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 Δ*nfsB*Δ*oat* 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

tase- or *O*-acetyltransferase-deficient strains suggest that nitro PAHs seem to be strongly associated with the genotoxicity observed in the urban and industrial regions (9).

To sensitively detect the genotoxicity of B[a]P and other PAHs in complex mixtures extracted from various polluted environmental sources, Matsui *et al* (10). have recently engineered *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) strain YG5161, which harbored plasmid pYG768 carrying *dinB* encoding *Escherichia coli* DNA polymerase IV in standard Ames tester strain TA1538. The DNA polymerase can bypass a variety of DNA lesions such as those induced by B[a]P (11,12). Thus, the expression of the polymerase enhances B[a]P-induced -2 frameshift events in CGC-GCGCG repetitive sequences in the *hisD* gene in strain TA1538, which reverse the phenotype from His^- to His^+ (13). In fact, the stain exhibits several times higher sensitivity to the genotoxicity of B[a]P than does strain TA1538 or another standard strain TA98 (10). Strain TA98 is the same as strain TA1538 but harbors plasmid pKM101 carrying *mucAB* encoding another Y-family DNA polymerase, i.e., DNA polymerase RI (14). Strain YG5161 also exhibits higher sensitivity to 10-azabenzo[a]pyrene (10-AzaB[a]P), 3-methylcholanthrene (3-MC) and 3-nitrobeno[a]pyrene (3-NB[a]P) than does strain TA1538 or TA98.

Despite the high sensitivity, strain YG5161 possesses a potential problem that is cross sensitivity to genotoxic nitroaromatics and aromatic amines. *S. typhimurium* has strong metabolic activation enzymes for nitroaromatics and aromatic amines, i.e., nitroreductase and *O*-acetyltransferase (15,16). The former is required for the reductive activation of nitroaromatics, and the latter is involved in the activation of *N*-hydroxy compounds derived from nitro- and amino-aromatics. Because of the potent enzyme activities, the genotoxicity of nitroaromatics and aromatic amines is very sensitively detected with *S. typhimurium* tester strains (17). As a consequence, the genotoxic PAHs can be veiled in complex mixtures extracted from various polluted environmental sources if genotoxic nitroaromatics or aromatic amines are contaminated.

In this study, we disrupted the *nfsB* and *oat* genes encoding nitroreductase and *O*-acetyltransferase, respectively, to decrease the cross sensitivity to nitro- and amino-aromatics. Introducing plasmid pYG768 into the *ΔnfsBΔoat* strain resulted in strain YG5185, which retained the high sensitivity to PAHs but exhibited much reduced sensitivity to nitro- and amino-aromatics. We propose that the novel strain YG5185 is useful to detect genotoxic PAHs in the complex mixtures specifically and sensitively.

Materials and Methods

Strains and plasmids: The strains and the plasmids used in this study are listed in Table 1.

Chemicals: The names, CAS registry numbers, abbreviations and sources of the chemicals assayed in this study are as follows: B[a]P (50-32-8), 7,12-dimethylbenz[a]anthracene (57-97-6, DMBA), 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (67730-11-4, Glu-P-1), and 2-aminoanthracene (613-13-8, 2-AA) from Wako Pure Chemicals (Osaka, Japan); 3-MC (56-49-5) and 1-aminoanthracene (610-49-1, 1-AA) form Sigma-Aldrich Japan K. K. (Tokyo, Japan); 1-nitropyrene (5522-43-0, 1-NP) and 1,8-dinitropyrene (42397-65-9, 1,8-DNP) from Tokyo Kasei Kogyo (Tokyo, Japan). 10-AzaB[a]P (189-92-4) and 1-nitrobenzo[a]pyrene (70021-99-7, 1-NB[a]P) were provided by Drs. Ken-ichi Saeki, Nagoya City University, Nagoya, Japan, and Kiyoshi Fukuhara, National Institute of Health Sciences, Tokyo, Japan, respectively. *N*-Ethyl-*N*′-nitro-N-nitrosoguanidine (4245-77-6, ENNG) is a laboratory stock.

Media: Luria-Bertani broth and agar were used for bacterial culture. Vogel-Bonner minimal agar plates and top agar were prepared as previously described, and used for the His^+ reversion assay with *S. typhimurium* (13). Nutrient broth (Difco, MI, U.S.A.) with ampicillin (AP, 50 μg/ml) was used for pre-cultures of strain YG5161 and YG5185 for the reversion assay.

