J. Radiat. Res., 48, 361–368 (2007)

Regular Paper

Frameshift Mutations Produced by 9-Aminoacridine in Wild-Type, *uvrA* and *recA* Strains of *Escherichia coli*; Specificity Within a Hotspot

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9-aminoacridine/*Escherichia coli*/*tonB*/G:C repeat/Minus frameshift.

The endogenous *tonB* gene of *Escherichia coli* was used as a target for 9-aminoacridine-induced mutations that were identified in *recA*$^-$ and *uvrA*$^-$ cells. The cytotoxicity of 9-aminoacridine was enhanced in the *uvrA* and *recA* strains compared to the wild-type strain, and the mutagenicity of 9-aminoacridine in the *uvrA* and *recA* strains was similar to that in the wild type. For all three strains, the most common mutations were minus frameshifts in repetitive G:C base-pairs followed by minus frameshfits in nonrepetitive G:C base-pairs. 9-aminoacridine-induced minus frameshifts in the wild-type strain were distributed with several hot and warm spots. These sites were also hot and warm spots for minus frameshifts in the *recA* and *uvrA* stains. Furthermore, they were hot and warm sites in a 9-aminoacridine-treated strain carrying the target *tonB* gene oriented in the opposite direction. 9-Aminoacridine is known to interact with DNA to form intercalations which are involved in minus frameshift mutagenesis. In this study, we therefore argue that 1) 9-aminoacridine can induce bulky DNA lesions which are excised by nucleotide excision repair and not involved in mutagenesis, 2) the presence or absence of a *recA*-dependent repair pathway does not influence the mutagenic effect of 9-aminoacridine, and 3) both leading strand and lagging strand replication equally produce minus frameshifts, therefore gene orientation is not an important determinant of the formation of hot and warm spots by 9-aminoacridine.

INTRODUCTION

Acridine derivatives are thought to produce frameshift mutations consisting of additions and deletions of one or two bases at repeated sequences.[1)] The specialized mutagenic effect of these planar aromatic compounds is believed to result from their affinity for DNA to form intercalation complexes.[2)] There are structures in which the planar ring systems of the polycyclic molecule lie sandwiched between adjacent base pairs of the double helix.

9-aminoacridine, one of the derivatives of acridine, induces frameshift mutations during DNA replication *in vivo*[1,3)] and *in vitro*.[4)] Strong specificity is exhibited for the deletion over the addition of a single G:C pair in a run of two or more adjacent G:C pairs.[3)] The mechanisms involved in frameshifts are believed to result from the binding to DNA noncovalently by intercalation.[5)] There are indications that the premutagenic lesions that cause frameshift mutations occur during DNA replication.[4,6,7)]

On the basis of the observation that 9-aminoacridine reverts one particular *his*$^-$ frameshift in *Salmonella* and not another,[8)] it has been suggested that there is a sequence specificity in the mode of action of 9-aminoacridine. Skopek and Hutchinson[3)] studied mutagenesis by 9-aminoacridine in the λ *CI* gene in an *E. coli* lysogen; of 23 mutants sequenced, 19 resulted from the deletion of a single G:C pair in a run of adjacent G:C pairs. There are two hotspots with 9 mutations resulting from one particular run of 5Gs and 8 resulting from another particular runs of 4Gs. However, the two remaining runs of 4Gs in the *CI* gene counted for only one and zero minus frameshifts, respectively. Thus the DNA sequence responsible for the formation of a hot spot may include some feature other than repetitive sequences alone.

Although aromatic hydrocarbons, including 9-aminoacridine, ICR181, and proflavin, do induce mutations in the Ames test and other bacterial assay systems, these

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doi:10.1269/jrr.07036

chemicals do not induce cancer in rodents and are categorized as non-carcinogens.[9–11] Thus, a class of planar aromatic hydrocarbons represents potent bacterial mutagens but not rodent carcinogens.[11] There have been a number of discussions in the literature regarding the mechanisms and relevance of mutagenic non-carcinogens, and whether this class of chemicals is consistent with the general theories of multistep carcinogenic mechanisms.[12–14]

In this study, we determined the mutational consequences of 9-aminoacridine-treatment in the endogenous *tonB* gene on the chromosome of *E. coli* wild-type, *recA56* (SOS repair-deficient), and Δ*uvrA*::Km (nucleotide excision repair-deficient) strains. The previous data for 9-aminoacridine-induced changes in a gene on a bacterial chromosome are for a *C*I repressor gene on a λ phage in lysogenic *E. coli* wild-type.[3] The result for *tonB* in *E. coli* is comparable to that recently published for spontaneous mutagenesis of the same gene of *E. coli*.[15] We also determined the mutations induced by 9-aminoacridine in an endogenous *tonB* gene having the opposite orientation with regard to the *oriC* replication origin[16] in order to see the correlation between the formation of a hot spot and direction of replication.

