研究成果の刊行に関する一覧表

雑誌

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Nitrogen-substitution effect on in vivo mutagenicity of chrysene

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Abstract

We have previously reported the in vivo mutagenicity of aza-polycyclic aromatic hydrocarbons (azaPAHs), such as quinoline, benzo[*f*]quinoline, benzo[*h*]quinoline, 1,7-phenanthroline and 10-azabenzo[*a*]pyrene. The 1,10-diazachrysene (1,10-DAC) and 4,10-DAC, nitrogen-substituted analogs of chrysene, were shown to exhibit mutagenicity in *Salmonella typhimurium* TA100 in the presence of rat liver S9 and human liver microsomes in our previous report, although DACs could not be converted to a bay-region diol epoxide, the ultimate active form of chrysene, because of their nitrogen atoms. In the present study, we tested in vivo mutagenicity of DACs compared with chrysene using the *lacZ* transgenic mouse (Muta™Mouse) to evaluate the effect of the nitrogen substitution. DACs- and chrysene-induced mutation in all of the six organs examined (liver, spleen, lung, kidney, bone marrow and colon). The mutant frequencies obtained with chrysene showed only small differences between the organs examined and ranged from 1.5 to 3 times the spontaneous frequency. The 4,10-DAC was more mutagenic than chrysene in all the organs tested. The highest *lacZ* mutation frequency was observed in the lung of 4,10-DAC-treated mice and it was 19 and 6 times the spontaneous frequency and the frequency induced by chrysene, respectively. The 1,10-DAC induced *lacZ* mutation in the lung with a frequency 4.3- and 1.5-fold higher than in the control and chrysene-treated mice, respectively, although the mutant frequencies in the other organs of 1,10-DAC-treated mice were almost equivalent to those of chrysene-treated mice. Not only chrysene but also DACs depressed the G:C to A:T transition and increased the G:C to T:A transversion in the liver and lung. These results suggest that the two types of nitrogen substitutions in the chrysene structure may enhance mutagenicity in the mouse lung, although they showed no difference in the target-organ specificity and the mutation spectrum.

Keywords: Aza-substitution; In vivo mutagenesis assay; Mutation spectrum

1. Introduction

We have been investigating the mutagenicity of aza-polycyclic aromatic hydrocarbons (azaPAHs) with

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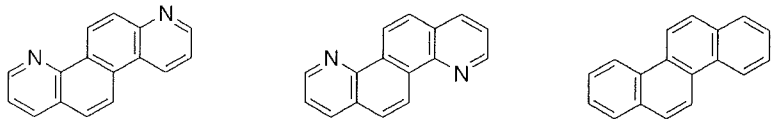

Fig. 1. Chemical structures of 1,10-DAC, 4,10-DAC and chrysene.

special attention to their metabolic activation mechanism. The 10-azabenzo[*a*]pyrene (10-azaBaP), a 10-aza-analog of benzo[*a*]pyrene (BaP), was reported to be as mutagenic as BaP in *Salmonella typhymurium* TA100 in the presence of PCB-treated rat liver S9 [1,2], although 10-azaBaP could not be converted to a bay-region diol epoxide, the ultimate mutagenic form of BaP [3,4], because of its nitrogen atom. We have previously reported that 10-azaBaP showed a higher mutagenicity than BaP in the Ames test using pooled human liver S9 [5]. However, in the in vivo mutagenesis assay system using the *lacZ* transgenic mouse (Muta^{TM}Mouse), 10-azaBaP was mutagenic only in the liver and colon and showed much less mutagenicity than BaP, which showed high mutagenicity in all of the organs tested [5]. Thus, 10-azaBaP interestingly showed differences in mutagenicity between the in vitro and in vivo assay systems.

We have also reported that quinoline, an aza-analog of naphthalene, one of simplest azaPAHs and a hepatocarcinogen [6,7], showed mutagenicity only in the liver of Muta^{TM}Mouse [8]. We also observed that it caused remarkable induction of G:C to C:G transversion [9] and suggested that it might be metabolically activated in the pyridine moiety to the ultimate mutagenic form [10]. Its active form was supposed to be an enamine epoxide (1,4-hydrated 2,3-epoxide), which would be responsible for the mutagenic modification of DNA [11–14]. Furthermore, three trycyclic azaPAHs, i.e., benzo[*f*]quinoline, benzo[*h*]quinoline and 1,7-phenanthroline, were shown to exhibit mutagenicity in Muta^{TM}Mouse in our previous report [15]. Benzo[*h*]quinoline and 1,7-phenanthroline were suggested to be converted to the ultimate genotoxic form in the pyridine moiety [16].

