56], what supports the notion that Cys326Ser polymorphism affects the repair ability of OGG1. Interestingly, case-control studies on the polymorphism of Ser326Cys suggest that OGG1-Cys326 may be a risk factor for a variety of human cancers [50,51,57–59]. It is reported, however, that 8-OH-G DNA glycosylase activities in human lymphocytes are not affected by the polymorphic status at codon 326 of the *OGG* gene [60]. Thus, further studies are needed to examine whether the polymorphism of Cys326Ser of OGG1 is a significant risk factor for human cancers associates with oxidative damage in DNA.

Another variant is OGG1-Gln46, which was first identified in human tumors but later found in DNA isolated from peripheral lymphocytes from a patient with squamous cell carcinoma of the lung in one case [49,61,62]. OGG1-Ser326 and -Cys326 have Arg at codon 46, and OGG1-Gln46 has Ser at codon 326. OGG1-Gln46 is apparently less active than OGG1-Ser326: the k_{cat}/K_m value of OGG1-Gln46 is about half of that of OGG1-Ser326 [63], and GST-OGG1-Gln46 suppresses the mutations induced by methylene blue plus visible light less effectively in *S. typhimurium* than does GST-OGG1-Ser326 or Cys326 [55]. GST-OGG1-Gln46 displays a weaker suppression than do other two forms in a *mutM mutY* mutant of *E. coli* (Fig. 2) and *S. typhimurium* [55]. Because of the scarcity, no epidemiological reports are available with OGG1-Gln46.

The third variant OGG1-His154 was found in gastric human cancer cell line MKN1and human kidney tumor [62,64]. OGG1-Ser326, -Cys326 and -Gln46 have Arg at codon 154, and OGG1-His154 has Arg and Ser at codon 46 and 326, respectively. OGG1-His154 is less effective than OGG1-Ser326 and OGG1-Gln46 for excision of 8-OH-G and Fapy [63]. Arg154 is a conserved amino acid in human, mouse and yeast OGG1 proteins, and is directly involved in the recognition of the cytosine residue opposite 8-OH-G in DNA. In fact, the change of Arg154 to His relaxes the requirement for a pyrimidine opposite the lesion [63]. Screening of OGG1-His154 has been conducted in Korean population [65]. It is suggested that OGG1-His154 may be a low/moderate-penetrance modifier for colorectal cancer development.

3. MUTYH

The human *MUTYH* gene consists of 16 exons and encodes a protein of 535 amino acids that displays 41% identity to the *E. coli* MutY [66]. The gene maps on the short arm of chromosome 1 between p32.1 and p34.3. Alternative splicing gives type 1 (535 amino acids) and type 2 (521 amino acids) proteins [67]. An N-terminal portion of MUTYH acts as an MTS, and the region near C-terminal has a NLS. The type 2 protein lacks the first exon containing the MTS and is transferring to the nucleus while the type 1 protein is transferred to the mitochondria [67]. MUTYH protein interacts with a number of proteins such as RPA, APE1, PCNA and MSH6, and the level of expression in the nucleus increases in S phase compared to early G1 [68–70].

In NCBI database, several SNPs with amino acid substitutions have been registered. A variation of G/C in exon 12 is the first polymorphism found in the *MUTYH* gene [71]. This nucleotide variation is associated with amino acid substitution of Gln to His. Thus, four major types of MUTYH, i.e., type 1-Gln324, type 1-His324, type 2-Gln310 and type 2-His310, are expressed in human cells. The adenine glycosylase activity on A:8-OH-G and the ability to suppress spontaneous mutations in an *E. coli mutM mutY* mutant are greater in type 2 protein than in type 1 protein. However, there is apparently no difference in the repair activities between the two types of polymorphic MUTYH proteins. The allele frequency of His324Gln, i.e., 24%, is not significantly different in the individuals affected with colorectal cancer from those in 100 unaffected controls in British families [31].