Construction of nitroreductase deficient strain: Plasmid pYG638 (Fig. 1A) was digested with *Sal*I and *Pvu*II (New England Biolabs, MA, U.S.A.) to remove the replication origin (18), and the 6.8-kb linear *Sal*I-*Sal*I DNA fragment containing the kanamycin-resistance (Km^r) gene between two flanking regions of the *nfsB* gene was purified with JET Sorb extraction kit (Genomed GmbH, Bad. Oeynhausen, Germany) after agarose gel electrophoresis. The purified DNA fragment was treated with T4 DNA ligase (Nippon Gene, Tokyo, Japan), and introduced into *Δoat* derivative of strain TA1538, i.e., strain YG7129, by electroporation (19,20). Colonies resistant to kanamycin were selected, and replacement of the *nfsB* gene with the DNA fragment carrying the Km^r gene was examined by PCR (primers; 5′-TGGAACTGCCTTTTACCGAACACT-3′ and 5′-CCCGGACATAATAGAAAACCGGT-3′) followed by 0.8% agarose gel electrophoresis.

Mutagenicity assay: The mutagenicity assay was carried out with a pre-incubation procedure (13). 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 1/15M phosphate buffer pH7.4 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 4–7 doses on triplicate plates with four

Table 1. Strains and plasmids used in this study

Strain	Genetic characteristic	Source
TA1538	*hisD3052*, *gal*, Δ(*chl*, *uvrB bio*) *rfa*	(13)
YG5161	the same as TA1538, but harbors pYG768; Ap^r	(10)
YG7129	the same as TA1538, but deficient in *oat*; Cm^r	(19)
YG7158	the same as YG7129, but deficient in *nfsB*; Cm^r Km^r	this study
YG5185	the same as YG7158, but harbors pYG768; Ap^r Cm^r Km^r	this study
plasmid		
pYG638	Derivative of pBR322 for the disruption of the *nfsB* gene; Km^r Ap^r (see Fig. 1)	(18)
pYG768	Derivative of pWSK29 with *dinB* gene; Ap^r	(34)

