(2006)

2. 学会発表

1) K. Yamauchi, S. Kakinuma, S. Sudo, S. Kito, Y. Oota, T. Nohmi, Y. Shimada, Mutation frequency of thymocytes after combined exposure of X-rays with N-ethyl-N-nitrosourea is dependent on X-ray-dose, 日本環境変異原学会第 35 回大会(2006, 11)
2) Y. Sakamoto, K. Masumura, S. Takahashi, D. Nakae, T. Nohmi, Dietary choline deficiency induces oxidative mutagenesis in the liver of *gpt* delta rats, 日本環境変異原学会第 35 回大会(2006, 11)
3) T. Nohmi, M. Ikeda, K. Masumura, Y. Sakamoto, B. Wang, M. Nenoi, K. Sakuma, I. Hayata, Combined genotoxicity of low-dose-rate radiation and tobacco-specific nitrosamine NNK, CODATA 2006 in Beijing, China (2006, 10)
4) 梅村隆志、岡野圭太、黒岩有一、田崎雅子、児玉幸夫、能美健彦、西川秋佳、広瀬雅雄、マウス肝発癌剤dicyclanilが誘発する*gpt* deltaマウス肝の酸化的DNA損傷およびin vivo変異原性、第65回日本癌学会学術総会(2006, 9)
5) 蒋麗、鐘毅、赤塚慎也、劉玉亭、増村健一、能美健彦、豊國伸哉、トランスジェニックマウス*gpt* deltaを用いた鉄ニトリロ三酢酸誘導の腎発がんモデルのDNA変異を検出、第65回日本癌学会学術総会(2006, 9)
6) 大森雅子、魏民、木下アンナ、柚木孝之、土井賢一郎、加藤あゆみ、増村健一、能美健彦、福島昭治、鰐渕英機、*gpt* deltaラット肝における1,4-ジオキサンの発がん性および変異原性、第65回日本癌学会学術総会(2006, 9)
7) 池田恵、増村健一、松井恵子、甲野裕之、佐久間慶子、田中卓治、能美健彦、*gpt* deltaトランスジェニックマウスの肺におけるNNK誘発突然変異に対するNobiletinの化学予防効果の解析、第65回日本癌学会学術総会(2006, 9)
8) 増村健一、中江大、坂元康晃、高橋正一、鰐淵英機、梅村隆志、広瀬雅雄、能美健彦、F344系およびSD系*gpt* deltaラットを用いたコリン欠乏アミノ酸食による内因性ラット肝発がんと突然変異誘発能の解析、第65回日本癌学会学術総会(2006, 9)
9) 坂元康晃、増村健一、黒岩有一、今井聖子、林宏行、西川秋佳、広瀬雅雄、津田洋幸、能美健彦、ヒトプロト型c-Ha-ras導入*gpt* deltaトランスジェニックラットを用いた化学発がん高感受性モデルにおける突然変異誘発能の解析、第65回日本癌学会学術総会(2006, 9)
10) 田崎雅子、黒岩有一、神吉けい太、児玉幸夫、能美健彦、梅村隆志、西川秋佳、ペンタクロロフェノール誘発マウス肝DNAの酸化的損傷ならびにin vivo変異原性に及ぼすp53の影響、第65回日本癌学会学術総会(2006, 9)
11) T. Nohmi, M. Ikeda, K. Masumura, Y. Sakamoto, B. Wang, M. Nenoi, K. Sakuma, I. Hayata, Evaluation of combined genotoxicity of low-dose-rate radiation and tobacco-specific nitrosamine NNK, The 49th Annual Meeting of the Japan Radiation Research Society in Sapporo (2006, 9)
12) 能美健彦、増村健一、マウス個体で観察される欠失変異と点突然変異の分子解析、日本放射線影響学会 第49回大会 (2006, 9)
13) 塩見尚子、野代勝子、鬼頭靖司、増村健一、能美健彦、塩見忠博、生殖細胞と体細胞における自然および放射線誘発突然変異発生率の比較研究 II、精細胞期照

射における放射線誘発突然変異、日本放射線影響学会 第49回大会 (2006, 9)

14) 山内一己、柿沼志津子、須藤聡美、鬼頭靖司、能美健彦、増村健一、島田義也、マウス胸腺リンパ腫における放射線とエチルニトロソウレアの複合影響、日本放射線影響学会 第49回大会 (2006, 9)

G. 知的所有権の取得状況

なし

別紙4

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Matsui K, Yamada M, Imai M, Yamamoto K, Nohmi T	Specificity of replicative and SOS-inducible DNA polymerases in frameshift mutagenesis: Mutability of *Salmonella typhimurium* strains overexpressing SOS-inducible DNA polymerases to 30 chemical mutagens	DNA Repair	5	465-478	2006
Yamada M, Matsui K, Nohmi T	Development of a bacterial hyper-sensitive tester strain for specific detection of the genotoxicity of polycyclic aromatic hydrocarbons	Genes & Environ.	28	23-30	2006
Yamada M, Nunoshiba T, Shimizu M, Grúz P, Kamiya H, Harashima H, Nohmi T	Involvement of Y-Family DNA polymerases in Mutagenesis by oxidized nucleotides in *Escherichia coli*	J. Bacteriol.	188	4992-4995	2006
Koyama N, Sakamoto H, Sakuraba M, Koizumi T, Takashima Y, Hayashi M, Matsufuji H, Yamagata K, Masuda S, Kinae N, and Honma M	Genotoxicity of acrylamide and glycidamide in human lymphoblastoid TK6 cells	Mutat. Res.	603	151-158	2006
Oka H, Ikeda K, Yoshimura H, Ohuchida A, Honma M	Relationship between p53 status and 5-fluorouracil sensitivity in 3 cell lines	Mutat. Res.	606	52-60	2006
Ishikawa, S, Sasaki Y, Kawaguchi S, Mochizuki M, Nagao M.	Characterization of genotoxicity of Kojic acid by mutagenicity in Salmonella and micronucleus induction in rodent liver	Genes & Environ,	28	31-37	2006
Misaki K, Matsui S, Matsuda T	Metabolic Enzyme Induction by HepG2 Cells Exposed to Oxygenated and Non-oxygenated Polycyclic Aromatic Hydrocarbons	Chem. Res. Toxicol.	20	277-283	2007

T Matsuda, H Yabushita, RA Kanaly, S Shibutani, and A Yokoyama	Increased DNA Damage in ALDH2-Deficient Alcoholics	Chem. Res. Toxicol.	19	1374-1378	2006
Ikeda M, Masumura K, Sakamoto Y, Wang B, Nenoi M, Sakuma K, Hayata I, Nohmi T	Combined genotoxic effects of radiation and a tobacco-specific nitrosamine in the lung of *gpt* delta transgenic mice.	Mutat Res.	626	15-25	2007
Umemura T, Kanki K, Kuroiwa Y, Ishii Y, Okano K, Nohmi T, Nishikawa A, Hirose M.	In vivo mutagenicity and initiation following oxidative DNA lesion in the kidneys of rats given potassium bromate	Cancer Sci.	97	829-835	2006
Jiang L, Zhong Y, Akatsuka S, Liu YT, Dutta KK, Lee WH, Onuki J, Masumura K, Nohmi T, Toyokuni S	Deletion and single nucleotide substitution at G:C in the kidney of *gpt* delta transgenic mice after ferric nitrilotriacetate treatment.	Cancer Sci.	97	1159-67	2006
Ikeda M, Masumura K, Matsui K, Kohno H, Sakuma K, Tanaka T, Nohmi T	Chemopreventive effects of nobiletin against genotoxicity induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the lung of *gpt* delta transgenic mice.	Genes & Environ.	28	84-91	2006

DNA REPAIR 5 (2006) 465–478

available at www.sciencedirect.com

SCIENCE @ DIRECT®

journal homepage: www.elsevier.com/locate/dnarepair

Specificity of replicative and SOS-inducible DNA polymerases in frameshift mutagenesis: Mutability of *Salmonella typhimurium* strains overexpressing SOS-inducible DNA polymerases to 30 chemical mutagens

Keiko Matsui[a], *Masami Yamada*[a], *Masaru Imai*[b], *Kazuo Yamamoto*[b], *Takehiko Nohmi*[a,*]

[a] *Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[b] *Department of Biomolecular Sciences, Graduate School of Life Sciences, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai, Miyagi Prefecture 980-8577, Japan*

ARTICLE INFO

Article history:
Received 16 August 2005
Received in revised form 26 November 2005
Accepted 15 December 2005
Published on line 7 February 2006

Keywords:
SOS-inducible DNA polymerases
Replicative DNA polymerase
Translesion DNA synthesis
Frameshift
Mutagens

ABSTRACT

DNA replication is frequently hindered because of the presence of DNA lesions induced by endogenous and exogenous genotoxic agents. To circumvent the replication block, cells are endowed with multiple specialized DNA polymerases that can bypass a variety of DNA damage. To better understand the specificity of specialized DNA polymerases to bypass lesions, we have constructed a set of derivatives of *Salmonella typhimurium* TA1538 harboring plasmids carrying the *polB*, *dinB* or *mucAB* genes encoding *Escherichia coli* DNA polymerase II, DNA polymerase IV or DNA polymerase RI, respectively, and examined the mutability to 30 chemicals. The parent strain TA1538 possesses CGCGCGCG hotspot sequence for −2 frameshift. Interestingly, the chemicals could be classified into four groups based on the mutagenicity to the derivatives: group I whose mutagenicity was highest in strain YG5161 harboring plasmid carrying *dinB*; group II whose mutagenicity was almost equally high in strain YG5161 and strain TA98 harboring plasmid carrying *mucAB*; group III whose mutagenicity was highest in strain TA98; group IV whose mutagenicity was not affected by the introduction of any of the plasmids. Introduction of plasmid carrying *polB* did not enhance the mutagenicity except for benz[a]anthracene. We also introduced a plasmid carrying *polA* encoding *E. coli* DNA polymerase I to strain TA1538. Strikingly, the introduction of the plasmid reduced the mutagenicity of chemicals belonging to groups I, II and III, but not the chemicals of group IV, to the levels observed in the derivative whose SOS-inducible DNA polymerases were all deleted. These results suggest that (i) DNA polymerase IV and DNA polymerase RI possess distinct but partly overlapping specificity to bypass lesions leading to −2 frameshift, (ii) the replicative DNA polymerase, i.e., DNA polymerase III, participates in the mutagenesis and (iii) the enhanced expression of *E. coli polA* may suppress the access of Y-family DNA polymerases to the replication complex.