MATERIALS AND METHODS

Strains and growth conditions

Escherichia coli strains KK1[17] and KK300[16] were isogenic, differing only in the orientation of the *tonB* gene, which is 731 bp in size, resides 4.6 kb counterclockwise from the *trp* operon, and has been used as a target for inducing mutations. In KK1, the *tonB* gene has the original orientation and the direction of transcription and DNA replication is the same. Thus, the nontranscribed strand is the template for lagging strand synthesis. In strain KK300, the direction of the *tonB* gene's transcription is the opposite to that of DNA replication. Thus, the nontranscribed strand is the template for leading strand synthesis. We refer to the *tonB* gene in KK1 as *tonBF*$^+$ and that in KK300 as *tonBR*$^+$. KK5 (*recA56*)[17] and KK6 (*uvrA6*)[18] are the derivatives of KK1, carrying a chloramphenicol-resistance gene about 1.9 kb upstream of the *tonB* gene on the chromosome.[17]

Luria Bertani broth (LB), LB-plates (LB + 1.5% agar), and 0.067 M phosphate buffer were used.[19,20] Ampicillin, tetracycline and chloramphenicol were included, if necessary, in LB and LB-plates at concentrations of 50, 10, and 30 μg/ml, respectively. For the selection of colicin B-resistant (ColB^R) mutants, colicin-plates were prepared using the *E. coli* CA18 strain as described.[21]

Reagents and chemicals

Enzymes and reagents used for DNA manipulation were purchased from TaKaRa (Kyoto, JAPAN) and Applied Biosystems (Foster City, CA). 9-aminoacridine (Sigma Chemicals Co., St Louis, Mo) was dissolved in dimethyl sulfoxide at 5 mM. It was stored in a freezer in small quantities covered with aluminum foil.

9-aminoacridine treatment and mutant selection

For the mutation assay, independent colonies of strains were grown in LB at 37°C overnight, diluted 100-fold in LB, and incubated at 37°C for an appropriate time to reach 2×10^8 cells/ml. A frozen solution of 9-aminoacridine was thawed immediately before use. 9-aminoacridine treatment was for 30 min at 37°C with shaking. After the treatment, some cells were diluted in phosphate buffer to measure the killing effects of 9-aminoacridine, the rest were washed twice in chilled phosphate buffer by centrifugation, resuspended in LB, and grown overnight at 37°C for mutant expression. Samples of these overnight cultures were plated on colicin plates and colonies were scored as ColB^R mutants after a 48-h incubation. Viable cells were scored on LB-plates after 18 h of incubation. The frequency of mutation was obtained by dividing the number of ColB^R colonies by the number of viable colonies. To collect *tonB* mutants for DNA sequencing, only one ColB^R colony was chosen from each colicin plate. To avoid photodynamic effects, 9-aminoacridine experiments were carried out under yellow light.

Sequencing of mutant DNA

For sequencing the *tonB* mutant, only one ColB^R colony was chosen from each colicin plate, an approach that ensured each mutant analyzed was of independent origin. DNA sequencing was carried out as described previously.[22] In brief, the DNA fragment containing the mutant *tonB* gene was amplified by PCR using appropriate primers from genomic DNA that had been extracted from the ColB^R mutant. After amplification, the concentration of the amplified DNA was determined from the intensity of the band of proper size on electrophoresis of 1-μl samples on 0.7% agarose gels. Mutant sequences were determined by the dideoxy chain termination method using an automated sequencer. Sequence-primers used, except primer 3.5, have been described previously.[22] The sequence of primer 3.5 was 5'-CGACTCTACGGGTTTGACAT-3', which hybridizes nucleotide 3364 to 3345 for KK1 and the derivative strains, and 5429 to 5448 for strain KK300.