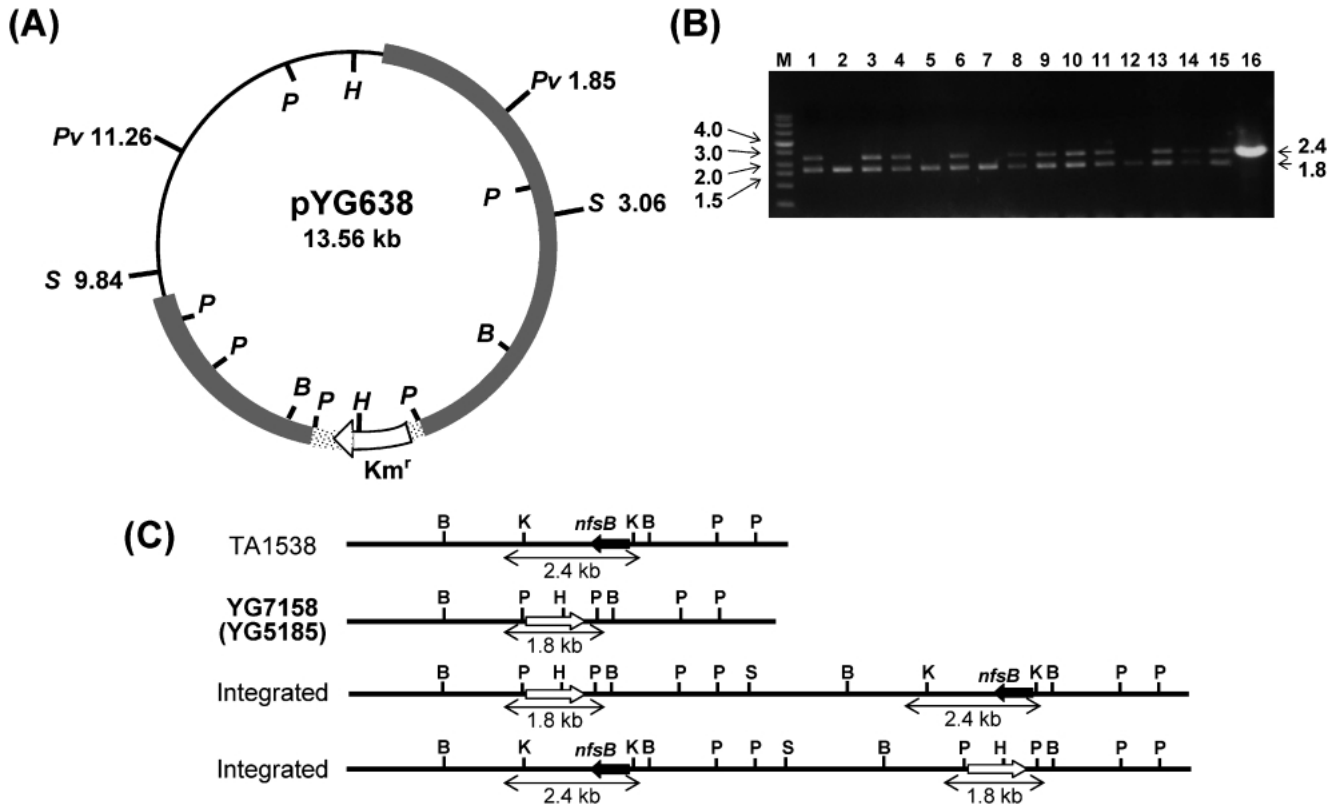

Fig. 1. Disruption of the *nfsB* gene. (A) Physical map of pYG638 (18). The thin and thick gray lines indicate DNA of plasmid pBR322 and the chromosome DNA derived from *S. typhimurium* TA1538, respectively. The dotted region shows the *Pst*I fragment derived from plasmid pUC-4k, which contains the Km^r-gene cassette, whose transcriptional direction is indicated by the arrow head. Symbols for restriction enzyme sites: B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I. A *Sal*I-*Sal*I fragment containing the Km^r cassette with the size of 6.78 kb was used for targeted disruption of the *nfsB* gene by pre-ligation method. (B) Result of PCR. PCR products were subjected to 0.8% agarose gel electrophoresis. M indicates size marker and lane numbers are indicated at the top of the gel image. Lanes 2, 5, 7 and 12 indicate the clone whose *nfsB* gene has been replaced with the Km^r gene and lane 16 shows the proper size of the band including the $nfsB^+$ gene. Other lanes exhibit the clones that have the $nfsB^+$ gene as well as the Km^r-gene fragment integrated into the chromosome. (C) Partial restriction maps of the *nfsB* gene and the surrounding chromosomal region in strain TA1538 and its Km^r recombinants. Closed and open arrows indicate the position and the transcriptional direction of the *nfsB* gene and the Km^r cassette, respectively. Thin arrows indicate the size of the bands amplified by PCR in (B).

strains, i.e., TA1538, YG7158, YG5161, YG5185, in parallel.

Results

Establishment of S. typhimurium strain YG5185: To reduce the cross sensitivity to aromatic amines and nitroaromatics, we disrupted the *nfsB* gene of *S. typhimurium* strain YG7129, which is the same as strain TA1538 but the *oat* gene is already disrupted (Table 1). After electroporation of the DNA fragments containing the Km^r gene instead of the *nfsB* gene into strain YG7129, Km^r colonies were selected. Chromosome

Table 2. Mutagenicity of 11 chemicals in *S. typhimurium* strains with and without plasmid carrying the *dinB* gene encoding *E. coli* DNA pol IV in the presence and the absence of the *nfsB* and *oat* genes

Chemicals	Group	S9	TA1538	YG5161	YG7158	YG5185	Dose
				+DNA pol IV	*ΔnfsBΔoat*	*ΔnfsBΔoat* +DNA pol IV	
10-AzaB[a]P	A	+	2 (1.0)	21 (10.5)	2 (1.0)	28 (14.0)	25 μg/plate
B[a]P	A	+	8 (1.0)	103 (12.9)	9 (1.1)	126 (15.8)	10 μg/plate
3-MC	A	+	8 (1.0)	51 (6.4)	5 (0.6)	48 (6.0)	10 μg/plate
2-AA	A	+	531 (1.0)	1,111 (2.1)	432 (0.8)	1,680 (3.2)	2 μg/plate
DMBA	A	+	5 (1.0)	15 (3.0)	4 (0.8)	9 (1.8)	5 μg/plate
ENNG	A	−	2 (1.0)	11 (5.5)	2 (1.0)	12 (6.0)	10 μg/plate
1-AA	B	+	18 (1.0)	53 (2.9)	5 (0.3)	14 (0.8)	10 μg/plate
1-NB[a]P	B	−	192 (1.0)	2,042 (10.6)	72 (0.4)	326 (1.7)	0.5 μg/plate
1-NP	C	−	145 (1.0)	230 (1.6)	23 (0.2)	29 (0.2)	1 μg/plate
1,8-DNP	C	−	130,560 (1.0)	218,040 (1.7)	2,040 (0.02)	3,120 (0.02)	25 ng/plate
Glu-P-1	C	+	41,240 (1.0)	45,550 (1.1)	1,710 (0.04)	1,670 (0.04)	100 ng/plate