A sequence variation of G/C was found in intron 1 of *MUTYH* (IVS1 + 5G/C) in a lung cancer cell line [72]. The variation from G to C induces an activation of cryptic splicing donor site and an alternative splicing in intron 1. The alternative splicing provides an extra sequence to 5′-side of the native open reading frame of type 2 protein and reduces the translation efficiency by 70%. Since this variation is identified in non-cancerous tissues of individuals as well, it can be regarded as an SNP rather than a somatic mutation.

A variation of A/G was found in intron 10 of *MUTHY* (IVS10-2A > G) of patients of Japanese familial gastric cancer [73]. The variant causes the production of aberrant mRNA encoding a truncated MUTYH protein. Unlike the wild-type MUTYH, the truncated protein is not localized in the nucleus. The correlation between IVS10-2A/G and gastric cancer was examined in a case-control study, but no significant difference in the distribution of the IVS10-2A > G variant was found between the cases and controls.

Important variants of *MUTYH* were found in the siblings affected with multiple adenoma and carcinoma of colon. They are compound heterozygotes of Tyr165Cys and Gly382Asp of *MUTYH* and most of the tumors display G:C to T:A transversions in the *APC* gene [31]. Introduction of mutations at the corresponding amino acids of *E. coli* MutY, i.e., Tyr82 and Gly253, reduces the glycosylase activities for A:8-OH-G and A:G substrates significantly [31,74]. Tyr82 is located in the pseudo-helix-hairpin-helix (HhH) motif and is predicted to function in mismatch specificity. Mouse MUTYH protein with an amino acid substitution (Gly365Asp), which corresponds to a human germ-line mutation of Gly382Asp, cannot suppress the elevated spontaneous mutations in MUTHY-null ES cells [75], and fails to prevent OGG1 from excising 8-OH-G opposite the generated abasic site [76]. Mouse MUTYH-Tyr150Cys shows a decrease in the rate of adenine removal from both substrates containing 8-OH-G:A and G:A pairs, while mouse MUTYH-Gly365Asp shows a decrease in the ability to catalyze adenine removal only with a G:A-containing substrate [77]. MUTYH mutants, Tyr165Cys and Gly382Asp, are devoid of glycosylase activity directed towards 8-OH-G:A [78]. Homozygous, but not heterozygous, *Mutyh* deficiency enhances intestinal tumorigenesis in $Apc^{Min/+}$ mice [79]. Thus, the inherited variations of Tyr165Cys and Gly382Asp of *MUTYH* is apparently associated with G:C to T:A mutation in colorectal tumors. This finding is significant because this is the first case to link between inherited deficiencies in the base excision repair pathway and human genetic disorders.

Further studies support the role of MUTYH in the colorectal adenoma and carcinoma predisposition [80–85]. In a screening of patients affected with colorectal adenomas, four were homozygous for truncated mutations, two were homozygous for Tyr165Cys and one was compound heterozygous for Tyr165Cys and Gly382Asp in the *MUTYH* gene [83]. The truncated mutations were caused by either Tyr90 to stop codon (one Pakistani case) or Glu466 to stop codon (three Indian cases). The missense mutations are derived from British Caucasian patients. The allele frequency of each variant may be different depending on ethnic groups. In other screening, 14 of 70 patients in familial adenomatous polyposis (FAP)/AAP with no detectable APC mutation and a family history compatible with recessive inheritance had biallelic germline MUTYH variants and 3 were heterozygotes [81]. The survey also identified a variation of ΔGGA1359 as a subpolymorphic form of MUTYH.