RESULTS

Survival and mutation frequencies in E. coli cells treated with 9-aminoacridine

We observed that exposure to different concentrations of 9-aminoacridine for 30 min had substantially different lethal effects on KK1 (wild-type), KK5 (*recA56*), and KK6 (*uvrA6*) as shown in Fig. 1. The mutagenicity of 9-aminoacridine, however, appeared similar in the three strains (Fig. 2). Ferguson and MacPhee[23,24] have reported that the *recA1* strain and *uvrB* strain of *Salmonella typhimurium* are almost

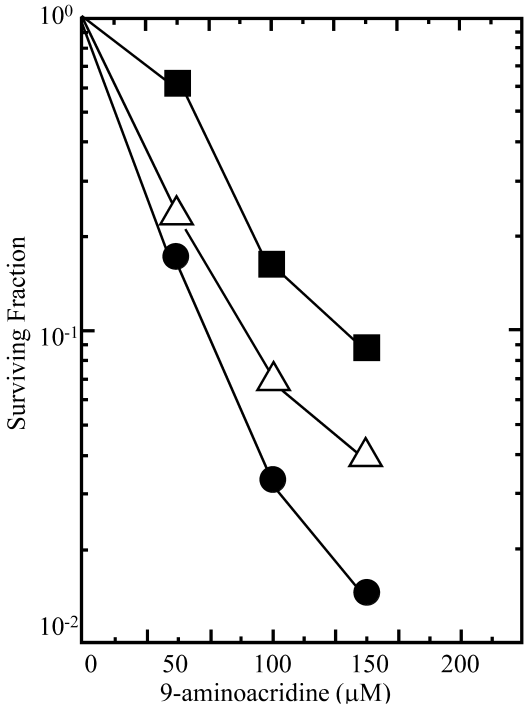

Fig. 1. Survival of *E. coli* strains exposed to different concentrations of 9-aminoacridine. The strains used were KK1 (filled square), KK6 (*uvrA*; open triangle), and KK5 (*recA*; filled circle). The curves represent the average of 3 independent experiments.

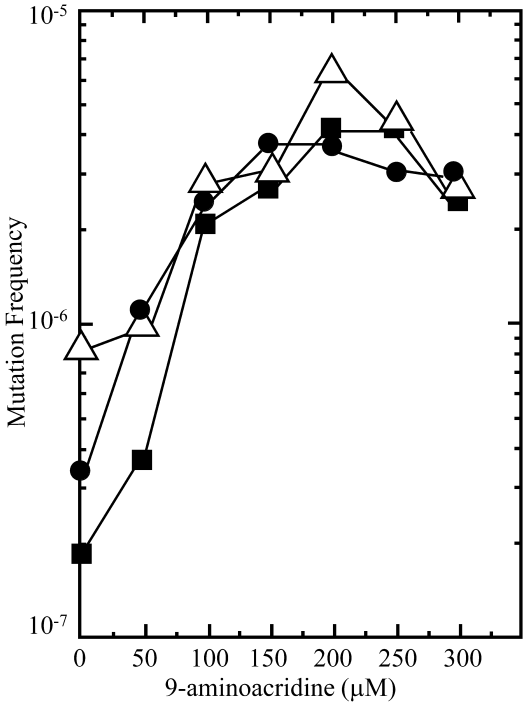

Fig. 2. Mutation frequencies of *E. coli* strains treated with different concentrations of 9-aminoacridine. The strains used are KK1 (filled square), KK6 (open triangle), and KK5 (filled circle). The curves represent the average of 3 independent experiments.