Each chemical was assayed with 4–7 doses on triplicate plates with four strains in parallel. The numbers of His^+ revertants per plate per μg are calculated at the doses indicated with arrows in Fig. 2. The numbers in parenthesis represent the values relative to the numbers of His^+ revertants per μg in TA1538. Differences of relative mutability more than two fold or less than half were regarded as significant effects of the introduction of plasmid pYG768 carrying *dinB* encoding DNA pol IV or the deletion of the *oat* and *nfsB* genes on the mutability. Group A: chemicals whose mutagenicity was enhanced by DNA pol IV but was not reduced by *ΔnfsΔoat*; Group B: chemicals whose mutagenicity was enhanced by DNA pol IV and was reduced by *ΔnfsΔoat*; Group C: chemicals whose mutagenicity was not enhanced by DNA pol IV but was reduced by *ΔnfsΔoat*.

DNA surrounding the *nfsB* gene was amplified from the colonies by PCR using primers flanking the *nfsB* gene, and the products were analyzed by agarose gel electrophoresis (Fig. 1B). If the chromosomal *nfsB* gene is correctly replaced with the introduced DNA fragments carrying the Km^r gene by recombination using the flanking homologous sequences, the Km^r colonies will exhibit single DNA bands of 1.8 kb. If no such true replacement occurs and the introduced DNA fragments are only integrated into the vicinity of the chromosomal *nfsB* gene, they will exhibit double bands of 2.4 kb (the intact *nfsB* gene) and 1.8 kb (the Km^r gene). As expected, some Km^r colonies exhibited single 1.8-kb bands while others exhibited double bands of 2.4 kb and 1.8 kb. We suggested that the Km^r colonies exhibited single 1.8-kb bands were deficient in the *nfsB* gene as well as the *oat* genes, and named the resulting strain YG7158. Plasmid pYG768 carrying the *dinB* gene encoding *E. coli* DNA polymerase IV was introduced and the resulting strain was referred to as YG5185, which was used for the subsequent mutation assays.

Specificity and sensitivity of strain YG5185 to genotoxic PAHs: We compared the sensitivity of strain YG5185 to 11 mutagens with those of three reference strains: the parent strain, i.e., TA1538, the derivative of strain TA1538 harboring plasmid pYG768, i.e., YG5161, and the *ΔnfsBΔoat* derivative of strain TA1538, i.e., YG7158 (Table 2). Introduction of plasmid pYG768 did not affect the spontaneous mutation frequencies in strains TA1538 and YG7158. Based on the order of sensitivity of the strains, we classified the chemicals into Group A to C as follows. For Group A compounds, i.e., 10-AzaB[a]P, B[a]P, 3-MC, 2-AA, DMBA and ENNG, deletion of the *nfsB* and *oat* genes did not reduce the sensitivity, and introduction of plasmid pYG768 substantially enhanced it (Fig. 2A). Thus, YG5185 exhibited similar or comparable sensitivity to Group A compounds as did strain YG5161, and the order of the sensitivity was YG5185 = YG5161 > TA1538 = YG7158. For Group B