---

* *Corresponding author*. Tel.: +81 3 3700 9873; fax: +81 3 3707 6950.
E-mail address: nohmi@nihs.go.jp (T. Nohmi).

1568-7864/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2005.12.010

1. Introduction

Cellular DNA is continuously exposed to a variety of endogenous and exogenous genotoxic agents. Although DNA repair mechanisms are in operational to remove DNA lesions, DNA polymerases have to often encounter the lesions that are escaped from the repair mechanisms. DNA damages, such as ultraviolet light (UV) photoproducts or carcinogen adducts, strongly block the progress of DNA replication, and thus daughter-strand gaps are generated downstream of the lesions [1]. In *Escherichia coli*, the single-stranded regions are recognized by RecA protein, which mediates recombination to fill in the gaps with a homologous DNA sequence that is derived from the replicated sister chromatids [2]. In addition, the binding of RecA to the single-stranded regions activates RecA protein itself to mediate the cleavage of LexA repressor, which triggers the expression of more than 30 genes in the chromosome. This damage-inducible gene expression is referred to as an SOS response [3]. Interestingly, some of DNA polymerases in *E. coli* (see below) are regulated as part of the SOS response, and the SOS-inducible DNA polymerases appear to be involved in translesion DNA synthesis (TLS), which directly bypasses the lesions to fill in the gaps [4–6]. Some TLS reactions they catalyze are error-prone, i.e., incorporating incorrect bases in the nascent strand, while others are error free [7]. Thus, filling in the gaps by TLS appears to contribute to mutagenesis as well as to DNA damage tolerance, while filling-in reactions by homologous recombination are supposed to be non-mutagenic.

In *E. coli*, there are five DNA polymerases, i.e., DNA polymerases I–V [8]. DNA polymerase I encoded by *polA* is the first DNA polymerase to be described and is involved in lagging strand DNA synthesis, i.e., processing of Okazaki fragment, DNA repair and initiation of ColE1 plasmids such as pBR322 [9,10]. DNA polymerase II encoded by *polB* is a damage (SOS)-inducible DNA polymerase [11,12] and is involved in the process to restart the synthesis of damaged DNA [13,14]. This polymerase is reported to bypass 2-acetylaminofluorene adducts, which results in −2 frameshift [15,16]. Unlike DNA polymerase I, which belongs to A-family DNA polymerase, DNA polymerase II is a member of B family, in which mammalian replicative DNA polymerases such as DNA polymerase delta are included [17]. DNA polymerase III holoenzyme, which is composed of multiple subunits, is responsible for the chromosome replication of *E. coli* and the catalytic subunit is encoded by *dnaE* (or *polC*) [18,19]. This enzyme is classified into C family, in which prokaryotic replicative DNA polymerases are categorized [8]. DNA polymerase IV and DNA polymerase V belong to Y family, whose members are mostly involved in TLS, and the expression of *dinB* and *umuDC* encoding DNA polymerase IV and DNA polymerase V, respectively, is regulated as part of the SOS response [20–24]. DNA polymerase IV is shown to be involved in −1 frameshift mutagenesis induced by 4-nitroquinoline N-oxide and benzo[a]pyrene [25,26], and DNA polymerase V is known to play important roles in mutagenesis induced by UV and a variety of genotoxic compounds [27,28]. However, current knowledge about the roles of replicative, i.e., DNA polymerase I and DNA polymerase III, and SOS-inducible specialized DNA polymerases, i.e., DNA polymerase II, DNA polymerase IV and DNA polymerase V, in mutagenesis is still limited because synthetic oligonucleotides bearing specific DNA lesions are required for in vitro and in vivo analyses to address the question.

Salmonella typhimurium is a Gram negative bacterium, whose genome sequence is 70–90% homologous to *E. coli* [29]. Some of *S. typhimurium* strains have been widely used to detect a variety of environmental mutagens and carcinogens as tester strains of Ames test [30]. One of such strain TA1538 possesses CGCGCGCG sequence in the *hisD* gene, which is a mutational hot spot for −2 (−CG) frameshift [31,32]. The strain bears a deep-rough *rfa* mutation, which increases the permeability to hydrophobic compounds such as polycyclic aromatic hydrocarbons (PAH) [33]. In addition, the strain is deficient in the capacity to excise bulky DNA adducts by the *uvrB* mutation, so that the DNA adducts are more likely to be bypassed rather than removed by repair enzymes [33].

In a previous study, we have systematically disrupted the genes of *S. typhimurium* TA1538 encoding SOS-inducible DNA polymerases, i.e., $polB_{ST}$, $dinB_{ST}$, $umuDC_{ST}$ and *samAB*, and concluded that different sets of DNA polymerases are engaged in lesion bypass in the CGCGCGCG sequence depending upon the environmental threats by chemicals [34]. We also proposed that not only SOS-inducible DNA polymerases but also the main replicative DNA polymerase, i.e., DNA polymerase III, plays important roles in −2 frameshift [34].

In this study, we generated a set of isogenic derivatives of *S. typhimurium* TA1538 by introducing plasmids carrying *polB*, *dinB* or *mucAB* encoding *E. coli* DNA polymerase II, DNA polymerase IV or DNA polymerase RI, respectively, and examined the mutability to 30 chemicals. We introduced the plasmid carrying *mucAB*, i.e., pKM101, instead of a plasmid carrying *E. coli umuDC*, because DNA polymerase RI is a homologue of *E. coli* DNA polymerase V [35], and the derivative of TA1538 harboring plasmid pKM101, i.e., strain TA98, has been widely used as a standard tester strain of Ames test [30]. We also introduced a plasmid carrying the *polA* gene of *E. coli* to strain TA1538 and examined the mutability to investigate the possible involvement of DNA polymerase I in TLS leading to frameshift. Intriguingly, the introduction of the *polA* plasmid completely suppressed the mutations depending on the activities of $dinB_{ST}$ and $umuDC_{ST}$ of *S. typhimurium*. Collectively, the present results suggest that (1) DNA polymerase IV and DNA polymerase RI has distinct but partly overlapping specificity to bypass lesions leading to −2 frameshift, (2) the replicative DNA polymerase, i.e., DNA polymerase III, substantially contributes to −2 frameshift and (3) the enhanced expression of *E. coli* polymerase I inhibits the access of Y-family DNA polymerases to the replication complex where TLS occurs. In addition, our results raise an interesting possibility that strain YG5161 harboring plasmid pYG768 carrying *dinB* could be a superior tester strain to strain TA98 to detect the mutagenicity of environmental PAHs.

2. Materials and methods

2.1. Strains and plasmids

The strains and plasmids used in this study are listed in Table 1. Strain YG5160 and strain YG5161 were constructed

Table 1 – *S. typhimurium* strains and plasmids

Strain or plasmid	Description	Source
Strains		
TA1535	*hisG46, gal, Δ (chl, uvrB, bio), rfa*	Maron and Ames [30]
TA1537	*hisC3076, gal, Δ (chl, uvrB, bio), rfa*	Maron and Ames [30]
TA1538	*hisD3052, gal, Δ (chl, uvrB, bio), rfa*	Maron and Ames [30]
TA98	As TA1538 but harbors plasmid pKM101	Maron and Ames [30]
YG5160	As TA1538 but harbors plasmid pYG787	This study
YG5161	As TA1538 but harbors plasmid pYG768	This study
YG6215	As TA1538 but $\Delta umuDC_{ST}$::Km^r, $\Delta samAB$::Cm^r, $\Delta dinB_{ST}$::Sp^r, $\Delta polB_{ST}$::Tc^r	Kokubo et al. [34]
Plasmids		
pKM101	Plasmid carrying the *mucAB* genes	Maron and Ames [30]
pYG768	Derivative of pWSK29 with the *E. coli dinB* gene	Kim et al. [36]
pYG787	Derivative of pWKS30 with the *E. coli polB* gene	Kokubo et al. [34]
pIMA-1	Derivative of pWKS30 with the *E. coli polA* gene	Imai and Yamamoto (unpublished)

by introduction of plasmid pYG787 carrying *polB* and plasmid pYG768 carrying *dinB*, respectively, into strain TA1538 [34,36]. Plasmid pIMA-1 carrying *E. coli polA* [9] was constructed by the insertion of a 3.5-kb fragment of the *polA* gene between EcoRI and SalI sites of plasmid pWKS30 (Imai and Yamamoto, unpublished). The direction of transcription of the *polA* gene in the plasmid is opposite to that of the *lacZ* gene. The plasmid could complement the killing sensitivity of a *polA* strain of *E. coli* to ultraviolet light and methyl methanesulfonate. Transformation was conducted by electroporation [37].

2.2. *Chemicals*

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

2.3. *Media*

Luria–Bertani broth and agar were used for bacterial culture [38]. Vogel–Bonner minimal agar plates and top agar were prepared as previously described, and used for the His$^+$ reversion assay with *S. typhimurium* [30]. Nutrient broth (Difco, MI, USA) with ampicillin (AP, 25 μg/ml) was used for pre-cultures of the strains for the reversion assay.

2.4. *Mutagenicity assay*

The mutagenicity assay was carried out with a pre-incubation procedure [30]. Briefly, 0.1 ml overnight culture was incubated with the chemicals dissolved in 0.1 ml solvent and 0.5 ml 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 to seven doses on duplicate plates with four strains, i.e., strain TA1538, TA98, YG5160 and YG5161, in parallel. In the series of experiments, we regarded the effects of introduction of plasmids, i.e., pYG787, pYG768 or pKM101, on the mutability of strain T1538 as significant when the transformed strains displayed more than and including 50% higher or lower mutability, compared to the parent strain TA1538.

3. Results

3.1. *Specificity of SOS-inducible DNA polymerases in frameshift induced by 30 chemicals*

To assign the role of SOS-inducible DNA polymerases in bypass of DNA lesions, we introduced plasmids carrying *E. coli polB*, *dinB* or *mucAB* encoding different SOS-inducible DNA polymerases to strain TA1538 and examined their mutability to 30 chemicals. The dose–response curves are presented in Fig. 2, and the numbers of revertants per microgram per plate of each chemical and strain are summarized in Table 3. To make the comparison easier, we also calculated the relative mutability of each derivative by assigning the number of revertants per microgram in strain TA1538 as 1.0. According to the mutagenicity, we classified the 30 chemicals into four groups as follows.