Table 1. Nature of 9-aminoacridine-induced *tonB* mutations[a]

Classes of Mutations	KK1 (*tonBF*$^+$)		KK300 (*tonBR*$^+$)		KK 5 (*tonBF*$^+$ *recA*)		KK 6 (*tonBF*$^+$ *uvrA*)	
	– 9AA[b]	+ 9AA	– 9AA	+ 9AA	– 9AA[c]	+9AA	–9AA[d]	+9AA
Base substitution	28 (27%)	4 (8%)	30 (42%)	6 (12.5%)	15 (28%)	2 (4%)	12 (32%)	3 (6 %)
G:C→A:T	4	0	13	1	9	0	5	1
A:T→G:C	1	0	1	2	0	0	1	0
G:C→T:A	12	2	8	1	4	2	2	1
G:C→C:G	0	2	0	0	0	0	0	0
A:T→T:A	8	0	8	2	1	0	3	1
A:T→C:G	3	0	0	0	1	0	1	0
Frameshift	13 (6%)	42 (84%)	12 (17%)	39 (81%)	3 (6%)	50 (91%)	2 (5%)	42 (87.5%)
– frameshift	12	36	9	34	2	50	2	39
+ frameshift	1	6	3	5	1	0	0	3
IS	22 (43%)	4 (8%)	22 (31%)	3 (6%)	34 (63%)	3 (5%)	19 (50%)	3 (6 %)
Deletion	12 (20%)	0 (–)	5 (7%)	0 (–)	1 (2%)	0 (0)	3 (8%)	0 (–)
Miscellaneous	4 (4%)	0 (–)	3 (4%)	0 (–)	0 (–)	0 (–)	2 (2%)	0 (–)
Total	79	50	72	48	53	55	38	48

[a]9-aminoacridine was added at 0 μM (– 9AA) and 200 μM (+ 9AA) for 30 min.
[b]Data from Tago *et al.*[15]
[c]Data, except for mutation frequency, from Kitamura *et al.*[25] In this case TM35 (*recA56*; AB1157 derivative) was used instead of KK5.
[d]Data, except for mutation frequency, from Kitamura *et al.*[25] In this case TM36 (*uvrA6*; AB1157 derivative) was used instead of KK6.

equally mutable in terms of minus frameshifts as the wild-type strain. Thus, it was suggested that 9-aminoacridine produces bulky lesions that are excised by the nucleotide excision repair system but may not be involved in mutagenesis.

9–aminoacridine-induced mutational spectra

A total of 50 ColB^R mutations from KK1 (wild-type), 55 from KK5 (*recA*), and 48 from KK6 (*uvrA*) were collected after treatment with 100–150 μM 9-aminoacridine and used for DNA sequencing. Their distribution by class is listed in Table 1 along with previously published results from untreated controls using the wild-type,[15] *recA*,[25] and *uvrA*[18] strains. 9-aminoacridine exclusively induced minus frameshifts; plus frameshifts and base substitutions were detected after the treatment, however, their numbers were not great enough for an evaluation of significance.

Table 2 shows the location and the types of minus frameshift mutations. Among 42, 50, and 42 frameshifts caused by 9-aminoacridine in the wild-type, *recA* and *uvrA* strains, respectively, 36, 50, and 39, respectively, were minus frameshifts. Among 16 minus frameshift sites recovered from the wild-type strain, we observed 14 G:C sites and 2 A:T sites, and 12 at runs of identical bases and 4 at non-runs of bases. Among 10 minus frameshift sites in the *recA* strain, we observed 9 G:C sites and 1 A:T site, and 6 at runs and 4 at

Table 2. Location and type of 9 aminoacridine-induced minus frameshift mutations in strains KK1 (*tonBF*$^+$), KK300 (*tonBR*$^+$), KK5 (*tonBF*$^+$ *recA56*), and KK6 (*tonBF*$^+$ *uvrA6*).

position[a]	change	sequence[b]	KK1	KK300	KK5	KK6
3012–3014	–C	TCAATGA[CCC]TTGA	2	1	0	0
3031	–C	TCGCCG[C]TTCCCCT	7	5	9	3
3034–3037[c]	–C	CCGCTT[CCCC]TGGCC	1	4	0	1
3082–3084[c]	–G	GTGGC[GGG]TCTGCT	10	8	17	19
3100–3101	–G	TACCTC[GG]TACATCA	1	0	0	0
3194–3195	–C	TTCAG[CC]GCCA	0	1	0	0
3353–3356	–C	TCAAA[CCC]GTAGA	0	1	0	0
3397–3398	–G	CGCACC[GG]CACGCC	1	0	4	0
3399	–C	ACGGCA[C]GGCACG	0	1	0	0
3474–3476	–A	GCATT[AAA]GCGTAA	1	0	0	0
3488–3489	–C	ATCAG[CC]GCAGTAT	0	1	0	0
3527–3529[c]	–G	TTGAA[GGG]CAGGTT	1	1	10	3
3593	–G	CCTCTCA[G]CCAAGC	1	2	0	0
3594–3595	–C	TCAG[CC]AAGCCT	1	0	0	0
3616	–G	TTTGA[G]CGTGA	1	0	2	0
3627–3631	–A	AGGTG[AAAAA]TGCG	0	0	0	2
3655	–G	TGA[G]CCGGGTAA	0	1	2	0
3658–3660	–G	AGCC[GGG]TAA	2	0	0	4
3664	–G	GGGTAA[G]CCAGGCA	2	4	3	5
3668–3669	–G	GCCA[GG]CAGT	3	0	0	0
3674–3676[c]	–G	GCAGT[GGG]ATTGTG	1	3	3	2
3698–3701	–A	TGTTT[AAAA]TTAA	1	1	0	0
Total			36	34	50	39