4. MTH1

MTH1, a counterpart of *E. coli* MutT, possesses 8-oxo-dGTPase activity, which degrades oxidized nucleotide 8-oxo-dGTP to sanitize the nucleotide pool [86]. When the cDNA for MTH1 is expressed in *E. coli mutT* cells, the elevated level of spontaneous A:T to C:G mutation frequency reverts to normal [87]. Although bacterial MutT does not hydrolyze oxidized dATP, MTH1 degrades 2-OH-dATP and 2-OH-ATP more efficiently than 8-oxodGTP [88]. The pool size of ATP in human cells is 100 times larger than dGTP pool, what supports the idea that the physiological substrates for MTH1 may be oxidized ATP or dATP [89]. The *MTH1* gene consists of five major exons and is mapped on 7p22 [87]. The mRNA is abundant in thymus, testis and the embryonic tissues. The *MTH1* gene produces four different polypeptides (p18, p21, p22, p26) through alternative splicing, alternative initiation of translation or SNP of splice site [90]. mRNA with a polymorphic alteration (GU to GC) at the beginning of exon 2C produces p26. Among them, p18 is the major form of the products and is identical to the purified 8-oxo-dGTPase in Jurkat cell [34,90]. The p18 protein is mainly localized in cytoplasm, and about 5% in mitochondrial matrix [91]. An alteration of MTH1 expression with the accumulation of 8-OH-G in DNA is observed in neurodegenerative diseases [92].

A G to A change at codon 83 of exon 4 of *MTH1* has been identified as an SNP, and this change results in the substitution of Val to Met [93]. Interestingly, this polymorphism is strongly linked with the polymorphism

(GU to GC) at exon 2C [90]. Met83-MTH1 has GC at exon 2C while Val83-MTH1 has GT at the site. 8-OH-dGTPase activity of MTH1-Met83 is more thermolabile than that of MTH1-Val83 [94]. The allele frequency of Val83 and Met83 in Japanese healthy volunteers is 0.91 and 0.09, respectively [90]. Met83 was detected in nine hereditary non-polyposis colorectal cancer (HNPCC) patients [93]. However, the same change was detected in 5 of 30 unrelated healthy individuals and it was concluded that the polymorphism is not associated with a marked HNPCC predisposition. MTH1is expressed aberrantly in the brains of patients with Parkinson's disease (PD) [95]. Val83Met polymorphism was studied in 73 patients with sporadic PD and 151 age-matched non-PD controls. The frequency of either Val83 or Met83 allele was not statistically different between PD patients (92.5 or 7.5%) and the controls (88.7 or 11.3%). MTH1 is not directly associated with ovarian cancer, either [96]. However, the polymorphism is suggested to be involved in the development of type 1 diabetes mellitus in the Japanese female population [97].

5. POLH (human DNA polymerase η)

During chromosome replication, 8-OH-G in DNA pairs with A as well as C, which may cause G:C to T:A transversions [12]. In mammalian cells, DNA pol δ, which is responsible for chromosome DNA replication, preferentially incorporates C opposite template 8-OH-G in the presence of PCNA [98]. However, its extension activity is not so strong, suggesting other DNA polymerases might have a role in the extension from 8-OH-G:C pair. Pol ζ in *S. cerevisiae* cannot bypass 8-oxoG in DNA, but can efficiently extend 8-OH-G:C pair that is formed by the incorporation of C opposite template 8-OH-G by DNA pol δ [99]. In yeast, DNA pol δ incorporates A opposite template 8-OH-G [45]. It is possible that more than one DNA polymerase is involved in translesion DNA synthesis (TLS) of 8-OH-G in DNA. Newly categorized Y-family DNA polymerase has strong TLS activity with low fidelity [100]. In humans, there are four DNA polymerases, i.e., DNA polymerase η, ι, κ and REV1, belonging to Y-family. Human DNA polymerase η and κ efficiently bypass 8-OH-G in DNA, and DNA polymerase η incorporates A and C opposite 8-OH-G with similar efficiency [101] while DNA polymerase κ preferentially incorporates A opposite 8-OH-G [102]. Human DNA polymerase ι correctly incorporates C opposite 8-OH-G but its bypass efficiency is very low [103]. Thus, among Y-family DNA polymerase, DNA polymerase η and κ may be involved in TLS of 8-OH-G in DNA.