Group I includes benzo[a]pyrene and other seven chemicals. The mutagenicity of these compounds was highest in strain YG5161 harboring plasmid pYG768 carrying *dinB* encoding DNA polymerase IV, followed by strain TA98 harboring plasmid pKM101 carrying *mucAB* encoding DNA polymerase RI. The mutagenicity of the chemicals to strain YG5160 harboring plasmid pYG787 carrying *polB* encoding DNA polymerase II was very similar to the parent strain TA1538 except for benzo[a]pyrene-7,8-dihydroepoxide and 1-aminoanthracene where introduction of plasmid pYG787 appeared to alleviate the mutagenicity by 50% and 40%, respectively. For benzo[a]pyrene, the ratio of the mutability of strain YG5161, TA98, YG5160 and TA1538 was 7:2:1:1. The compounds in this group are derivatives of benzo[a]pyrene except for 3-methylcholanthrene, 1-aminoanthracene and 2-aminoanthracene.

Group II includes ENNG and other four chemicals. The mutagenicity of these compounds was almost equally high in strain YG5161 and strain TA98. The introduction of plasmid pYG787 carrying *polB* did not enhance the mutagenicity. Rather, plasmid pYG787 seemed to reduce the mutagenicity of 6-aminochrysene by 60%. The ratio of the mutability of strain YG5161, TA98, YG5160 and TA1538 was 20:19:1:1 for ENNG. The compounds in this group are PAHs and the derivative except for ENNG.

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

Chem. no.	Chemical	CAS registry numbers	Sources[a]
1	Benzo[a]pyrene-7,8-dihydroepoxide	36504-65-1	Mi
2	Benzo[a]pyrene diol epoxide	58917-67-2	Mi
3	10-Azabenzo[a]pyrene	189-92-4	1
4	Benzo[a]pyrene	50-32-8	W
5	3-Nitro-benzo[a]pyrene	70021-98-6	4
6	3-Methylcholanthrene	56-49-5	S
7	1-Aminoanthracene	610-49-1	S
8	2-Aminoanthracene	613-13-8	W
9	7,12-Dimethylbenz[a]anthracene (DMBA)	57-97-6	W
10	6-Aminochrysene	2642-98-0	S
11	1-Nitro-benzo[a]pyrene	70021-99-7	4
12	Benzo[a]pyrene-4,5-dihydroepoxide	64437-52-1	Mi
13	N-Ethyl-N′-nitro-N-nitrosoguanidine (ENNG)	4245-77-6	5
14	1-Nitropyrene	5522-43-0	T
15	1,8-Dinitropyrene	42397-65-9	T
16	6-Nitro-benzo[a]pyrene	63041-90-7	4
17	1-Nitro-6-azabenzo[a]pyrene	138835-35-5	4
18	3-Nitro-6-azabenzo[a]pyrene	138835-36-6	4
19	Furylfuramide	3688-53-7	W
20	Aflatoxin B1	1162-65-8	S
21	Benzo[a]pyrene-7,8-tetrahydroepoxide	36504-67-3	Mi
22	Acridine orange	65-61-2	Me
23	Benz[a]anthracene	56-55-3	S
24	2-Nitrofluorene	607-57-8	T
25	2-[2-(Acetylamino)-4-[bis-(2-methoxy-ethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole (PBTA-1)	194590-84-6	3
26	2-Amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1)	67730-11-4	W
27	Aminophenylnorharman	219959-86-1	3
28	N-Hydroxyacetylaminofluorene (N-OH-AAF)	53-95-2	Mi
29	4-Nitroquinoline-1-oxide (4-NQO)	56-57-5	T
30	2-Acetylaminofluorene	53-96-3	T

[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 follwing 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); laboratory stock (5).

Group III includes 1-nitropyrene and other 10 chemicals. The mutagenicity of these compounds was highest in strain TA98. Introduction of plasmid pYG768 carrying *dinB* displayed moderate (less than three-fold) enhancing effects on the mutagenicity of this group of chemicals. The introduction of plasmid pYG787 carrying *polB* enhanced the mutagenicity of benz[a]anthracene three-fold reproducibly, although it had no enhancing effects on other chemicals. For the mutagenicity of 1,8-dinitropyrene, 1-nitro-6-azabenzo[a]pyrene and furylfuramide, plasmid pYG787 reduced the mutagenicity by half. The ratio of the mutability of strain TA98, YG5161, YG5160 and TA1538 was 16:1:1:1 for 1-nitropyrene. The compounds in this group include structurally unrelated compounds such as furylfuramide, aflatoxin B1 and acridine orange.

Group IV includes 2-acetylaminofluorene and other five compounds. The characteristic of this group was that the mutagenicity was not enhanced by the introduction of any of the plasmids encoding SOS-inducible DNA polymerases. The ratio of the mutability of strain YG5161, TA98, YG5160 and TA1538 was 1:1:1:1 for 2-acetylaminofluorene. The compounds in this group are aromatic amines except for 4-NQO.

3.2. −1 *Frameshit and base substitutions by benzo[a]pyrene and ENNG promoted by DNA polymerase IV and DNA polymerase RI*

Since DNA polymerase IV encoded by *dinB* appeared to promote −2 frameshift induced by benzo[a]pyrene (group I chemical) and ENNG (group II chemical), we examined the possibility whether the polymerase also promotes other types of mutations, i.e., −1 frameshift and base substitutions, by the chemicals. To this end, we took advantage of other *S. typhimurium* strains, i.e., TA1537 and TA1535, which detects mutagens that cause −1 frameshift in CCC sequence in the *hisC* gene and base substitutions in GGG sequence in the *hisG* gene, respectively [30]. For benzo[a]pyrene-induced mutagenesis, the introduction of plasmid pYG768 carrying *dinB* into strain TA1537 slightly enhanced the mutagenicity, but the effect of enhancing mutagenesis was much lower compared to the effect of plasmid pKM101 carrying *mucAB* encoding DNA polymerase RI (Fig. 3A). For base substitutions, DNA polymerase IV seemed inactive and virtually no enhancement was observed in strain TA1535 with plasmid pYG768. In contrast, DNA polymerase RI actively promoted the base substitution mutations. As has been observed

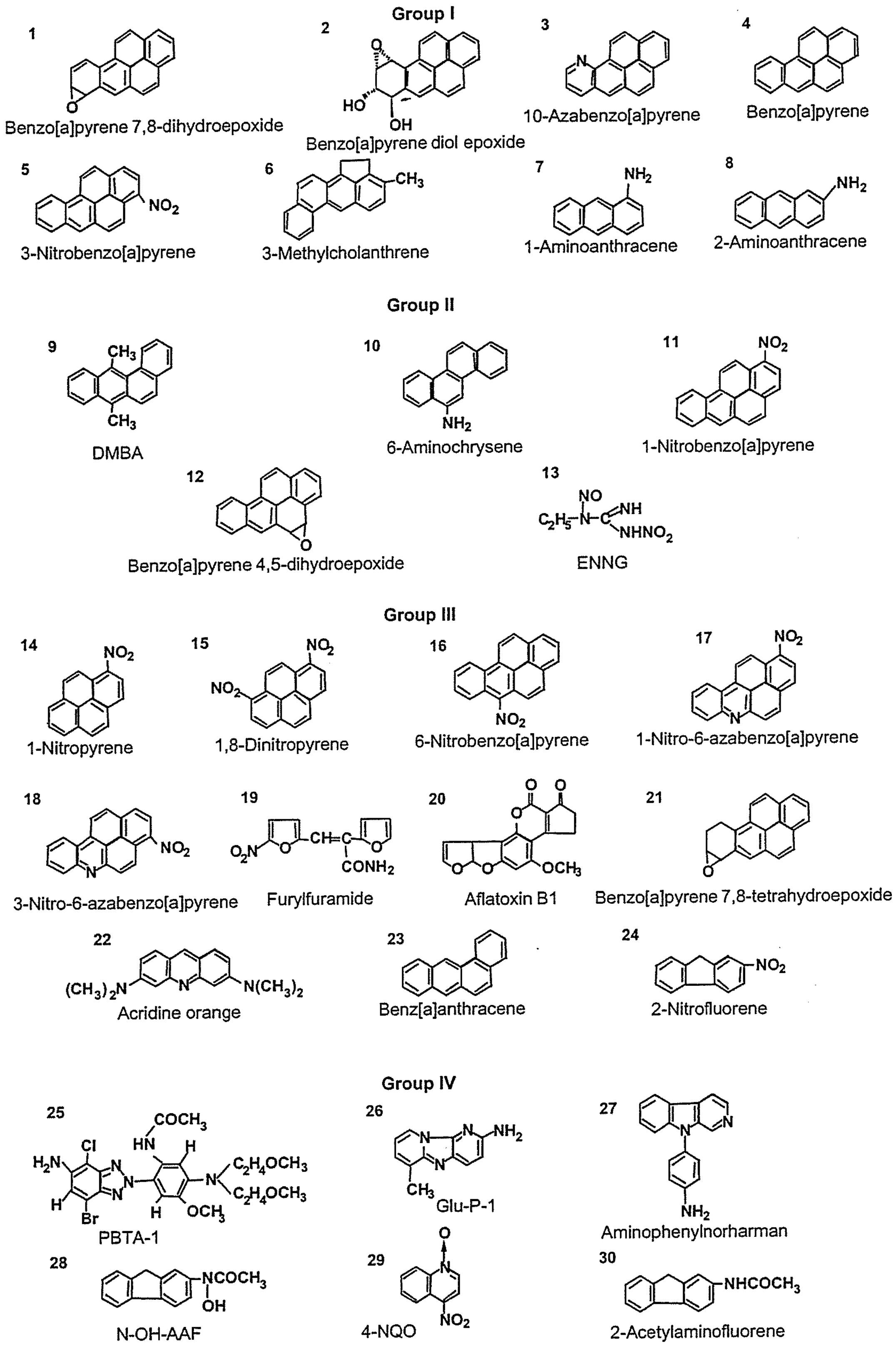

Fig. 1 – Structures of the chemical mutagens used in this study.

in −2 frameshift, introduction of plasmid pYG787 carrying *polB* had almost no effects on any types of mutations induced by benzo[a]pyrene. These results suggest that the efficiency of error-prone bypass across lesions by DNA polymerase IV strongly depends on the types of mutations and the sequence context surrounding the lesions. For ENNG-induced mutagenesis, both DNA polymerase IV and DNA polymerase RI appeared to promote −1 frameshift and base substitutions substantially (Fig. 3B). Introduction of plasmid pYG787 carrying *polB* had almost no effects on the mutability. Unlike ENNG-induced mutagenesis, −2 frameshift, −1 frameshift and base substitutions induced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) were not enhanced by the introduction of either plasmid pYG768 or pKM101 (data not shown). These results suggest that both DNA polymerase IV and DNA polymerase RI bypass ethyl, but not methyl, adducts in DNA leading to −2 and −1 frameshifts and base substitutions.