[a]Numbering is in accordance with Kitamura and Yamamoto[18] in which 2993 is the first A of the ATG start codon and 3727 is the last A of the TAA stop codon of the *tonB* gene.
[b]Sequences shown are from the non–transcribed strand and the template for lagging strand synthesis in the *tonBF*$^+$ configuration and for leading strand synthesis in the *tonBR*$^+$ configuration.
[c]Mutational sites where both + and – frameshifts were observed.

non-runs. Among 6 minus frameshift sites in the *uvrA* strain, we observed 6 G:C sites and 0 A:T sites, and 4 at runs and 2 at non-runs. Thus, we argue that 9-aminoacridine-induced minus frameshift mutations occurred at G:C sites and at runs of identical bases (Table 2).

Minus frameshifts induced by 9-aminoacridine in the wild-type strain were not equally distributed along the entire *tonB* gene but rather distributed with hot spots at nucleotide 3031 (one C site) and at nucleotide 3082–3084 (three Gs run site). These sites were also the hot spots for minus frameshifts in the *recA* and *uvrA* stains. In the *recA* strain, nucleotide 3527–3529 (a run of three Gs) is also a hot spot generated by 9-aminoacridine. This is a warm spot in the *uvrA* strain (three incidences). Concerning sites at 3664 (one G site) and 3674–3676 (three Gs), several minus frameshifts were observed in all strains tested, indicating warm spots. Thus, except for 3527–3529 which is a hot spot for the *recA* strain but not wild-type or *uvrA* strain, the hot spots were common to the wild-type, *recA* and *uvrA* strains. On the other hand, formation of hot spot at 3527–3529 in *recA* strain cannot be interpreted at present. Table 2 shows the very strong preference for mutagenesis with 9-aminoacridine at runs of G:C base pairs (23 of 36 minus frameshifts in KK1), consistent with a previous study.[3] 9-aminoacridine also induced minus frameshifts at nonrun G:C base pairs in the endogenous *tonB* gene (11 cases among 36).

It is mentioned that 9-aminoacridine can induce minus frameshifts as well as plus frameshifts.[1] The spectra of mutations induced by 9-aminoacridine *in vitro*[4] and *in vivo*[3] indicated a predominance of minus frameshifts and a low level of plus frameshifts. The location and type of plus frameshift mutations in the endogenous *tonB* gene indicated that 9-aminoacridine could induce plus frameshifts at G:C sites, suggesting an effect of intercalation (Table 3). Consistent with previous results,[3] 9-aminoacridine induced fewer plus frameshifts than minus frameshifts (Tables 1 and 3).

The effect of replication direction of 9-aminoacridine induced frameshifts

Concerning hot spots of minus frameshifts induced by 9-aminoacridine, we observed two, at 3031 and 3082–3084, and several warm spots in KK1 (Table 2). In this case, the target *tonB* gene is in the original orientation and the direction of transcription and DNA replication is the same. To see whether the formation of hot and warm spots seen in KK1 is dependent on the direction in which the DNA is replicated, the mutagenic effects of 9-aminoacridine were observed in strain KK300, in which the orientation of the *tonB* gene is reversed.[16] The frequency of mutation with 100 μM of 9-aminoacridine in KK300 was 1.79×10^{-6}, which is about 10-fold higher than the spontaneous frequency, 2.06×10^{-7}. As a reference, the corresponding values in KK1 were 1.86×10^{-7} and 2.06×10^{-6}, respectively (Fig. 2). There were no significant differences in the frequency of mutation between strains KK1 (*tonBF*$^+$) and KK300 (*tonBR*$^+$).