1,10-Diazachrysene (1,10-DAC) and 4,10-DAC are diaza-analogs of chrysene (Fig. 1), consisting of two quinoline moieties, and have structures similar to 10-azaBaP. We have previously reported that these DACs showed mutagenicity in Ames tests in the presence of rat liver S9 or human liver microsomes [17], although formation of the bay-region diol epoxide from DACs seemed impossible because of their nitrogen atoms. DACs have not been found in our living environments, but these are expected to be useful compounds to investigate the nature of mutagenicity in azaPAHs.

In the present study, we undertook to investigate the in vivo mutagenicity of DACs in comparison with chrysene by the in vivo mutation assay system using the *lacZ* transgenic mouse (Muta^{TM}Mouse) to evaluate the nitrogen-substitution effect in the chrysene skeleton on their mutagenicity.

2. Materials and methods

2.1. Materials

Chrysene (CAS Registry No. 218-01-9) and phenyl-β-D-galactoside (P-gal) were purchased from Sigma Chemical Co. (St. Louis, MO), proteinase K and olive oil from Wako Pure Chemicals (Osaka) and RNase from Boehringer Mannheim. The 1,10-DAC (CAS Registry No. 218-21-3) and 4,10-DAC (CAS Registry No. 218-34-8) were synthesized in this laboratory according to the reported methods [18].

2.2. In vivo mutagenesis assays using Muta^{TM}Mouse

2.2.1. Animals and treatments

Male Muta^{TM}Mice, at 7–8 weeks of age, were supplied by COVANCE Research Products (PA, USA) and acclimatized for 1 week before use. Chrysene and 4,10-DAC dissolved in olive oil (10 mL/kg body weight)

were injected intraperitoneally into four mice each at a single dose of 200 mg/kg once a week for 4 consecutive weeks (800 mg/kg in total). The 1,10-DAC dissolved in olive oil (10 mL/kg body weight) was injected into four mice at a single dose of 100 mg/kg similarly (400 mg/kg in total). Four control mice were given 10 mL olive oil/kg.

2.2.2. Tissues and DNA isolation

All mice were killed by cervical dislocation 7 days after the last administration of test chemicals. The liver, spleen, lung, kidney, bone marrow and colon were immediately extirpated, frozen in liquid nitrogen and stored at −80 °C until DNA extraction. The genomic DNA was extracted from each tissue by the phenol/chloroform method according to the Muta™Mouse/PS Mutation Assay Manual (Corning Hazleton, 1995). The isolated DNA, which was precipitated with ethanol, was air-dried and dissolved in an appropriate volume (20–200 μL) of TE-4 buffer (10 mM Tris–HCl at pH 8.0 containing 4 mM EDTA) at room temperature overnight. The DNA solution thus prepared was stored at 4 °C.

2.2.3. In vitro packaging

The lambda gt10/*lacZ* vector could be efficiently recovered by in vitro packaging reactions [19]. Our homemade packaging extract (HM) consisting of sonic extract (SE) of *Escherichia coli* NM759 and freeze–thaw lysate (FTL) of *E. coli* BHB2688 was prepared according to the method of Gunther et al. [20]. As a general procedure for handling the HM extract, approximately, 5 μg DNA was mixed with 15 μL of FTL and 30 μL of SE and incubated at 37 °C for 90 min. Then SE and FTL were added again and the mixture was incubated for another 90 min. The reaction was terminated by the addition of an appropriate volume of SM buffer (50 mM Tris–HCl at pH 7.5, 10 mM $MgSO_4$, 100 mM NaCl and 0.01% gelatin) and the mixture was stored at 4 °C. By this procedure, the lambda gt10 vector to form an infectious phage was efficiently rescued from genomic DNA.