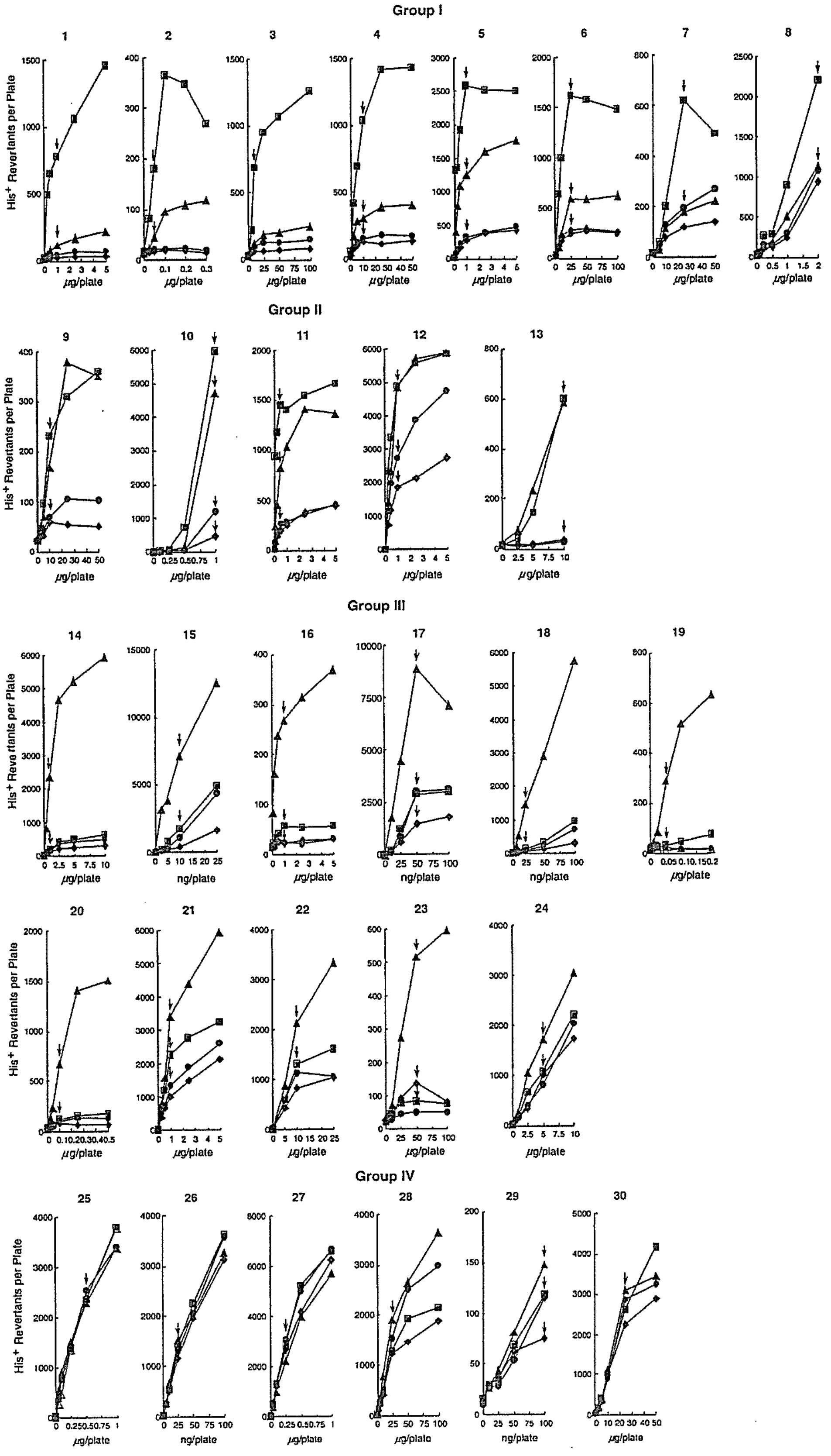

Table 3 – Mutagenicity of 30 chemicals in *S. typhimurium* strains harboring plasmids carrying genes encoding *E. coli* SOS DNA polymerases

Number	Chemical	Group	S9	TA1538 no plasmid	TA98 pKM101 (DNA pol RI)	YG5160 pYG787 (DNA pol II)	YG5161 pYG768 (DNA pol IV)
1	Benzo[a]pyrene-7,8-dihydroepoxide	I	–	44 (1.0)	113 (2.6)	22 (0.5)	776 (17.6)
2	Benzo[a]pyrene diol epoxide	I	–	420 (1.0)	920 (2.2)	320 (0.8)	3620 (8.6)
3	10-Azabenz[a]pyrene	I	+	9 (1.0)	12 (1.3)	6 (0.7)	68 (7.6)
4	Benzo[a]pyrene	I	+	15 (1.0)	30 (2.0)	13 (0.9)	104 (6.9)
5	3-Nitro-benzo[a]pyrene	I	–	316 (1.0)	1244 (3.9)	277 (0.9)	2580 (8.2)
6	3-Methylcholanthrene	I	+	10 (1.0)	23 (2.3)	11 (1.1)	65 (6.5)
7	1-Aminoanthracene	I	+	8 (1.0)	7 (0.9)	5 (0.6)	25 (3.1)
8	2-Aminoanthracene	I	+	540 (1.0)	564 (1.0)	468 (0.9)	1102 (2.0)
9	DMBA	II	+	7 (1.0)	17 (2.4)	6 (0.9)	23 (3.3)
10	6-Aminochrysene	II	+	1200 (1.0)	4693 (3.9)	461 (0.4)	5955 (5.0)
11	1-Nitro-benzo[a]pyrene	II	–	524 (1.0)	1640 (3.1)	400 (0.8)	2896 (5.5)
12	Benzo[a]pyrene-4,5-dihydroepoxide	II	–	2724 (1.0)	4836 (1.8)	1856 (0.7)	4900 (1.8)
13	ENNG	II	–	3 (1.0)	58 (19.3)	4 (1.3)	60 (20)
14	1-Nitropyrene	III	–	154 (1.0)	2354 (15.3)	112 (0.7)	194 (1.3)
15	1,8-Dinitropyrene	III	–	110100 (1.0)	708300 (6.4)	39300 (0.4)	171600 (1.6)
16	6-Nitro-benzo[a]pyrene	III	–	24 (1.0)	268 (11.2)	21 (0.9)	58 (2.4)
17	1-Nitro-6-azabenzo[a]pyrene	III	–	60500 (1.0)	178180 (2.9)	29700 (0.5)	58020 (1.0)
18	3-Nitro-6-azabenzo[a]pyrene	III	–	3560 (1.0)	57640 (16.2)	2480 (0.7)	5240 (1.5)
19	Fulylfuramide	III	–	480 (1.0)	5760 (12.0)	220 (0.5)	620 (1.3)
20	Aflatoxin B1	III	+	990 (1.0)	6680 (6.7)	750 (0.8)	1160 (1.2)
21	Benzo[a]pyrene-7,8-tetrahydroepoxide	III	–	1332 (1.0)	3404 (2.6)	1000 (0.8)	2252 (1.7)
22	Acridine orange	III	+	113 (1.0)	234 (2.1)	83 (0.7)	131 (1.2)
23	Benz[a]anthracene	III	+	1 (1.0)	10 (10.0)	3 (3.0)	2 (2.0)
24	2-Nitrofluorene	III	–	162 (1.0)	341 (2.1)	203 (1.3)	215 (1.3)
25	PBTA-1	IV	+	5074 (1.0)	4568 (0.9)	4768 (0.9)	4720 (0.9)
26	Glu-P-1	IV	+	52800 (1.0)	61120 (1.2)	46480 (0.9)	56640 (1.1)
27	Aminophenylnorharman	IV	+	12352 (1.0)	8880 (0.7)	10688 (0.9)	11456 (0.9)
28	N-OH-AAF	IV	–	62 (1.0)	76 (1.2)	49 (0.8)	52 (0.8)
29	4-NQO	IV	–	1150 (1.0)	1470 (1.3)	750 (0.7)	1180 (1.0)
30	2-Acetylaminofluorene	IV	+	114 (1.0)	124 (1.1)	89 (0.8)	105 (0.9)

Each chemical was assayed with four to seven doses on duplicate plates with four strains in parallel. The assays with chemical nos. 2, 4, 7, 8, 10, 12, 15, 17, 23 and 29 were repeated to confirm the initial results. The numbers of His^+ revertants per plate per microgram of each strain are calculated at the doses indicated with arrows in Fig. 2. The numbers in parentheses represent the values relative to the numbers of His^+ revertants per microgram in TA1538 (no plasmid). Difference of the relative mutability two-fold or more was regarded as significant effects of the introduction of plasmids on the mutability.

Group I: the chemicals whose mutagenicity was highest in strain YG5161 harboring plasmid pYG768 carrying *dinB* (DNA pol IV).

Group II: the chemicals whose mutagenicity was equally high in both strain YG5161and strain TA98 harboring plasmid pKM101 carrying *mucAB* (DNA pol RI).

Group III: the chemicals whose mutagenicity was highest in strain TA98.

Group IV: the chemicals whose mutagenicity was not substantially modulated by the introduction of any of the plasmids.