The changes in sequence detected in KK300 are summarized in Tables 1–3. The distribution and type of minus frameshifts induced by 9-aminoacridine in the *tonB* gene were not markedly different from those for KK1 (Table 2). Minus frameshifts in KK300 and KK1 were seen equally at hot spot sites, 3031 and 3082–3084, and warm spots, 3664 and 3674–3676 (Table 2). The results suggested that the DNA lesions generated by 9-aminoacridine are mutated equally or similarly by leading and lagging DNA replication.

As a record, the location and types of base substitutions induced by 9-aminoacridine have been presented as online supplemental Table S1 at http://www.biology.tohoku.ac.jp/~kazuo/niranjan07/.

Table 3. Location and type of 9 aminoacridine–induced plus frameshift mutations in strains KK1 (*tonBF*$^+$), KK300 (*tonBR*$^+$), KK5 (*tonBF*$^+$ *recA56*), and KK6 (*tonBF*$^+$ *uvrA6*).

position[a]	change	sequence	KK1	KK300	KK5	KK6
3034–3037[b]	+C	CGCTT[CCCC]TGGCCGA	0	0	0	1
3082–3084[b]	+G	TTGTGGC[GGG]TCTGCT	0	1	0	0
3179–3180	+C	AACCG[CC]ACAAGC	1	0	0	0
3206–3207	+C	ACCGGAG[CC]GGTGGT	2	0	0	0
3462–3463	+G	GGCTTCA[GG]ACCA	3	0	0	0
3527–3529[b]	+G	TTGAA[GGG]CAGGTT	0	2	0	2
3674–3676[b]	+G	GGCAGT[GGG]ATTGT	0	2	0	0
Total			6	5	0	3

[a]Numbering is in accordance with Kitamura and Yamamoto[18] in which 2993 is the first A of the ATG start codon and 3727 is the last A of the TAA stop codon of the *tonB* gene.
[b]Mutational sites where both + and – frameshifts were observed.

DISCUSSION

Similar to a previous report[3] using the gene λ*CI* in lysogenic *E. coli*, the present experiment showed that 9-aminoacridne induced single base pair deletions in the endogenous *tonB* gene of wild-type *E. coli*; no increase in large deletions or base substitutions occurred, and only a very slight increase in single base additions was observed. The DNA sequences most subject to deletion were runs of G over runs of C and a very weak preference for runs of A (Tables 2 and 4). In the present study, we also found that, upon treatment with 9-aminoacridine, the *recA* and *uvrA* strains showed essentially the same mutability and same specificity with regard to minus frameshifts as the wild-type strain (Fig. 2 and Tables 2 and 3, respectively). Ferguson and MacPhee[23,24] have reported that the *recA1* strain and *uvrB* strain of *Salmonella typhimurium* are almost equally mutable in terms of minus frameshifts as the wild-type strain. It is argued that 9-aminoacridine binds to DNA noncovalently by intercalation.[5] Thus, 9-aminoacridine-intercalation can induce minus frameshifts at repeats of G to essentially the same extent in the *recA*, *uvrA,* and wild-type strains.

We observed that 9-aminoacridine was more cytotoxic to the *uvrA* strain than the wild-type strain (Fig. 1). It is known that 9-aminoacridine inhibits DNA replication *in vitro*.[4,26] It has also been shown that 9-aminoacridine does not induce *recA*-*lexA*-dependent SOS responses.[27] We observed no increase of *recA* gene expression during 9-aminoacridine treatment (data not shown). As mentioned above, 9-aminoacridine induces frameshifts in the wild-type, *recA* and *uvrA* strains to an almost equal extent. Thus, the results indicated that 9-aminoacridine-induced lethal lesions might lead to the inhibition of DNA replication and differential effects on survival in uvrA and recA strains, but might not be involved in induction of the SOS response and induction of frameshift mutagenesis.