As for 8-OH-dGTP incorporation, the oxidized dGTP is a very poor substrate for calf thymus DNA polymerase δ, with insertion efficiency more than 10^4-fold lower than for dGTP incorporation [98]. It prefers to incorporate 8-OH-dGTP opposite template C. 8-OH-dGTP is also very poorly incorporated into DNA by T7 DNA polymerase exo$^-$, HIV reverse transcriptase, *E. coli* DNA polymerase II and Klenow exo$^-$ [104]. In contrast, human DNA polymerase β incorporates 8-OH-dGTP with an efficiency of 10–20% of normal dGTP incorporation and favors to incorporate it opposite template A [105]. Human DNA polymerase η also efficiently incorporates 8-OH-dGTP opposite A and incorporates 2-OH-dATP opposite G, T or C [106]. Thus, DNA polymerase η and β may be involved in the incorporation of 8-OH-dGTP into DNA, and the incorporation opposite template A may cause A:T to C:G transversions.

Human DNA polymerase η is encoded by *POLH* (or *XPV*), which is defective in patients of xeroderma pigmentosum variant (XP-V), an inherited disorder with increased sensitivity to sunlight-induced skin cancers [43,44]. The *POLH* gene includes 11 exons and is located in chromosome 6p21.1-6p12 [107]. The gene encodes a protein of 713 amino acids. Human DNA polymerase η bypasses across TT dimer by inserting correct AA opposite the dimer, and is localized in replication sites during S phase and accumulates at the site of stalled replication forks [108,109]. Human DNA polymerases η and ι co-localize with proliferating cell nuclear antigen (PCNA) and their direct interactions have been demonstrated [110–113]. In addition, human DNA polymerase η as well as ι and κ, interacts with REV1, suggesting that REV1 may be a scaffold that recruits TLS polymerases [114–116].

More than 20 alleles of *POLH* have been identified in the cell lines derived from XP-V patients [43,44,107,117]. Most of them cause premature termination of XPV protein, and truncation occurs within domains of finger, palm, thumb and little finger, which are common to all Y-family DNA polymerases. Most of the truncated proteins are less than 410 amino

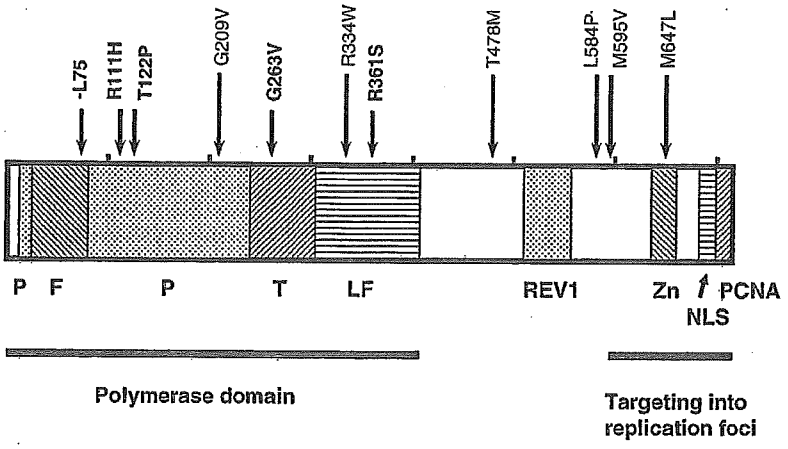


Fig. 3. Pictorial representation of several alleles of human DNA polymerase η. The positions of alleles in bold letters are due to Broughton et al. [117] and other are from NCBI database. Truncated mutations are not shown. P, F, T and LF in the polymerase domain represent the domains of palm (P), finger (F), thumb (T) and little finger (LF) deduced from Ling et al. [122]. REV1 represents the region necessary for interaction with REV1 [114]. Zn, NLS and PCNA stand for putative zinc finger motif, nuclear localization signal and PCNA-interacting motif, respectively [118].