Fig. 2 – Responses of *S. typhimurium* tester strains to 30 chemical mutagens. The chemicals are: benzo[a]pyrene-7,8-dihydroepoxide (1); benzo[a]pyrene diol epoxide (2); 10-azabenzo[a]pyrene (3); benzo[a]pyrene (4); 3-nitro-benzo[a]pyrene (5); 3-methylcholanthrene (6); 1-aminoanthracene (7); 2-aminoanthracene (8); DMBA (9); 6-aminochrysene (10); 1-nitro-benzo[a]pyrene (11); benzo[a]pyrene-4,5-dihydroepoxide (12); ENNG (13); 1-nitropyrene (14); 1,8-dinitropyrene (15); 6-nitro-benzo[a]pyrene (16); 1-nitro-6-azabenzo[a]pyrene (17); 3-nitro-6-azabenzo[a]pyrene (18); furylfuramide (19); aflatoxin B1 (20); benzo[a]pyrene-7,8-tetrahydroepoxide (21); acridine orange (22); benz[a]anthracene (23); 2-nitrofluorene (24); PBTA-1 (25); Glu-P-1 (26); aminophenylnorharman (27); N-OH-AAF (28); 4-NQO (29); 2-acetylaminofluorene (30). The strains used are: TA1538 (circles ●); YG5160 (diamonds ♦); YG5161 (squares ■); TA98 (triangles ▲). The arrow indicates the dose that was used for the calculation of His^+ revertants per microgram per plate in Table 3.

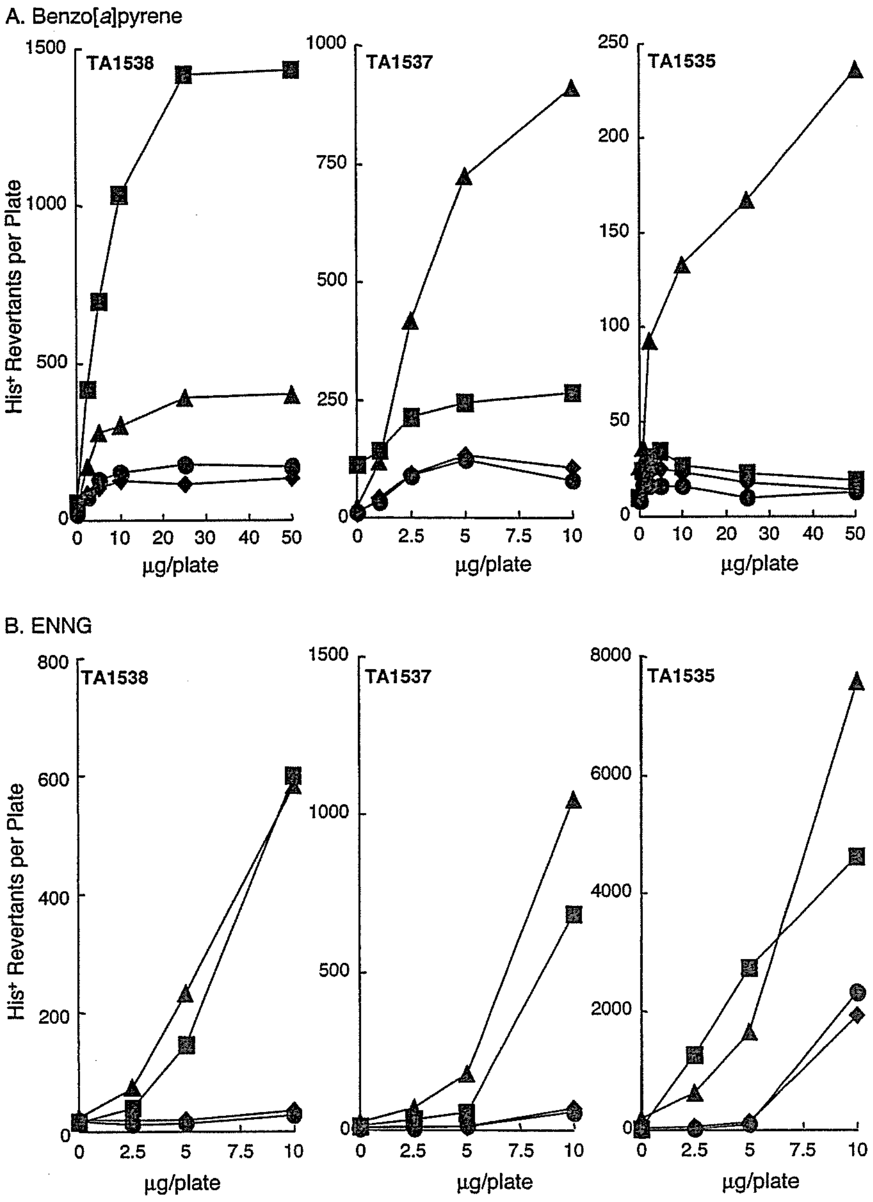

Fig. 3 – Responses of S. *typhimurium* tester strains to benzo[a]pyrene and ENNG. Benzo[a]pyrene plus S9 mix (A) or ENNG (B) was assayed with four to six doses on duplicate plates with four strains, i.e., the parent strain without plasmids (circles ●), the derivative harboring plasmid pYG787 carrying *polB* (diamonds ♦), the derivative harboring plasmid pYG768 carrying *dinB* (squares ■) and the derivative harboring plasmid pKM101 carrying *mucAB* (triangles ▲) in parallel. The parent strains were S. *typhimurium* TA1538, TA1537 and TA1535 for the detection of −2 frameshift, −1 frameshift and base substitutions, respectively, and they were transformed with one of three plasmids, i.e., pYG787, pYG768 and pKM101.

3.3. *Effects of the introduction of plasmid carrying polA encoding E. coli DNA polymerase I on the mutability of strain TA1538*

The mutagenicity of the compounds of group IV was not influenced by introduction of any of the plasmids carrying genes encoding SOS-inducible DNA polymerases (Fig. 2). This suggests the involvement of replicative DNA polymerases, i.e., DNA polymerase I and/or DNA polymerase III, in the mutagenesis. To examine the possible involvement of DNA polymerase I, we introduced plasmid pIMA-1 carrying *polA* encoding DNA polymerase I to strain TA1538 and its derivative YG6215, which lacks all the genes encoding SOS-inducible DNA polymerases [34], and compared the mutability to the group IV compounds, i.e., PBTA-1, Glu-P-1, aminophenylnorharman, 4-NQO and 2-acetylaminofluorene (Fig. 4). We also examined the mutagenicity of 2-aminofluorene, a derivative of 2-acetylaminofluorene. The introduction of plasmid pIMA-1 did not affect the mutability of strain TA1538 and YG6215 to the group IV compounds and 2-aminofluorene. We also examined the mutability of strain TA1538 and YG6215 harboring plasmid pIMA-1 carrying *polA* to other chemicals belong to group I, i.e., benzo[a]pyrene, 10-azabenzo[a]pyrene, 3-methylcholanthrene and 1-aminoanthracene, group II,

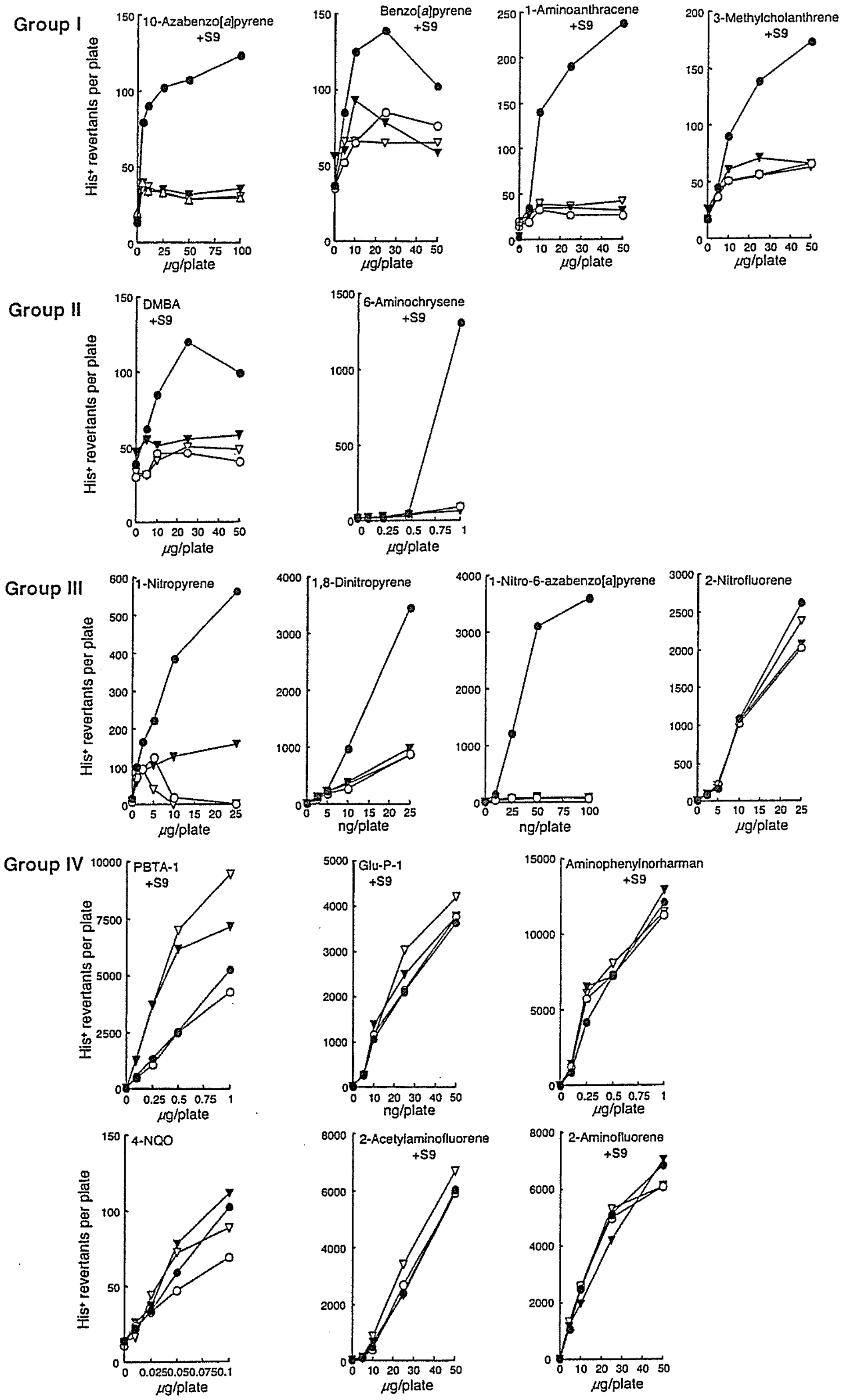

Fig. 4 – Effects of introduction of plasmid pIMA-1 carrying *polA* on the mutability of strain TA1538 and YG6215 to chemicals. The strains used were *S. typhimurium* TA1538 harboring the vector pWKS30 (closed circles ●), TA1538 harboring plasmid pIMA-1 (open circles ○), YG6215 harboring the vector pWKS30 (closed triangles ▼) and YG6215 harboring plasmid pIMA-1 (open triangles ▽). Each chemical was assayed with five to six doses on triplicate plates with four strains in parallel. The dose response curves of PBTA-1 plus S9 was unique in that strain YG6215 lacking all SOS-inducible DNA polymerases displayed higher mutability than strain TA1538 [34].

i.e., DMBA and 6-aminochrysene and group III, i.e., 1-nitropyrene, 1,8-dinitropyrene, 1-nitro-6-azabenzo[a]pyrene and 2-nitrofluorene. Surprisingly, the introduction of plasmid pIMA-1 reduced the mutability of strain TA1538 to the level of strain YG6215 harboring the vector plasmid (Fig. 4). Only exception was 2-nitrofluorene where the mutability of strain TA1538 and YG6215 was not affected by the introduction of plasmid pIMA-1 as in the case of group IV compounds. The dose–response curves of strain YA1538 harboring plasmid pIMA-1 almost completely overlapped those of strain YG6215 harboring the vector plasmid. The mutability of strain YG6215 was not affected by the introduction of plasmid pIMA-1 carrying *polA* except for 1-nitropyrene where strain YG6215 as well as strain TA1538 exhibited an enhanced killing sensitivity to the chemical when the *polA* plasmid was introduced.