It has been mentioned that 9-aminoacridine induces minus frameshift mutations *in vivo* mainly at repetitive sequences.[1,3] Therefore, we quantified the number of minus frameshifts (as a percentage) as a function of base composition and the number of bases available for mutation in a given repeat length (Table 4). The occurrence of minus frameshifts in KK300 (*tonBR*$^+$) was also quantified (Table 4 in parentheses). First, the number of minus G frameshifts induced by 9-aminoacridine increased linearly from nonrepetitive Gs (13% of 116 sites), to runs of two Gs (7% of 34 sites) and then runs of three Gs (58% of 4 sites). This can compare to minus C frameshifts, two of which occurred at runs of four Cs (2 sites) and two of which occurred at runs of three Cs (2 sites). We therefore argue that interaction with 9-aminoacridine in a repeat of G:C base pairs stabilizes a loop-out C residue more efficiently than a loop-out G residue. Second, very few minus A frameshifts and no minus T frameshifts were observed, indicating no interaction of the A:T base pair with 9-aminoacridine.[28] Third, we observed essentially the same results when the target *tonB* gene was oriented in the opposite direction in terms of DNA replication (Table 4). The sites of mutagenic action of 9-aminoacridine are associated with the DNA replication fork.[4,6,7] Thus, at the replication

Table 4. The effects of composition and repeat length on occurrence of minus frameshifts caused by 9-aminoacridine

	% occurrence in *tonBF*$^+$ (in *tonBR*$^+$)/target size[a]							
Repeat length	A		G		C		T	
1	0/88	(0/88)	13/116	(21/116)	15/89	(18/89)	0/79	(0/79)
2	0/24	(0/24)	7/34	(0/34)	1/48	(5/48)	0/24	(0/24)
3	1/6	(0/6)	58/4	(35/4)	2/2	(6/2)	0/7	(0/7)
4	1/6	(3/6)	–[b]		2/2	(12/2)	–	
5	2/2	(0/2)	–		–		–	
Total	4/126	(3/126)	78/154	(56/154)	20/141	(42/141)	0/110	(0/110)

[a]Each column indicates the % occurrence of minus frameshifts in *tonBF*$^+$ and *tonBR*$^+$ (in parentheses) strains divided by the total number of identical bases occurring in repeats of the size indicated. In the *tonBF*$^+$ configuration in the wild-type, *recA*$^-$, and *uvrA*$^-$ strains, 36, 50 and 39 minus frameshifts, respectively, were detected, therefore 125 minus frameshifts were detected (Tables 1 and 2). For example, there are two 4C repeats at 3034–3037 and 3241–3244, and minus frameshifts numbered one in the wild-type and one in the *uvrA* strain (Table 1). Thus, % occurrence at 4Cs was 2/125 = 2% and two 4C sites exist in the *tonB* gene, giving 2/2. Four frameshifts were observed in KK300 (*tonBR*$^+$) at the 4C site (Table 2), thus giving 12/2.

[b]A dash indicates the absence of a given repeat length for that base.

fork, 9-aminacridine intercalates with DNA to stabilize loop-out C residues irrespective of the mode of replication, leading strand replication or lagging strand replication. Previously, a preference for the lagging strand was observed with respect to the induction of frameshift mutations by N-2-acetylaminofluorene.[29] However, the study did not directly address the intrinsic fidelity of replication because it focused on the probability of bypassing DNA lesion at one particular site.

We observed several hot and warm spots at runs of 3 Gs irrespective of the direction of replication (Tables 2 and 4). However, two runs of 3Cs and even two runs of 4Cs counted for only 2% and 0% of minus frameshifts, respectively, in the *tonBF*$^+$ configuration and 6 and 12%, respectively in the *tonBR*$^+$ configuration (Table 4). Thus, the DNA sequence responsible for the formation of a hot spot may include some additional feature other than repetitive sequences.

9-aminoacridine can produce mutations in most simple gene mutation assays but does not produce cancer in mice and is therefore categorized as a non-carcinogenic or equivocal substance.[9,10] Many chemicals among those considered chemical mutagens are classified as non-carcinogens. Thus the way non-carcinogenic mutagens, including 9-aminoacridine, exert their effects requires further investigation.

ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan

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Received on April 6, 2007
Revision received on May 20, 2007
Accepted on May 31, 2007
J-STAGE Advance Publication Date: July 5, 2007