2.2.4. Mutation assays

2.2.4.1. lacZ mutant frequency determination. The positive selection for *lacZ* mutants was performed as previously reported [21–23]. Briefly, the phage

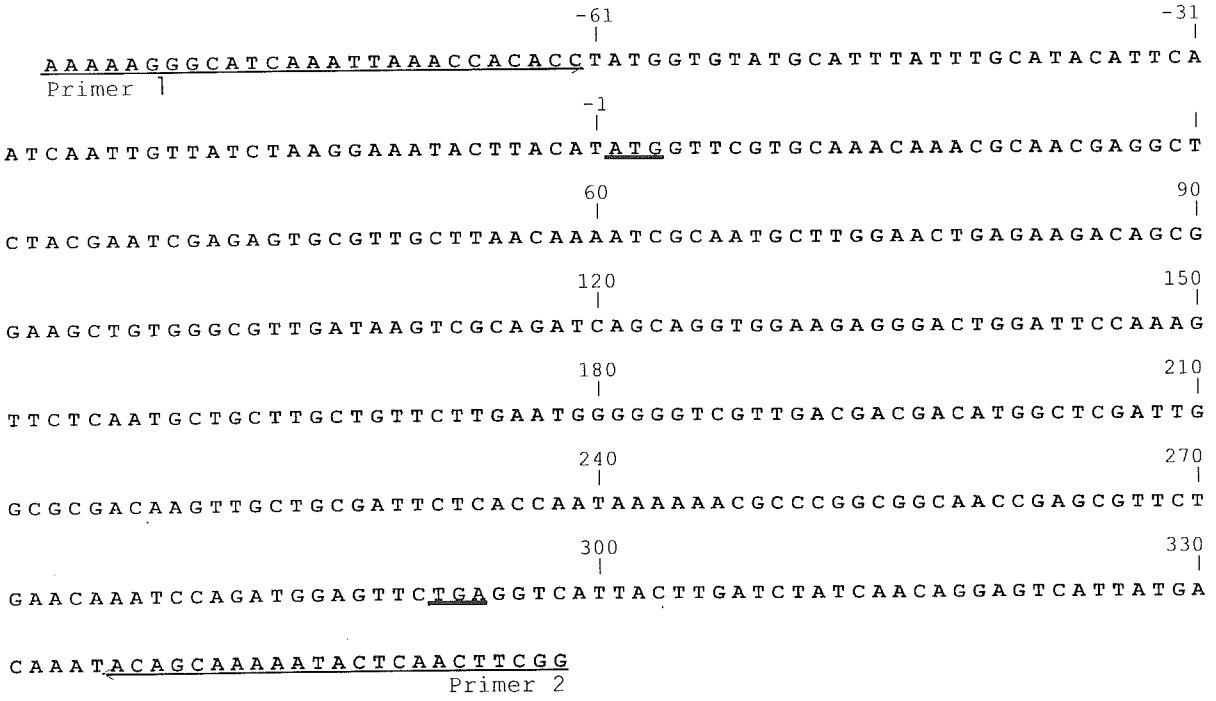

Fig. 2. Sequence map of the *cII* gene; primers used for PCR amplification and sequencing are shown by arrows. The PCR gives 446 bp products that involve the entire (294 bp) *cII* gene. Initiation and stop codons are underlined.

Table 1
Mutant frequencies induced by 1,10-DAC, 4,10-DAC and chrysene in six organs of Muta™Mouse