4. Discussion

DNA polymerase III holoenzyme replicates the chromosome of *E. coli* with high fidelity but its progress is occasionally blocked by DNA lesions, and DNA polymerase V encoded by *umuDC* bypasses the lesions to assist the chromosome replication, which mostly results in base substitutions [10,28]. Less is known, however, about the mechanisms of translesion bypass leading to frameshift [39–41]. To assign the role of each SOS-inducible DNA polymerases of *E. coli* in TLS leading to frameshift, we have introduced plasmids carrying the genes encoding SOS-inducible DNA polymerases to strain TA1538 possessing CGCGCGCG sequence in the *hisD* gene as a −2 frameshift hot spot [31], and examined the mutability to 30 chemical mutagens. The *mucB* gene is expressed 13,000 molecules per cell from plasmid pKM101 when LexA repressor is inactivated [42], and the expression level is much higher than the chromosomal expression level of the *umuC* gene, i.e., about 200 molecules per cell upon SOS induction [43]. Similarly, the *dinB* gene is expressed 25,000–50,000 molecules per cell from plasmid pYG768 in the presence of DNA damage, which is 10–20 times higher than the expression level of *dinB* from the chromosome upon SOS induction [25]. The exact expression levels of DNA polymerase I and DNA polymerase II from plasmid pIMA-1 and pYG787, respectively, are unclear, but they could be at least two to three times higher than those from the chromosome because both plasmids are derivatives of low-copy-number plasmid pWKS30 [44]. DNA polymerase I and DNA polymerase II encoded by *polA* and *polB*, respectively, are expressed 400 and 30–50 molecules per cell from the chromosome and the expression of *polB* is enhanced about seven-fold by DNA damage [10,45]. Thus, we assume the expression levels are about 1000 for DNA polymerase I, 500–1000 for DNA polymerase II, 10–20 for DNA polymerase III [10], 25,000–50,000 for DNA polymerase IV and 13,000 for DNA polymerase RI per cell when the chromosome DNA is damaged by chemicals. Because of the high levels of expression of these DNA polymerases from the plasmids, we could observe distinct enhancing effects on the mutability of strain TA1538 to 30 chemicals (Fig. 2, Table 3). Based on the mutagenicity, we classified the chemicals into four groups as follows.

Group I includes eight chemicals that exhibited highest mutagenicity in strain YG5161 harboring plasmid pYG768 carrying *dinB*. The introduction of plasmid pKM101 also enhanced the mutagenicity of some compounds, but the extent of the enhancement was much less compared to the effects of plasmid pYG768. In fact, the introduction of plasmid pKM101 into strain TA1538 did not enhance the mutagenicity of 1-aminoanthracene and 2-aminoanthracene while plasmid pYG768 enhanced the mutagenicity more than two times. These results suggest that DNA polymerase IV encoded by *dinB* efficiently bypasses DNA lesions induced by group I compounds in CGCGCGCG sequence leading to −2 frameshift. This is consistent with our previous results that deletion of endogenous *dinB* gene of *S. typhimurium*, i.e., $dinB_{ST}$, significantly reduced the mutability of strain TA1538 to 10-azabenzo[a]pyrene, benzo[a]pyrene, 1-aminoanthracene and 2-aminoanthracene [34]. DNA polymerase IV could have specificity to bypass across guanine bases modified with the polycyclic aromatics, leading to −2 frameshift. In contrast to −2 frameshift, the introduction of plasmid pYG768 did not enhance base substitutions induced by benzo[a]pyrene (Fig. 3A). *E. coli* DNA polymerase IV is reported to bypass N^2-guanine adducts of benzo[a]pyrene diol epoxide in vitro with higher efficiency and high fidelity compared to DNA polymerase II and DNA polymerase V [46]. Thus, we suggest that DNA polymerase IV correctly incorporate cytosine opposite the adducted guanine in the GGG sequence in the *hisG* gene, thereby leading to no base substitutions. Correct insertion of cytosine opposite the adducted guanine in the CGCGCGCG sequence in *hisD*, however, may induce a two-bp slippage leading to −2 frameshift [47].

Group II includes five chemicals that displayed equally high mutagenicity to strain YG5161 and strain TA98. They are derivatives of PAHs, i.e., DMBA and benzo[a]pyrene-4,5-dihydroepoxide, an aromatic amine, i.e., 6-aminochrysene, a nitroaromatic, i.e., 1-nitrobenzo[a]pyrene and an alkylating agent, i.e., ENNG. The results that strains YG5161 and TA98 exhibited similar mutability to the compounds suggest that lesions induced by group II compounds can be bypassed by either DNA polymerase IV or DNA polymerase RI at similar efficiency. In fact, the previous study indicates that deletion of either $dinB_{ST}$ or $umuDC_{ST}$ reduced the mutagenicity of DMBA and 6-aminochrysene [34]. These compounds appear to require the presence of at least two specialized DNA polymerases to bypass the lesions. Of group II compounds, ENNG is exceptional because it is a simple alkylating agent that induces a mutagenic guanine base, i.e., O^6-ethylguanine [48]. Since the lesion in DNA directs the incorporation of thymine as well as cytosine during DNA synthesis, it was expected that this compound enhanced base substitutions in strain TA1535 (Fig. 3 B). It was a big surprise, however, that the simple alkylating agent was also capable of inducing −1 and −2 frameshifts in the repetitive sequences in strains TA1537 and TA1538, respectively, and that the bypass reactions leading to frameshifts appeared to be mediated by DNA polymerase IV or DNA polymerase RI. We also observed that the introduction of plasmids pYG768 and pKM101 enhanced the mutability of strain TA1538 against ethylnitrosourea (ENU), which induces O^6-ethylguanine in DNA (unpublished results). Eckert and Hile reported that frameshift errors are generated during in vitro DNA synthesis of ENU-treated template single-stranded DNA by mammalian DNA alpha-primase and DNA polymerase beta

[49]. Since humans possess Y-family DNA polymerases, i.e., DNA polymerase eta, iota, kappa and REV1 [8], it seems worth examining the abilities to bypass O^6-ethylguanine in repetitive sequences leading to frameshifts.

Since the newly established strain YG5161 harboring plasmid pYG768 exhibited higher sensitivity to groups I and II compounds than the standard Ames tester strain TA98 harboring plasmid pKM101, strain YG5161 could be useful for the sensitive detection of environmental mutagens and carcinogens such as benzo[a]pyrene and its derivatives. Actually, 10-azabenzo[a]pyrene, benzo[a]pyrene and 3-nitrobenzo[a]pyrene are present in polluted air and soot of combustion of coal [50,51], and some of them are identified in cigarette smoke [52].

Group III includes 1-nitropyrene and other 10 compounds. They include structurally unrelated compounds such as furylfuramide, aflatoxin B1 and acridine orange. The previous study indicated that deletion of *umuDC*$_{ST}$ significantly reduced the mutagenicity of 1-nitropyrene, 1,8-dinitropyrene, 1-nitro-6-azabenzo[a]pyrene and 3-nitro-6-azabenzo[a]pyrene [34], which are all included in this group. DNA adducts induced by group III compounds appeared to be more efficiently bypassed by DNA polymerase RI leading to −2 frameshift, compared to DNA polymerase IV or DNA polymerase II. Of the compounds, the mutagenicity of 3-nitro-6-azabenzo[a]pyrene is reduced by more than 95% by the deletion of *umuDC*$_{ST}$ [34]. Since the endogenous DNA polymerase V encoded by *umuDC*$_{ST}$ is capable to bypass DNA adduct(s) induced by this compound, the exogenous expression of DNA polymerase RI from plasmid pKM101 merely enhanced the mutagenicity by less than three-fold. Although DNA polymerase II encoded by *polB* had virtually no enhancing effects or rather suppressing effects on the mutagenicity of the chemicals examined, the introduction of plasmid pYG787 carrying *polB* specifically and repeatedly enhanced the mutagenicity of benz[a]anthracene, which is a potent carcinogen (Table 3). This compound induces adducts in guanine N^2 and adenine N6 atoms upon metabolic activation [53,54]. Thus, guanine N^2-adducts by the active metabolites of benz[a]anthracene in the CG repetitive sequence could be bypassed by DNA polymerase II leading to −2 frameshift. Since DNA polymerase II is a member of B-family DNA polymerase, its mammalian counterpart such as DNA polymerase delta may have an ability to bypass the adducts in the repetitive sequences.