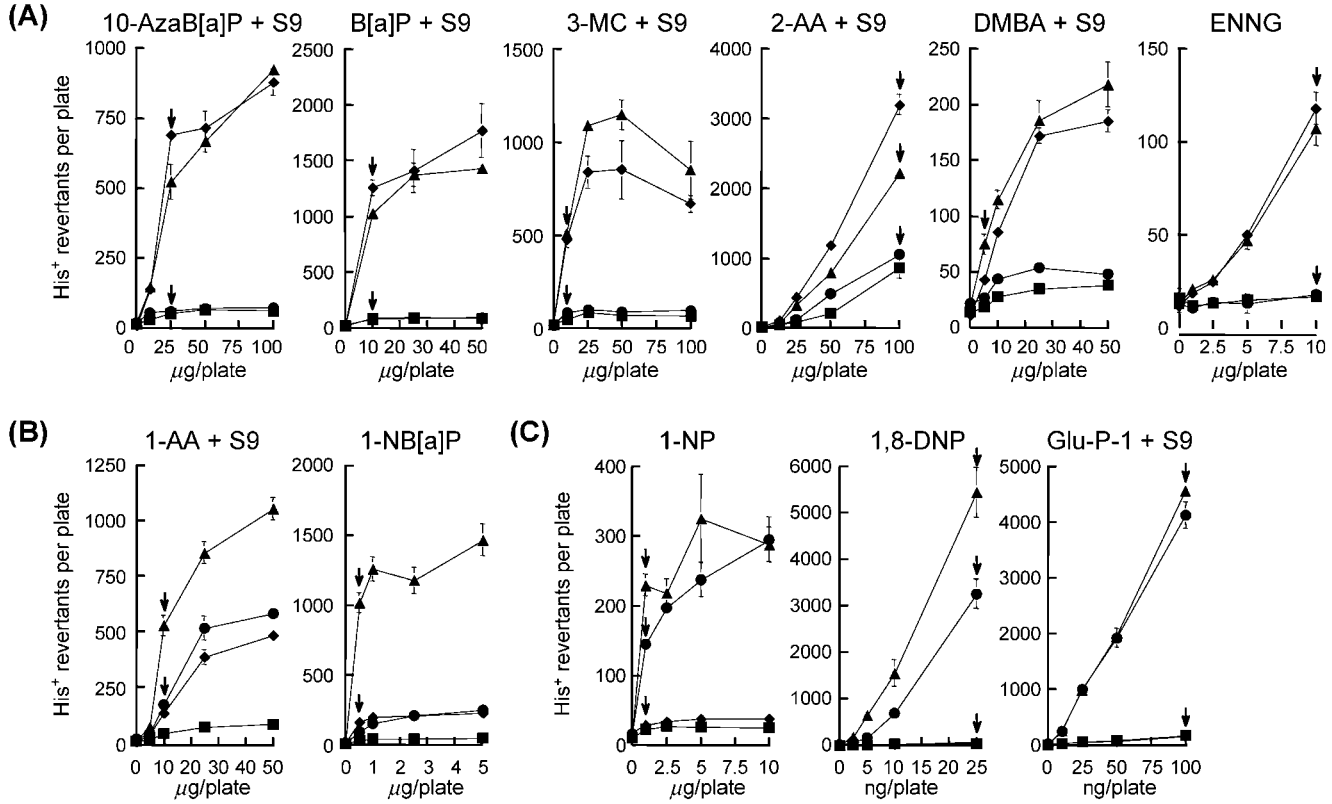

Fig. 2. Mutagenic responses of *S. typhimurium* newly constructed strains in the Ames test. The test chemicals are indicated on each panel. The chemicals are categorized into three Groups A, B and C, which are mentioned in the text and Table 2. Symbols are: ●, TA1538; ▲, YG5161; ■, YG7158; ◆, YG5185. The arrow indicates the dose that was used for the calculation of His^+ revertants per μg per plate in Table 2.

compounds, i.e., 1-AA and 1-NB[a]P, deletion of the *nfsB* and *oat* genes substantially reduced the sensitivity, and introduction of plasmid pYG768 enhanced it (Fig. 2B). Thus, the order of the sensitivity was YG5161 > TA1538 > YG5185 > YG7158. For Group C compounds, i.e., 1-NP, 1,8-DNP and Glu-P-1, deletion of the *nfsB* and *oat* genes severely reduced the sensitivity, and introduction of plasmid pYG768 did not substantially enhance it (Fig. 2C). The order of the sensitivity was YG5161 = TA1538 > YG5185 = YG7158.

Discussion

Genetically engineered Ames tester strains have been proven to be useful in environmental genotoxicology due to their extreme sensitivity and the mechanistic information they can provide (17). For example, *S. typhimurium* strain YG1021, 1024, 1026 and 1029 have been widely used for environmental research for their hypersensitivity to the genotoxic action of nitroarenes and aromatic amines (21). The strains harbor plasmids carrying *nfsB* or *oat*, conferring high enzymatic activities of nitroreductase or *O*-acetyltransferase in strain TA98 or TA100 (15,16,22,23). Strains YG7104 and YG7108, whose repair systems for the damage by alkylating agents are disrupted, exhibit hyper sensitivity to alkylating agents, such as methyl methanesulfonate and dimethylnitrosamine (24,25), and they are used for mechanistic analyses for chemical mutagenesis and carcinogenesis (26–28). Strain YG3001, YG3002 and YG3003 are highly sensitive to oxidative mutagens due to the lack of the *mutM* gene encoding 8-hydroxyguanine DNA glycosylase (29), and are shown to be useful for the studies on oxidative DNA damage (30,31).