Tissue	Treatment	*lacZ* assay				*cII* assay			
		Individual animal data			Average ± S.D.	Individual animal data			Average ±S.D.
		No. of phages analyzed	No. of mutants	MF × 10^6	MF × 10^6	No. of phages analyzed	No. of mutants	MF × 10^6	MF × 10^6
Liver	Control (olive oil)	463000	36	77.8		1547000	31	20.0	
		173000	12	69.4		602000	20	33.2	
		1899000	247	130.1		1618000	47	29.0	
		396000	30	75.8	88.2 ± 24.3	1398000	22	15.7	24.5 ± 7.0
	1,10-DAC	391000	77	196.9		1299000	90	69.3	
		1126000	127	112.8		989000	40	40.4	
		371500	63	169.6		1805000	72	39.9	
		244000	36	147.5	156.7 ± 30.8*	1095000	23	21.0	42.7 ± 17.3
	4,10-DAC	192000	72	375.0		731000	79	108.1	
		355500	193	542.9		1446000	182	125.9	
		506500	225	444.2		2080000	233	112.0	
		503000	308	612.3	493.6 ± 90.9**	1751000	311	177.6	130.9 ± 27.8**
	Chrysene	452500	98	216.6		1025000	51	49.8	
		322500	88	272.9		1220000	81	66.4	
		362500	88	242.8		1343000	68	50.6	
		522000	89	170.5	225.7 ± 37.6**	1799000	60	33.4	50.0 ± 11.7*
Spleen	Control (olive oil)	866000	47	54.3		966000	14	14.5	
		1145000	59	51.5		1180000	25	21.2	
		870000	36	41.4		1024000	34	33.2	
		653500	23	35.2	45.6 ± 7.7	1039000	15	14.4	20.8 ± 7.7
	1,10-DAC	1700500	150	88.2		1550000	70	45.2	
		769000	77	100.1		909000	59	64.9	
		1252000	125	99.8		2119000	129	60.9	
		1316000	131	99.5	96.9 ± 5.0**	1837000	110	59.9	57.7 ± 7.5**
	4,10-DAC	469500	109	232.2		550000	56	101.8	
		1506000	525	348.6		1754000	260	148.2	
		1410000	374	265.2		2071000	268	129.4	
		1115000	271	243.0	272.3 ± 45.7**	1438000	219	152.3	132.9 ± 19.9**
	Chrysene	2225000	339	152.4		470500	12	25.5	
		638000	66	103.4		970000	72	74.2	
		1433500	202	140.9		2313000	102	44.1	
		247500	34	137.4	133.5 ± 18.2**	612000	13	21.2	41.3 ± 20.9
Lung	Control (olive oil)	310000	10	32.3		376000	14	37.2	
		296500	28	94.4		263500	11	41.7	
		646000	63	97.5		2016000	73	36.2	
		1402000	73	52.1	69.1 ± 27.8	1429000	23	16.1	32.8 ± 9.9
	1,10-DAC	1911000	539	282.1		1855000	255	137.5	
		1691000	342	202.2		1921000	151	78.6	
		1242000	430	346.2		1807000	183	101.3	
		1826500	652	357.0	296.9 ± 61.7**	2152000	281	130.6	112.0 ± 23.6**
	4,10-DAC	979500	1402	1431.3		1112000	530	476.6	
		1489000	1659	1114.2		1747000	771	441.3	
		1497000	2343	1565.1		1900000	1093	575.3	

Table 1 (*Continued*)

Tissue	Treatment	*lacZ* assay				*cII* assay			
		Individual animal data			Average ± S.D.	Individual animal data			Average ±S.D.
		No. of phages analyzed	No. of mutants	MF × 10^6	MF × 10^6	No. of phages analyzed	No. of mutants	MF × 10^6	MF × 10^6
		1673000	1702	1017.3	1282.0 ± 224.0**	1847000	1005	544.1	509.3 ± 53.0**
	Chrysene	1033000	121	117.1		1577000	61	38.7	
		1526000	371	243.1		2067000	231	111.8	
		1101000	241	218.9		1566000	115	73.4	
		871000	177	203.2	195.6 ± 47.5**	1254000	69	55.0	69.7 ± 27.2
Kidney	Control (olive oil)	447000	36	80.5		546000	21	38.5	
		741500	61	82.3		925000	23	24.9	
		1726000	62	35.9		2183000	70	32.1	
		1948000	72	37.0	58.9 ± 22.5	2244000	57	25.4	30.2 ± 5.6
	1,10-DAC	1609000	214	133.0		2570000	109	42.4	
		1510000	138	91.4		2278000	127	55.8	
		1410000	114	80.9		2621000	96	36.6	
		945000	119	125.9	107.8 ± 22.1*	1658000	67	40.4	43.8 ± 7.2*
	4,10-DAC	1378000	175	127.0		1695500	115	67.8	
		2101000	412	196.1		2526000	270	106.9	
		1447000	235	162.4		2217000	153	69.0	
		1355000	402	296.7	195.5 ± 63.3*	2014000	232	115.2	89.7 ± 21.5**
	Chrysene	1177000	161	136.8		2304000	101	43.8	
		1583000	239	151.0		2415000	157	65.0	
		1187500	241	202.9		2224000	115	51.7	
		1785000	257	144.0	158.7 ± 26.0**	2968000	147	49.5	52.5 ± 7.8**
Bone marrow	Control (olive oil)	421000	20	47.5		580000	12	20.7	
		1034000	48	46.4		1094000	25	22.9	
		1080000	61	56.5		1202000	38	31.6	
		1352000	48	35.5	46.5 ± 7.4	906000	17	18.8	23.5 ± 4.9
	1,10-DAC	1131000	78	69.0		1406000	57	40.5	
		764500	86	112.5		916000	39	42.6	
		1209000	108	89.3		1525000	58	38.0	
		732000	72	98.4	92.3 ± 15.8**	858000	48	55.9	44.3 ± 6.9**
	4,10-DAC	503500	147	292.0		779000	90	115.5	
		621000	239	384.9		1074000	191	177.8	
		1010000	200	198.0		1075000	104	96.7	
		829000	196	236.4	277.8 ± 70.2*	945000	124	131.2	130.3 ± 30.0**
	Chrysene	757500	65	85.8		1179000	20	17.0	
		819000	55	67.2		1046000	46	44.0	
		799500	49	61.3		1331000	33	24.8	
		853500	60	70.3	71.1 ± 9.1*	1392000	25	18.0	25.9 ± 10.9
Colon	Control (olive oil)	369500	17	46.0		1058000	43	40.6	
		1064000	77	72.4		1052000	40	38.0	
		660000	63	95.5		840000	36	42.9	
		676000	55	81.4	73.8 ± 18.0	1187000	33	27.8	37.3 ± 5.8
	1,10-DAC	306500	41	133.8		1715000	85	49.6	
		207000	25	120.8		1250000	57	45.6	