Group IV includes PBTA-1 and other five compounds. PBTA-1 is a potent aromatic amine mutagen in a polluted river [55]. The characteristic of this group of compounds is that the introduction of any of the plasmids encoding SOS-inducible DNA polymerase had no enhancing effects on the mutagenicity. In the previous study, we reported that the mutagenicity of PBTA-1, Glu-P-1, aminophenylnorharman, N-OH-AAF, 4-NQO and 2-acetylaminofluorene, which are all belong to group IV in this study, are not reduced by the deletions of any of *S. typhimurium* genes encoding SOS-inducible DNA polymerases [34]. Thus, we suggested that the replicative DNA polymerase, i.e., DNA polymerase III holoenzyme, is responsible for the translesion bypass across DNA adducts induced by the chemicals in the CG repetitive sequence leading to −2 frameshift [34]. Although we cannot strictly rule out the possibility that DNA polymerase I is involved in the translesion, we prefer the possibility that DNA polymerase III holoenzyme is responsible for the bypass reactions because the introduction of plasmid pIMA-1 carrying *polA* did not enhance the mutagenicity (Fig. 4). The group IV compounds are all aromatic amines except for 4-NQO and some of them are proved to induce guanine C8 adducts in DNA [56–58]. Thus, we suggest that DNA polymerase III holoenzyme efficiently skips over guanine C8 adducts by aromatic amines in certain sequence context such as CGCGCGCG, thereby inducing −2 frameshift. Involvement of the replicative DNA polymerase may make this repetitive sequence a hot spot for frameshift mutagenesis.

In the previous study, we systematically disrupted one or all the genes of *S. typhimurium* strain TA1538 encoding SOS-inducible DNA polymerases and examined the mutability to chemical mutagens [34]. It is in contrast with the present study where the expression of SOS-inducible DNA polymerases is enhanced. As expected, most of the chemicals exhibited contrastive responses. They displayed enhanced mutagenicity in the presence of enhanced expression of the DNA polymerase, and diminished mutagenicity in the absence of the gene encoding the polymerase. Curiously, some compounds exhibited unexpected mutagenicity in the plasmid-bearing strains and the deletion strains. For example, 3-nitrobenzo[a]pyrene, 1-nitrobenzo[a]pyrene and 2-nitrofluorene were classified into groups I, II and III, respectively (Table 3, Fig. 1). The mutagenicity was enhanced by the introduction of plasmid pYG768 carrying *dinB* encoding DNA polymerase IV and/or pKM101 carrying *mucAB* encoding DNA polymerase RI. However, the mutagenicity of these compounds was not reduced by deletion of any of the genes encoding SOS-inducible DNA polymerases (class IV compounds in the previous study [34]). These results suggest that DNA polymerase III holoenzyme is responsible for the translesion bypass across the lesions induced by the chemicals in physiological conditions, but the Y-family DNA polymerases can take over the translesion reactions when the expression levels are enhanced. In other words, DNA polymerase III holoenzyme, DNA polymerase IV and DNA polymerase RI share, at least in part, the specificity to bypass the lesions and the polymerase actually involved in the translesion depends upon the cellular expression levels or the concentrations of the DNA polymerase in the replication complex.

To examine the possible involvement of DNA polymerase I in the frameshift mutagenesis, we introduced plasmid pIMA-1 carrying *polA* to strain TA1538 and strain YG6215, in which all the genes encoding SOS-inducible DNA polymerase are deleted [34]. Strikingly, the introduction of plasmid pIMA-1 sharply reduced the mutability of strain TA1538 against groups I, II and III compounds to that of strain YG6215 (Fig. 4). The dose–response curves of strain TA1538 harboring plasmid pIMA-1 with benzo[a]pyrene, 10-azabenzo[a]pyrene, 1-aminoanthracene, 3-methylcholanthrene (group I), DMBA, 6-aminochrysene (group II), 1,8-DNP and 1-nitro-6-azabenzo[a]pyrene (group III) almost overlapped those of strain YG6215 harboring plasmid pIMA-1 or the vector plasmid pWKS30. Because the mutability of strain TA1538 harboring plasmid pIMA-1 appears to be similar to that of strain YG6215 harboring the vector, we suggest that the enhanced expression of DNA polymerase I prevents the access of the Y-family DNA polymerases, i.e., DNA polymerase IV and DNA polymerase V, to the replication complex where translesion bypass actually occurs. It is known that all five DNA polymerases in E. coli interact with the beta-subunit of DNA polymerase III holoenzyme [59–61]. Thus, there should be some competition for the polymerases to interact with the beta clamp. The beta-subunit assembles in a donut-like shape as a dimer and tethers DNA polymerase to a template/primer DNA, thereby preventing a falling off of polymerase from template DNA [62]. We speculate the order of the affinity of each DNA polymerase to the beta clamp or the replication complex may be DNA polymerase III > DNA polymerase I > DNA polymerase II > DNA polymerase IV = DNA polymerase V. This assumption is based on the observation that the introduction of plasmid pIMA-1 reduced the mutagenicity of compounds of groups I, II and III, which require the presence of the Y-family DNA polymerases for the maximum mutagenesis, but not group IV, whose mutagenicity is depended upon DNA polymerase III holoenzyme (Fig. 4). In addition, Foster suggested that DNA polymerase II may be dominant over DNA polymerase IV in the replication complex because the *dinB* mutator effects are more pronounced in the stationary-phase mutagenesis when the *polB* gene is deleted [63]. At present, we do not know which of DNA polymerase I or DNA polymerase II has a higher affinity to the replication complex. We prefer the possibility, however, that DNA polymerase I is dominant over DNA polymerase II because it is involved in lagging strand DNA synthesis during the chromosome replication. An alternative explanation for the suppressive effects of plasmid pIMA-1 (Fig. 4) is that DNA polymerase I expressed from the plasmid bypasses the lesions induced by the chemicals of groups I–III in an error-free manner, thereby reducing the mutagenicity. However, we think it less likely because the suppressive effects would vary with chemicals or lesions if DNA polymerase I mediated the error-free TLS. Each DNA polymerase including DNA polymerase I should have specificity to bypass the lesions. Hence, the introduction of pIMA-1 would suppress the mutagenicity of some compounds efficiently but not others. In fact, the strong suppressive effects were observed with almost all the compounds of groups I–III we examined. This is in contrast with the suppressive effects of DNA polymerase II expressed from plasmid pYG787, which reduced the mutagenicity of some of the compounds of groups I–III with various efficiencies (see below more detail). Thus, we prefer the possibility that DNA polymerase I expressed from the plasmid inhibits the access of the Y-family DNA polymerases to the replication complex. Nevertheless, it is important to examine whether a catalytically dead mutant of DNA polymerase I exhibits the suppressive effects on the mutagenicity of groups I–III chemicals to distinguish the possibilities.

In contrast to the clear suppressive effects by plasmid pIMA-1 carrying *polA*, the suppressive effects of plasmid pYG787 carrying *polB* on strain TA1538 were moderate. The introduction of plasmid pYG787 reduced the mutagenicity of benzo[a]pyrene-7,8-dihydroepoxide and 1-aminoanthracene (group I), 6-aminochrysene (group II), 1,8-dinitropyrene, 1-nitro-6-azabenzo[a]pyrene and furylfuramide (group III) by 40–60% (Table 3). DNA polymerase II may mediate the error-free translesion across DNA adducts induced by these compounds. In the previous study, deletion of *polB*$_{ST}$ reduced the −2 frameshift mutations induced by benzo[a]pyrene-7,8-tetrahydroepoxide, 3-methycholanthrene, 1-nitropyrene, 1,8-nitropyrene, 1-nitro-6-azabenzo[a]pyrene and 3-nitro-6-azabenzo[a]pyrene by 30–60% [34]. Thus, it seems that the enhanced expression as well as the lack of expression diminished the mutagenicity of 1,8-dinitropyrene and 1-nitro-6-azabenzo[a]pyrene. In other words, DNA polymerase II could have an optimal cellular concentration to enhance the translesion DNA synthesis leading to −2 frameshift. This is contrast to DNA polymerase IV and DNA polymerase RI, which enhance the mutagenesis when the levels of their expression are elevated. E. coli DNA polymerase II is reported to be involved in the immediate recovery of DNA synthesis after UV irradiation [13,14]. It is tempting to speculate that it 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 polymerase III holoenzyme encountered the lesion and stopped the replication. If the expression level of DNA polymerase II was enhanced, it might promote error-free bypass reactions across the lesions while the lack of DNA polymerase II might lead to poor translesion DNA synthesis by DNA polymerase IV or DNA polymerase V.

In summary, our results suggest that DNA polymerase IV and DNA polymerase RI possess distinct but partly overlapping specificity to bypass lesions leading to −2 frameshift, and also that the replicative DNA polymerase, i.e., DNA polymerase III holoenzyme, participates in the bypass reactions in the CG repetitive sequence. Although DNA polymerase III holoenzyme is responsible for the translesion, the Y-family DNA polymerase may take over the primer termini, thereby enhancing the bypass reactions, when the expression of the polymerases is enhanced. Based on the suppressive effects of plasmid pIMA-1, we speculate that the order of DNA polymerases in E. coli to access to the replication complex could be DNA polymerase III > DNA polymerase I > DNA polymerase II > DNA polymerase IV = DNA polymerase V. Our results also raise an interesting possibility that strain YG5161 harboring plasmid pYG768 is a sensitive tester strain to identify the mutagenicity of environmental PAHs.

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. 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] G.C. Walker, SOS-regulated proteins in translesion DNA synthesis and mutagenesis, Trends Biochem. Sci. 20 (1995) 416–420.

[4] H. Echols, M.F. Goodman, Fidelity mechanisms in DNA replication, Annu. Rev. Biochem. 60 (1991) 477–511.

[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] Z. Wang, Translesion synthesis by the UmuC family of DNA polymerases, Mutat. Res. 486 (2001) 59–70.

[8] 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.

[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] 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.

[16] 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.

[17] P.M. Burgers, E.V. Koonin, E. Bruford, L. Blanco, K.C. Burtis, M.F. Christman, W.C. Copeland, E.C. Friedberg, F. Hanaoka, D.C. Hinkle, C.W. Lawrence, M. Nakanishi, H. Ohmori, L. Prakash, S. Prakash, C.A. Reynaud, A. Sugino, T. Todo, Z. Wang, J.C. Weill, R. Woodgate, Eukaryotic DNA polymerases: proposal for a revised nomenclature, J. Biol. Chem. 276 (2001) 43487–43490.

[18] 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.

[19] 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.

[20] H. Ohmori, E.C. Friedberg, R.P. Fuchs, M.F. Goodman, F. Hanaoka, D. Hinkle, T.A. Kunkel, C.W. Lawrence, Z. Livneh, T. Nohmi, L. Prakash, S. Prakash, T. Todo, G.C. Walker, Z. Wang, R. Woodgate, The Y-family of DNA polymerases, Mol. Cell 8 (2001) 7–8.

[21] 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.

[22] 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.

[23] 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.

[24] 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.