To expand this line of research, we have previously established *S. typhimurium* strain YG5161 by introduction of plasmid carrying *dinB* encoding *E. coli* DNA polymerase IV into strain TA1538 to increase the sensitivity to genotoxic PAHs (10). Because of the error-prone nature of DNA polymerase IV, strain YG5161 exhibited higher sensitivity to PAHs such as B[a]P, 10-AzaB[a]P and 3-NB[a]P than did standard Ames strain TA1538 or TA98. Nevertheless, strain YG5161 has a potential defect as a bio-detector of genotoxic PAHs in complex mixtures, which is the cross sensitivity to other classes of genotoxic compounds, i.e., nitroaromatics and aromatic amines. Since these compounds are ubiquitously present in the environment and their genotoxicity in *S. typhimurium* is extremely amplified by the presence of intracellular metabolic activation enzymes, i.e., nitroreductase and *O*-acetyltran-

sferase, they can veil the potential genotoxicity of PAHs in the complex mixtures (17). For example, 1,8-DNP could be dominantly detected as a principle genotoxic compound in complex mixtures if 1,8-DNP and B[a]P were present at a weight ratio of 1:1,000. This is because the genotoxicity of 1,8-DNP, i.e., the numbers of His^+ revertants per plate per μg, is more than 15,000 times higher than B[a]P in strain TA1538 (Table 2). Despite the potent genotoxicity in *S. typhimurium*, 1,8-DNP is categorized into Group 2B (possible human carcinogens) by International Agency for Research on Cancer (IARC) while B[a]P is classified into Group 2A (probable human carcinogens) (32). Hence, we found it important to increase the specificity of tester strains to genotoxic PAHs.

Here, we disrupted the *nfsB* and *oat* genes of strain TA1538, introduced plasmid pYG768 carrying *dinB* into the *ΔnfsBΔoat* strain and established novel *S. typhimurium* strain YG5185 (Table 1). When compared the sensitivity of strain YG5185 and YG5161, they exhibited comparative sensitivity to Group A compounds where four out of six compounds, i.e., 10-azaB[a]P, B[a]P, 3-MC and DMBA, were PAHs (Table 2, Fig. 2A). Thus, strain YG5185 appears to be able to detect the genotoxic PAHs with similar high sensitivity as does strain YG5161. The remaining two compounds in Group A are 2-AA and ENNG. It cautions that the compounds that are more sensitively detected by strain YG5161 or YG5185 compared with strain TA1538 are not necessarily PAHs. They can be aromatic amines or alkylating agents.

For Group B compounds, i.e., 1-AA and 1-NB[a]P, the genotoxicity was significantly reduced by the deletion of *nfsB* and *oat*, as in the case of Group C compounds (Fig. 2B and C). Unlike Group C compounds, however, the genotoxicity of 1-AA and 1-NB[a]P was three to 10 times enhanced by the introduction of plasmid carrying *dinB* (Fig. 2B). Actually, the genotoxicity of two compounds was more sensitively detected with strain YG5161 compared with standard strain TA98 (10). Thus, it seems that DNA lesions induced by 1-AA and 1-NB[a]P are more efficiently bypassed by DNA polymerase IV in an error-prone manner than by DNA polymerase RI encoded by *mucAB* carried by plasmid pKM101 in strain TA98. Interestingly, the genotoxicity of 1-AA was reduced by more than 70% by the deletion of *nfsB* and *oat* while the genotoxicity of 2-AA was not (Table 2, Fig. 2A and B). These results suggest that the intracellular metabolic activation mechanisms are markedly different between two aromatic amino compounds despite the structural similarity.

In Group C compounds, the deletion of *nfsB* and *oat* reduced the genotoxicity of 1-NP by more than 85% and those of 1,8-DNP and Glu-P-1 by more than 95% (Table 2). Strain YG5185 exhibited much lower sensitivity to the genotoxicity of nitroaromatics and aromatic amine than did strain YG5161 (Fig. 2C). Thus, we concluded that strain YG5185 more specifically detects the genotoxicity of PAHs than does strain YG5161. Strain YG5185 could help and facilitate the successful isolation of genotoxic PAHs in complex mixtures. We have to point out, however, that the genotoxicity of 1,8-DNP and Glu-P-1 in strain YG5185 is still more than 25 times and 10 times higher than that of B[a]P. Hence, there is a possibility that genotoxic nitroaromatics or aromatic amines can be detected as principle mutagens if the complex mixtures are heavily contaminated with nitroaromatics or aromatic amines.