Table 1 (*Continued*)

Tissue	Treatment	*lacZ* assay				*cII* assay			
		Individual animal data			Average ± S.D.	Individual animal data			Average ±S.D.
		No. of phages analyzed	No. of mutants	MF × 10^6	MF × 10^6	No. of phages analyzed	No. of mutants	MF × 10^6	MF × 10^6
		988000	91	92.1		1396000	74	53.0	
		577000	75	130.0	119.2 ± 16.3*	951000	62	65.2	53.3 ± 7.3*
	4,10-DAC	469000	42	89.6		734000	48	65.4	
		369500	211	571.0		1425000	292	204.9	
		1004000	245	244.0		1089000	152	139.6	
		395000	188	475.9	345.1 ± 189.5	954000	187	196.0	151.5 ± 55.7*
	Chrysene	266500	29	108.8		980000	44	44.9	
		379000	52	137.2		1034000	68	65.8	
		1223000	222	181.5		1612000	78	48.4	
		615000	93	151.2	144.7 ± 26.2**	1178000	44	37.4	49.1 ± 10.4

Significantly different from the control group.
* $P < 0.05$.
** $P < 0.01$.

solution was absorbed to *E. coli* C (*lac*⁻ *galE*⁻) at room temperature for 20–30 min. For titration, appropriately diluted phage–*E. coli* solution was mixed with LB top agar (containing 10 mM $MgSO_4$) and plated onto dishes containing bottom agar. The remaining phage–*E. coli* solution was mixed with LB top agar containing phenyl-β-D-galactoside (3 mg/mL) and plated as described above. The mutant frequency (MF) was calculated by the following formula:

mutant frequency = (total number of plaques on selection plates/total number of plaques on titer plates) × dilution factor.

The significance of differences in the mutant frequency between the treated and control groups was analyzed by using Student's *t*-test and Welch's *t*-test in combination with the *F*-test.

2.2.4.2. cII mutant frequency determination. In the present study, we examined the mutagenicity in the lambda *cII* gene, which is also integrated as a lambda vector gene, which serves as another selective marker as reported previously in the *lacI* transgenic BigBlue mouse [24]. The positive selection for *cII* mutants was performed according to the method of Jakubczak et al. [24] with a slight modification as previously reported [9]. Briefly, the phage solution was absorbed to *E. coli* G1225 (*hfl*⁻) at room temperature for 20–30 min. For titration, appropriately diluted phage–*E. coli* solution was mixed with LB top agar (containing 10 mM $MgSO_4$) and plated onto dishes containing bottom agar and the plates were incubated at 37 °C for 24 h. The remaining phage–*E. coli* solution was mixed with LB top agar and plated onto dishes containing bottom agar. The plates were incubated at 25 °C for 48 h for selection of *cII* mutants. The wild-type phage, recovered from Muta™Mice, has a *cI*⁻ phenotype, which permits plaque formation with the *hfl*⁻ strain at 37 °C but not at 25 °C. The mutant frequency was calculated by the following formula:

mutant frequency = (total number of plaques on selection plates/total number of plaques on titer plates) × dilution factor.