[25] 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. Genom. 266 (2001) 207–215.

[26] 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.

[27] G.C. Walker, Bryn bridges and mutagenesis: exploring the intellectual space, Mutat. Res. 485 (2001) 69–81.

[28] 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.

[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] D.M. Maron, B.N. Ames, Revised methods for the *Salmonella* mutagenicity test, Mutat. Res. 113 (1983) 173–215.

[31] 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.

[32] T. Nohmi, M. Yamada, M. Matsui, K. Matsui, M. Watanabe, T. Sofuni, Involvement of *umuDCST* 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.

[33] 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.

[34] K. Kokubo, M. Yamada, Y. Kanke, T. Nohmi, Roles of replicative and specialized DNA polymerases in frameshift mutagenesis: mutability of *Salmonella typhimurium* strains lacking on eor all of SOS-inducible DNA polymerases to 26 chemicals, DNA Rep. (Amst.) 4 (2005) 1160–1171.

[35] M. Goldsmith, L. Sarov-Blat, Z. Livneh, Plasmid-encoded MucB protein is a DNA polymerase (pol RI) specialized for lesion bypass in the presence of MucA′, RecA, and SSB, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11227–11231.

[36] 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.

[37] 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.

[38] J.H. Miller, A short Course in Bacterial Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1992.

[39] 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.

[40] K. Bebenek, T.A. Kunkel, Frameshift errors initiated by nucleotide misincorporation, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 4946–4950.

[41] 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.

[42] C. Venderbure, A. Chastanet, F. Boudsocq, S. Sommer, A. Bailone, Inhibition of homologous recombination by the plasmid MucA'B complex, J. Bacteriol. 181 (1999) 1249–1255.

[43] R. Woodgate, D.G. Ennis, Levels of chromosomally encoded Umu proteins and requirements for in vivo UmuD cleavage, Mol. Gen. Genet. 229 (1991) 10–16.

[44] R.F. Wang, S.R. Kushner, Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*, Gene 100 (1991) 195–199.

[45] Z. Qiu, M.F. Goodman, The *Escherichia coli* polB locus is identical to dinA, the structural gene for DNA polymerase II. Characterization of Pol II purified from a polB mutant, J. Biol. Chem. 272 (1997) 8611–8617.

[46] 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.

[47] 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.

[48] R.B. Setlow, E.H. Cao, N.C. Delihas, Enzymology of repair of DNA adducts produced by N-nitroso compounds, IARC Sci. Publ. (1984) 561–570.

[49] K.A. Eckert, S.E. Hile, Alkylation-induced frameshift mutagenesis during in vitro DNA synthesis by DNA polymerases alpha and beta, Mutat. Res. 422 (1998) 255–269.

[50] K. Yamada, T. Suzuki, A. Kohara, M. Hayashi, A. Hakura, T. Mizutani, K. Saeki, Effect of 10-aza-substitution on benzo[a]pyrene mutagenicity in vivo and in vitro, Mutat. Res. 521 (2002) 187–200.

[51] H. Tokiwa, N. Sera, A. Nakashima, K. Nakashima, Y. Nakanishi, N. Shigematu, Mutagenic and carcinogenic significance and the possible induction of lung cancer by nitro aromatic hydrocarbons in particulate pollutants, Environ. Health Perspect. 102 (Suppl. 4) (1994) 107–110.

[52] S.S. Hecht, Tobacco smoke carcinogens and breast cancer, Environ. Mol. Mutagen. 39 (2002) 119–126.

[53] H.Y. Kim, A.S. Wilkinson, C.M. Harris, T.M. Harris, M.P. Stone, Minor groove orientation for the (1S,2R,3S,4R)-N2-[1-(1,2,3,4-tetrahydro-2,3,4-trihydroxybenz[a]anthracenyl)]-2′-deoxyguanosyl adduct in the N-ras codon 12 sequence, Biochemistry 42 (2003) 2328–2338.

[54] Z. Li, P.J. Tamura, A.S. Wilkinson, C.M. Harris, T.M. Harris, M.P. Stone, Intercalation of the (1R,2S,3R,4S)-N6-[1-(1,2,3,4-tetrahydro-2,3,4-trihydroxybenz[a]anthracenyl)]-2′-deoxyadenosyl adduct in the N-ras codon 61 sequence: DNA sequence effects, Biochemistry 40 (2001) 6743–6755.

[55] 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.

[56] M. Nagao, T. Sugimura, Carcinogenic factors in food with relevance to colon cancer development, Mutat. Res. 290 (1993) 43–51.

[57] Y. Totsuka, T. Takamura-Enya, R. Nishigaki, T. Sugimura, K. Wakabayashi, Mutagens formed from beta-carbolines with aromatic amines, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 802 (2004) 135–141.

[58] G.W. Hsu, J.R. Kiefer, D. Burnouf, O.J. Becherel, R.P. Fuchs, L.S. Beese, Observing translesion synthesis of an aromatic amine DNA adduct by a high-fidelity DNA polymerase, J. Biol. Chem. 279 (2004) 50280–50285.

[59] F.J. Lopez de Saro, M. O'Donnell, Interaction of the beta sliding clamp with MutS, ligase, and DNA polymerase I, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8376–8380.

[60] K.A. Bunting, S.M. Roe, L.H. Pearl, Structural basis for recruitment of translesion DNA polymerase Pol IV/DinB to the beta-clamp, EMBO J. 22 (2003) 5883–5892.

[61] B.P. Dalrymple, K. Kongsuwan, G. Wijffels, N.E. Dixon, P.A. Jennings, A universal protein–protein interaction motif in the eubacterial DNA replication and repair systems, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 11627–11632.

[62] P.T. Stukenberg, P.S. Studwell-Vaughan, M. O'Donnell, Mechanism of the sliding beta-clamp of DNA polymerase III holoenzyme, J. Biol. Chem. 266 (1991) 11328–11334.

[63] P.L. Foster, Adaptive mutation in *Escherichia coli*, Cold Spring Harb. Symp. Quant. Biol. 65 (2000) 21–29.

Genes and Environment

Vol. 28, No. 1, February, 2006

Development of a Bacterial Hyper-sensitive Tester Strain for Specific Detection of the Genotoxicity of Polycyclic Aromatic Hydrocarbons

Masami Yamada, Keiko Matsui and Takehiko Nohmi

Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, JAPAN

Genes and Environment, Vol. 28, No. 1 pp. 23–30 (2006)

Regular Article

Development of a Bacterial Hyper-sensitive Tester Strain for Specific Detection of the Genotoxicity of Polycyclic Aromatic Hydrocarbons

Masami Yamada, Keiko Matsui and Takehiko Nohmi[1]
Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, JAPAN

(Received December 16, 2005; Accepted January 10, 2006)

Benzo[a]pyrene (B[a]P), one of polycyclic aromatic hydrocarbons (PAHs), is a ubiquitous environmental pollutant and a potent mutagen and carcinogen. To sensitively detect the genotoxicity of PAHs in complex mixtures extracted from environmental pollutants, *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) strain YG5161 is engineered by introduction of plasmid pYG768 carrying the *dinB* gene encoding *Escherichia coli* DNA polymerase IV into standard Ames tester strain *S. typhimurium* TA1538 (Matsui *et al.*, DNA Repair in press). Strain YG5161 exhibits higher sensitivity to the genotoxicity of B[a]P and other PAHs than do strain TA1538 and TA98. As the conventional Ames tester strains do, however, strain YG5161 also detects the mutagenicity of aromatic amines and nitroaromatics with high sensitivity, which may veil the genotoxicity of PAHs in complex mixtures. *S. typhimurium* possesses strong enzyme activities of nitroreductase and *O*-acetyltransferase, which mediate the metabolic activation of aromatic amines and nitroaromatics and enhance the potent genotoxicity. In this study, we disrupted the *nfsB* and *oat* genes encoding the activation enzymes in strain TA1538 to reduce the cross sensitivity, and introduced plasmid pYG768 into the *ΔnfsBΔoat* strain. The resulting strain YG5185 retained similar high mutability to various chemicals including PAHs as did strain YG5161 and substantially decreased the sensitivity to 1-nitropyrene, 1,8-dnitropyrene and 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1). We propose that the novel tester strain YG5185 is useful to specifically and sensitively detect the genotoxic PAHs in complex mixtures from various polluted environmental sources.

Key words: genotoxicity, polycyclic aromatic hydrocarbons, complex mixture, *dinB*, translesion DNA synthesis

Introduction

The ambient air and soil of urban centers and other areas can be polluted with potentially carcinogenic and genotoxic chemicals including polycyclic aromatic hydrocarbons (PAHs), most of which are emitted into the atmosphere as a result of incomplete combustion of fossil fuels associated with motor vehicles, industrial activities and home heating (1). In fact, the pollution of air and soil with PAHs is a serious problem in many countries all over the world (2). In Asia, the Chinese government assessed the state of soil contamination on the Beijing outskirts where great changes are undergoing due to the rapid urbanization and industrial development, and concluded that the pyrogenic origins, especially traffic exhausts, are the dominant sources of PAHs (3). In Korea, it is reported that typical soils from agricultural areas contained PAHs at similar level to those in soils from highly industrialized countries (4). In Japan, concentrations of particles of diameter under 1 μm with attached PAHs were measured in various locations in Tokyo and the major polluted places were main traffic roads, highways, and street tunnels (5). In Europe, 20 PAHs and 12 polychlorinated biphenyls (PCBs) in forest soils of Germany were physico-chemically determined, and PAHs were more dominantly detected than PCBs (6). In England, soil samples have been collected from the same plot in 1893, 1944 and 1987 for analysis of PAHs, and it is revealed that the surface soil had been enriched in all PAH compounds, particularly in benzo[a]pyrene (B[a]P) (7). Even in the Southern Hemisphere where pollution levels seem to be lower than those in the northern one, studies of pollution seem to be urgently necessary. In Chile, some persistent toxic substances (PTS) in soils were analyzed, which led to the conclusion that environmental PTS levels are relatively low but PAHs may be of concern in some areas of basin (8). In Brazil, Ames genotoxicity assay was carried out with and without metabolic activation for air samples at four sites in urban area, and higher mutagenic activity was identified at the sites with heavier vehicle traffic. The results using nitroreduc-

---

[1] Correspondence to: Takehiko Nohmi, Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel: +81-3-3700-9873, Fax: +81-3-3707-6950, E-mail: nohmi@nihs.go.jp