acids, and the truncation is induced by nonsense mutations creating stop codon, frameshifts or deletions. However, three truncated proteins derived from cells of XP86VI (Gln521stop), XP1AB (Thr548stop) and XP37BR (556frameshift) have sizes of more than 520 amino acids. Extracts of these cells possess TLS activity with 10–50% of that of normal extracts [117]. Despite the in vitro TLS activity, the patients having C-terminal truncating mutations display symptoms of skin tumors. It is suspected that these C-terminal truncated mutants are not localized in the nucleus because polymerase η missing the last 120 amino acids is not localized in the nucleus and not localized in nuclear foci after UV irradiation [118]. Besides truncating mutations, several alleles cause substitutions of amino acids, i.e., Arg111His, Thr122Pro, Gly263Val and Arg361Ser, or deletion of one amino acid (−Leu75) (Fig. 3). Two of them, i.e., −Leu75 and Gly263Val, were tested for the in vitro TLS activity and the mutations abolished the activity in cell extracts [117]. In addition to alleles identified in cell lines from XP-V patients, six SNPs with amino acid substitutions have been deposited in NCBI database. Two of them, i.e., Gly209Val and Arg334Trp, are localized in N-terminal polymerase domain, and Gly209 is conserved in human DNA polymerase ι and DNA polymerase η of *S. cerevisiae*. Arg334 is not a conserved amino acid. Other four, i.e., Thr478Met, Leu584Pro, Met595Val and Met647Leu, are located in C-terminal part of DNA polymerase η. Among them, Met647 is located within putative Zn finger motifs and is conserved in mouse and Drosophila DNA polymerase η. The allele frequency of Met647Leu is about 8%. Neither the biochemical nature nor the epidemiological studies of the six SNPs are reported.

Besides *POLH*, SNPs of the *REV1* gene and the *POLI* gene encoding DNA polymerase ι are suggested to be associated with lung cancer risk [119]. A SNP of Phe257Ser of REV1 exhibits an association with squamous cell carcinoma risk and a SNP of Thr706Ala of DNA polymerase ι is associated with adenocarcinoma and squamous cell carcinoma risk, in particular in individuals of ages more than 61 years old. The mouse counterpart of *POLI*, *Poli* is identified as a candidate for the pulmonary adenoma resistance 2 (*Par2*) gene, which plays a role in the resistance to urethane-induced lung adenoma/adenocarcinoma. These results raise the possibility that SNPs of TLS polymerases might cause the inter-individual differences in the probability of mutagenesis and carcinogenesis in humans.

6. Conclusion

Polymorphisms of three human DNA repair enzymes, i.e., OGG1, MUTYH and MTH1, and human

DNA polymerase η have been reviewed. It is obvious that several variant forms of MUTYH and DNA polymerase η are associated with susceptibility to colorectal cancer and skin cancer, respectively. The variant forms of MUTYH, i.e., MUTYH-165Cys and MUTYH-382Asp, are devoid of glycosylase activity towards 8-oxo-G:A, and thus the variant cells could be vulnerable to oxidative damage. However, a question remains as to why the tumors are induced in a specific organ, i.e., colon. Oxidative damage may play a critical role in carcinogenesis in colon. DNA polymerase η can efficiently bypass thymine dimer in an error-free manner, thereby reducing the chance of error-prone bypass of the photoproduct. Although the role of DNA polymerase η in oxidative mutagenesis and carcinogenesis is not known, in vitro studies suggest that this polymerase may be involved in bypass of 8-OH-G in DNA and incorporation of 8-OH-dGTP during DNA replication. The variant forms of DNA polymerase η might affect the sensitivity of cells to oxidative mutagenesis in certain genetic backgrounds such as mismatch repair defective cells [120,121]. Significance of the polymorphisms of OGG1 and MTH1 is not clear yet although OGG1-Ser326Cys may be a risk factor for human cancers. A more detailed analysis of distribution of the polymorphism in various human populations is required to examine the possible roles in oxidative mutagenesis and carcinogenesis.