The significance of differences in the mutant frequency between the treated and control groups was analyzed by using Student's *t*-test and Welch's *t*-test in combination with the *F*-test.

2.2.5. Sequencing of mutants

The entire lambda *cII* region was amplified directly from mutant plaques by Taq DNA polymerase (Takara Shuzo, Tokyo, Japan) with primers P1, 5′-AAAAAGGGCATCAAATTAAACC-3′ and P2, 5′-CCGAAGTTGAGTATTTTTGCTGT-3′ as previously reported [9] (Fig. 2). A 446 bp PCR product was puri-

fied with a microspin column (Amersham Pharmacia, Tokyo, Japan) and then used for a sequencing reaction with the Ampli Taq cycle sequencing kit (PE Biosystems, Tokyo, Japan) using the primer P1. The reaction product was isolated by ethanol precipitation and analyzed with the ABI PRISM™ 310 genetic analyzer (PE Biosystems). In this study, about 40 mutants were subjected to sequence analysis in each group both in the liver and lung.

3. Results

3.1. Mutant frequencies by 1,10-DAC, 4,10-DAC and chrysene

Chrysene and its diaza-analogs, 1,10-DAC and 4,10-DAC, were tested for in vivo mutagenicity using *lacZ* transgenic mice (Muta™Mice). Chrysene and 4,10-DAC were injected at the total dose of 800 mg/kg

Table 2
Sequences of *cII* mutations in the liver of 1,10-DAC-treated Muta™Mouse

Mutant no.	Position	Mutation	Sequence			Amino acid change
C1	117	G to T	TCG	CAG	ATC	Gln to His
C2	42	G to T	ATC	GAG	AGT	Glu to Asp
C3	126	G to T	AGC	AGG	TGG	Arg to Ser
C4	40–43	−GA	GAG	AGT	GCG	Frameshift
C5	166	G to T	CTT	GCT	GTT	Ala to Ser
C6	233	T to A	ATT	CTC	ACC	Leu to His
C7	132	G to T	TGG	AAG	AGG	Lys to Asn
C8	89	C to T	ACA	GCG	GAA	Ala to Val
C9	11	C to TA	CGT	GCA	AAC	Frameshift
C10	178	T to A	GAA	TGG	GGG	Trp to Arg
C11	197	A to G	GAC	GAC	ATG	Asp to Gly
C12	179	G to T	GAA	TGG	GGG	Trp to Leu
C13	29	C to A	GAG	GCT	CTA	Ala to Asp
C14	101	G to T	GTG	GGC	GTT	Gly to Val
C15	89	C to T	ACA	GCG	GAA	Ala to Val
C16	117	G to C	TCG	CAG	ATC	Gln to His
C17	40	G to A	ATC	GAG	AGT	Glu to Lys
C18	294	A to C	TTC	TGA	–	Stop to Cys
C19	150	G to T	CCA	AAG	TTC	Lys to Asn
C20[a]	294	A to C	TTC	TGA	–	Stop to Cys
C21	211	G to A	TTG	GCG	CGA	Ala to Thr
C22	111	G to T	GAT	AAG	TCG	Lys to Asn
C23	123	C to A	ATC	AGC	AGG	Ser to Arg
C24	160	C to A	ATG	CTG	CTT	Leu to Met
C25	173	T to C	GTT	CTT	GAA	Leu to Pro
C26	125	G to C	AGC	AGG	TGG	Arg to Thr
C27	−3	C to G	tta	cat	ATG	Base substitution in the 5′-flanking region
C28	29	C to A	GAG	GCT	CTA	Ala to Asp
C29[a]	111	G to T	GAT	AAG	TCG	Lys to Asn
C30	38	T to A	CGA	ATC	GAG	Ile to Asn
C31	40	G to T	ATC	GAG	AGT	Glu to Stop
C32	163	C to T	CTG	CTT	GCT	Leu to Phe
C33	79	G to T	ACT	GAG	AAG	Glu to Stop
C34	57	C to A	CTT	AAC	AAA	Asn to Lys
C35	89	C to T	ACA	GCG	GAA	Ala to Val
C36	179–184	−G	TGG	GGG	GTC	Frameshift
C37	113	C to A	AAG	TCG	CAG	Ser to Stop
C38	89	C to G	ACA	GCG	GAA	Ala to Gly
C39	123	C to G	ATC	AGC	AGG	Ser to Arg
C40	125	G to A	AGC	AGG	TGG	Arg to Lys