During the strain construction, we noticed that *S. typhimurium* TA1538/1,8-DNP could have mutations in the genes other than the *oat* gene. This is because introduction of plasmid pYG768 carrying *dinB* did not enhance the sensitivity of the strain to B[a]P while introduction of the same plasmid into YG7158 (*ΔnfsBΔoat* strain) or YG7129 (*Δoat* strain) enhanced it more than 10 times. It may not be surprising that strain TA1538/1,8-DNP has unexpected mutations because it was generated by random mutagenesis with 1,8-DNP (33). To avoid such confusion by extra mutations, we specifically disrupted the *oat* and *nfsB* genes by a targeting method, i.e., the pre-ligation method, which has been developed in this laboratory (20).

In summary, we established novel *S. typhimurium* strain YG5185 by introduction of plasmid carrying *dinB* encoding *E. coli* DNA polymerase IV into *ΔnfsBΔoat* derivative of standard Ames tester strain TA1538. The newly constructed strain exhibited higher sensitivity to the genotoxic compounds including PAHs but reduced sensitivity to nitroaromatics and aromatic amines. We propose that strain YG5185 is useful to detect the genotoxic PAHs in complex mixtures extracted from various polluted environmental sources.

Acknowledgments: We wish to thank Drs. Ken-ichi Saeki, Nagoya City University, Nagoya and Kiyoshi Fukuhara, National Institute of Health Sciences, Tokyo for their generous gift of 10-AzaB[a]P and 1-NB[a]P, respectively. This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

References

1 Katsouyanni K, Pershagen G. Ambient air pollution exposure and cancer. Cancer Causes Control. 1997; 8: 284–91.

2 Menichini E. Urban air pollution by polycyclic aromatic hydrocarbons: levels and sources of variability. Sci Total Environ. 1992; 116: 109–35.