Acknowledgement

We thank Dr. Takashi Kohno, Biology Division, National Cancer Center Research Institute, Tokyo, Japan, for valuable comments and helpful suggestions on the manuscript.

References

[1] A.L. Jackson, L.A. Loeb, The contribution of endogenous sources of DNA damage to the multiple mutations in cancer, Mutat. Res. 477 (2001) 7–21.
[2] R.A. Floyd, Measurement of oxidative stress in vivo, in: K.J.A. Daview, F. Ursini (Eds.), The Oxygen Paradox, Cleup University Press, Cleup, 1995, pp. 89–103.
[3] B.N. Ames, Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases, Science 221 (1983) 1256–1264.
[4] D.I. Feig, T.M. Reid, L.A. Loeb, Reactive oxygen species in tumorigenesis, Cancer Res. 54 (1994) 1890s–1894s.
[5] H. Kasai, Chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis, Free Radic. Biol. Med. 33 (2002) 450–456.
[6] M.L. Michaels, J.H. Miller, The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine), J. Bacteriol. 174 (1992) 6321–6325.
[7] R.A. Floyd, C.A. Lewis, P.K. Wong, High-pressure liquid chromatography—electrochemical detection of oxygen free radicals, Methods Enzymol. 105 (1984) 231–237.
[8] T.A. Kunkel, The high cost of living. American Association for Cancer Research Special Conference: endogenous sources of mutations, Fort Myers, Florida, USA, 11–15 November 1998, Trends Genet. 15 (1999) 93–94.
[9] M. Kouchakdjian, V. Bodepudi, S. Shibutani, M. Eisenberg, F. Johnson, A.P. Grollman, D.J. Patel, 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 (1991) 1403–1412.
[10] Y. Oda, S. Uesugi, M. Ikehara, S. Nishimura, Y. Kawase, H. Ishikawa, H. Inoue, E. Ohtsuka, NMR studies of a DNA containing 8-hydroxydeoxyguanosine, Nucleic Acids Res. 19 (1991) 1407–1412.
[11] L.A. Lipscomb, M.E. Peek, M.L. Morningstar, S.M. Verghis, E.M. Miller, A. Rich, J.M. Essigmann, L.D. Williams, X-ray structure of a DNA decamer containing 7,8-dihydro-8-oxoguanine, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 719–723.
[12] S. Shibutani, M. Takeshita, A.P. Grollman, Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG, Nature 349 (1991) 431–434.
[13] M.L. Michaels, C. Cruz, A.P. Grollman, J.H. Miller, Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 7022–7025.
[14] H. Maki, M. Sekiguchi, MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis, Nature 355 (1992) 273–275.
[15] M. Sekiguchi, MutT-related error avoidance mechanism for DNA synthesis, Genes Cells 1 (1996) 139–145.
[16] S. Boiteux, J.P. Radicella, The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis, Arch. Biochem. Biophys. 377 (2000) 1–8.
[17] S.D. Bruner, D.P. Norman, G.L. Verdine, Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA, Nature 403 (2000) 859–866.
[18] K. Shinmura, J. Yokota, The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis, Antioxid. Redox. Signal. 3 (2001) 597–609.
[19] M.M. Greenberg, Z. Hantosi, C.J. Wiederholt, C.D. Rithner, Studies on N4-(2-deoxy-D-pentofuranosyl)-4,6-diamino-5-formamidopyrimidine (Fapy.dA) and N6-(2-deoxy-D-pentofuranosyl)-6-diamino-5-formamido-4-hydroxypyrimidine (Fapy.dG), Biochemistry 40 (2001) 15856–15861.