[a] Ascribable to the same mutation obtained in an identical mouse.

intraperitoneally, based on the tolerance dose. The total dose of 1,10-DAC was 400 mg/kg because 1,10-DAC showed more toxicity than 4,10-DAC and chrysene in a preliminary test. The mutant frequencies observed with the DNA preparations extracted from the six organs at 7 days after the last injection are shown in Table 1. More than 10 mutant plaques were analyzed in all organs.

The spontaneous mutant frequencies observed in the control group were similar among the six organs in both *lacZ* and *cII* assays, in the rate ranges of 46×10^{-6} to 88×10^{-6} and 21×10^{-6} to 37×10^{-6}, respectively. These results were comparable to our previous studies [5,8–10,15,25].

All the test compounds significantly increased the mutant frequencies in all the tested organs in the *lacZ*

Table 3
Sequences of *cII* mutations in the liver of 4,10-DAC-treated Muta^{TM}Mouse

Mutant no.	Position	Mutation	Sequence			Amino acid change
D1	113	C to T	AAG	TCG	CAG	Ser to Leu
D2	86	C to A	AAG	ACA	GCG	Thr to Lys
D3	65	C to A	ATC	GCA	ATG	Ala to Glu
D4	106	G to T	GTT	GAT	AAG	Asp to Tyr
D5	101	G to T	GTG	GGC	GTT	Gly to Val
D6	101	G to C	GTG	GGC	GTT	Gly to Ala
D7	29–30	CT to TC	GAG	GCT	CTA	Ala to Val
D8	74	G to T	CTT	GGA	ACT	Gly to Val
D9	115	C to A	TCG	CAG	ATC	Gln to Lys
D10[a]	113	C to T	AAG	TCG	CAG	Ser to Leu
D11	42	G to T	ATC	GAG	AGT	Glu to Asp
D12	119	T to A	CAG	ATC	AGC	Ile to Asn
D13	150	G to T	CCA	AAG	TTC	Lys to Asn
D14	190	G to T	GTT	GAC	GAC	Asp to Tyr
D15	103	G to T	GGC	GTT	GAT	Val to Phe
D16	106	G to T	GTT	GAT	AAG	Asp to Tyr
D17	101	G to A	GTG	GGC	GTT	Gly to Asp
D18	196	G to A	GAC	GAC	ATG	Asp to Asn
D19	226	G to C	GCT	GCG	ATT	Ala to Pro
D20	214	C to T	GCG	CGA	CAA	Arg to Stop
D21	47	C to G	AGT	GCG	TTG	Ala to Gly
D22	91	G to T	GCG	GAA	GCT	Glu to Stop
D23	86	C to G	AAG	ACA	GCG	Thr to Arg
D24	132	G to T	TGG	AAG	AGG	Lys to Asn
D25	123	C to A	ATC	AGC	AGG	Ser to Arg
D26	196	G to C	GAC	GAC	ATG	Asp to His
D27	52	C to T	TTG	CTT	AAC	Leu to Phe
D28	64	G to C	ATC	GCA	ATG	Ala to Pro
D29	163	C to T	CTG	CTT	GCT	Leu to Phe
D30[a]	132	G to T	TGG	AAG	AGG	Lys to Asn
D31	125	G to A	AGC	AGG	TGG	Arg to Lys
D32	196	G to A	GAC	GAC	ATG	Asp to Asn
D33	179–184	−G	TGG	GGG	GTC	Frameshift
D34	148	A to C	CCA	AAG	TTC	Lys to Gln
D35	117	G to T	TCG	CAG	ATC	Gln to His
D36	141	G to T	GAC	TGG	ATT	Trp to Cys
D37	103	G to T	GGC	GTT	GAT	Val to Phe
D38	160	C to A	ATG	CTG	CTT	Leu to Met
D39	125–126	GG to TT	AGC	AGG	TGG	Arg to Ile
D40	166	G to C	CTT	GCT	GTT	Ala to Pro