3 Ma LL, Chu SG, Wang XT, Cheng HX, Liu XF, Xu XB.

Polycyclic aromatic hydrocarbons in the surface soils from outskirts of Beijing, China. Chemosphere. 2005; 58: 1355–63.
4 Nam JJ, Song BH, Eom KC, Lee SH, Smith A. Distribution of polycyclic aromatic hydrocarbons in agricultural soils in South Korea. Chemosphere. 2003; 50: 1281–9.
5 Sakai R, Siegmann HC, Sato H, Voorhees AS. Particulate matter and particle-attached polycyclic aromatic hydrocarbons in the indoor and outdoor air of Tokyo measured with personal monitors. Environ Res. 2002; 89: 66–71.
6 Krauss M, Wilcke W, Zech W. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls in forest soils: depth distribution as indicator of different fate. Environ Pollut. 2000; 110: 79–88.
7 Jones KC, Stratford JA, Tidridge P, Waterhouse KS, Johnston AE. Polynuclear aromatic hydrocarbons in an agricultural soil: long-term changes in profile distribution. Environ Pollut. 1989; 56: 337–51.
8 Barra R, Popp P, Quiroz R, Bauer C, Cid H, von Tumpling W. Persistent toxic substances in soils and waters along an altitudinal gradient in the Laja River Basin, Central Southern Chile. Chemosphere. 2005; 58: 905–15.
9 Vargas VM. Mutagenic activity as a parameter to assess ambient air quality for protection of the environment and human health. Mutat Res. 2003; 544: 313–9.
10 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 (Amst). 2006; in press.
11 Shen X, Sayer JM, Kroth H, Ponten I, O'Donnell M, Woodgate R *et al*. 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. 2002; 277: 5265–74.
12 Ohmori H, Friedberg EC, Fuchs RP, Goodman MF, Hanaoka F, Hinkle D *et al*. The Y-family of DNA polymerases. Mol Cell. 2001; 8: 7–8.
13 Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983; 113: 173–215.
14 Goldsmith M, Sarov-Blat L, Livneh Z. 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. 2000; 97: 11227–31.
15 Watanabe M, Nohmi T, Ishidate M, Jr. New tester strains of *Salmonella typhimurium* highly sensitive to mutagenic nitroarenes. Biochem Biophys Res Commun. 1987; 147: 974–9.
16 Watanabe M, Ishidate M, Jr., Nohmi T. A sensitive method for the detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of *Salmonella typhimurium* strains TA98 and TA100. Mutat Res. 1989; 216: 211–20.
17 Josephy PD, Gruz P, Nohmi T. Recent advances in the construction of bacterial genotoxicity assays. Mutat Res. 1997; 386: 1–23.
18 Yamada M, Espinosa-Aguirre JJ, Watanabe M, Matsui K, Sofuni T, Nohmi T. Targeted disruption of the gene encoding the classical nitroreductase enzyme in *Salmonella typhimurium* Ames test strains TA1535 and TA1538. Mutat Res. 1997; 375: 9–17.
19 Espinosa-Aguirre JJ, Yamada M, Matsui K, Watanabe M, Sofuni T, Nohmi T. New *O*-acetyltransferase-deficient Ames *Salmonella* strains generated by specific gene disruption. Mutat Res. 1999; 439: 159–69.
20 Yamada M, Hakura A, Sofuni T, Nohmi T. New method for gene disruption in *Salmonella typhimurium:* construction and characterization of an *ada*-deletion derivative of *Salmonella typhimurium* TA1535. J Bacteriol. 1993; 175: 5539–47.
21 Einisto P, Watanabe M, Ishidate M, Jr., Nohmi T. Mutagenicity of 30 chemicals in *Salmonella typhimurium* strains possessing different nitroreductase or *O*-acetyltransferase activities. Mutat Res. 1991; 259: 95–102.
22 Watanabe M, Ishidate M, Jr., Nohmi T. Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated *O*-acetyltransferase levels. Mutat Res. 1990; 234: 337–48.
23 Hagiwara Y, Watanabe M, Oda Y, Sofuni T, Nohmi T. Specificity and sensitivity of *Salmonella typhimurium* YG1041 and YG1042 strains possessing elevated levels of both nitroreductase and acetyltransferase activity. Mutat Res. 1993; 291: 171–80.
24 Yamada M, Sedgwick B, Sofuni T, Nohmi T. Construction and characterization of mutants of *Salmonella typhimurium* deficient in DNA repair of O^6-methylguanine. J Bacteriol. 1995; 177: 1511–9.
25 Yamada M, Matsui K, Sofuni T, Nohmi T. New tester strains of *Salmonella typhimurium* lacking O^6-methylguanine DNA methyltransferases and highly sensitive to mutagenic alkylating agents. Mutat Res. 1997; 381: 15–24.
26 Aiub CA, Coelho EC, Sodre E, Pinto LF, Felzenszwalb I. Genotoxic evaluation of the organophosphorous pesticide temephos. Genet Mol Res. 2002; 1: 159–66.
27 Ishikawa S, Mochizuki M. Mutagenicity and cross-linking activity of chloroalkylnitrosamines, possible new antitumor lead compounds. Mutagenesis. 2003; 18: 331–5.
28 Fujita K, Nakayama K, Yamazaki Y, Tsuruma K, Yamada M, Nohmi T *et al*. Construction of *Salmonella typhimurium* YG7108 strains, each coexpressing a form of human cytochrome P450 with NADPH-cytochrome P450 reductase. Environ Mol Mutagen. 2001; 38: 329–38.
29 Suzuki M, Matsui K, Yamada M, Kasai H, Sofuni T, Nohmi T. Construction of mutants of *Salmonella typhimurium* deficient in 8-hydroxyguanine DNA glycosylase and their sensitivities to oxidative mutagens and nitro compounds. Mutat Res. 1997; 393: 233–46.
30 Kim SR, Kokubo K, Matsui K, Yamada N, Kanke Y, Fukuoka M *et al*. Light-dependent mutagenesis by benzo[a]pyrene is mediated *via* oxidative DNA damage. Environ Mol Mutagen. 2005; 46: 141–9.
31 Kim SR, Matsui K, Yamada M, Kohno T, Kasai H, Yokota J *et al*. Suppression of chemically induced and spontaneously occurring oxidative mutagenesis by three

alleles of human *OGG1* gene encoding 8-hydroxyguanine DNA glycosylase. Mutat Res. 2004; 554: 365–74.

32 Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. ''IARC Group 2B carcinogens'' reported in cigarette mainstream smoke. Food Chem Toxicol. 2001; 39: 183–205.

33 McCoy EC, Anders M, Rosenkranz HS. The basis of the insensitivity of *Salmonella typhimurium* strain TA98/1,8-DNP_6 to the mutagenic action of nitroarenes. Mutat Res 1983; 121: 17–23.

34 Kim SR, Maenhaut-Michel G, Yamada M, Yamamoto Y, Matsui K, Sofuni T *et al*. 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 USA. 1997; 94: 13792–7.