[a] Ascribable to the same mutation obtained in an identical mouse.

assay and/or *cII* assay. The 4,10-DAC showed the highest mutagenicity among the test compounds, and the highest *lacZ* mutant frequency of 4,10-DAC, observed in the lung, was 19-, 6- and 4-fold over the spontaneously, chrysene- and 1,10-DAC-induced frequencies, respectively. The highest *lacZ* mutant frequency of 1,10-DAC was also observed in the lung. Mutant frequencies obtained with chrysene were not different between the organs examined and ranged from 1.5- to 3-fold over the spontaneous frequency. The mutant frequencies in the *cII* assay showed a tendency similar to those in the *lacZ* assay.

3.2. Mutation spectra of DACs and chrysene in the liver and lung

Thirty-six and 38 control mutants in the liver and lung, respectively, were subjected to sequence analysis, together with 37 and 39 chrysene-induced mutants, 40 and 43 induced mutants of 1,10-DAC and 40

Table 4
Sequences of *cII* mutations in the liver of chrysene-treated Muta™Mouse

Mutant no.	Position	Mutation	Sequence			Amino acid change
B1	210	G to T	CGA	TTG	GCG	Leu to Phe
B2	214	C to T	GCG	CGA	CAA	Arg to Stop
B3	107	A to G	GTT	GAT	AAG	Asp to Gly
B4	150	G to T	CCA	AAG	TTC	Lys to Asn
B5	113	C to T	AAG	TCG	CAG	Ser to Leu
B6	220	G to T	CAA	GTT	GCT	Val to Phe
B7	132	G to T	TGG	AAG	AGG	Lys to Asn
B8	34	C to T	CTA	CGA	ATC	Arg to Stop
B9	40	G to A	ATC	GAG	AGT	Glu to Lys
B10	272–273	−A	TCT	GAA	CAA	Frameshift
B11	190–198	−GAC	GAC	GAC	GAC	Deletion
B12	107	A to G	GTT	GAT	AAG	Asp to Gly
B13	42	G to T	ATC	GAG	AGT	Glu to Asp
B14	125	G to T	AGC	AGG	TGG	Arg to Met
B15	57	C to A	CTT	AAC	AAA	Asn to Lys
B16	141	G to C	GAC	TGG	ATT	Trp to Cys
B17[a]	190–198	−GAC	GAC	GAC	GAC	Frameshift
B18	74	G to A	CTT	GGA	ACT	Gly to Glu
B19	86	C to G	AAG	ACA	GCG	Thr to Arg
B20	196	G to C	GAC	GAC	ATG	Asp to His
B21	91	G to T	GCG	GAA	GCT	Glu to Stop
B22	178	T to G	GAA	TGG	GGG	Trp to Gly
B23	212	C to A	TTG	GCG	CGA	Ala to Glu
B24	127	T to A	AGG	TGG	AAG	Trp to Arg
B25	88	G to C	ACA	GCG	GAA	Ala to Pro
B26	196	G to A	GAC	GAC	ATG	Asp to Asn
B27	129	G to T	AGG	TGG	AAG	Trp to Cys
B28	88	G to A	ACA	GCG	GAA	Ala to Thr
B29	99–101	−G	GTG	GGC	GTT	Frameshift
B30	212	C to A	TTG	GCG	CGA	Ala to Glu
B31	124	A to T	AGC	AGG	TGG	Arg to Trp
B32	179–184	−G	TGG	GGG	GTC	Frameshift
B33	91	G to T	GCG	GAA	GCT	Glu to Stop
B34	101	G to A	GTG	GGC	GTT	Gly to Asp
B35	89	C to T	ACA	GCG	GAA	Ala to Val
B36	89	C to A	ACA	GCG	GAA	Ala to Glu
B37	163	C to G	CTG	CTT	GCT	Leu to Val

[a] Ascribable to the same mutation obtained in an identical mouse.