DNA Sequence Determinants of Carcinogen-Induced Frameshift Mutagenesis†

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ABSTRACT: In order to examine the mechanisms of induced frameshift mutagenesis, we constructed double-stranded DNA plasmids which contain single *N*-2-acetylaminofluorene (AAF) adducts at specified positions within a run of contiguous guanine residues. The length of the homopolymeric run and the nature of the bases flanking the contiguous sequence were systematically varied. Monomodified plasmids were introduced into SOS-induced *Escherichia coli*, and −1 frameshift mutations were scored by means of a phenotypic assay. A strong positional effect of the DNA adduct within the contiguous sequence was observed irregardless of the nature of the flanking bases: the AAF-induced mutation frequency was 20–200-fold higher at the 3′-end of the contiguous sequence than at the 5′-end. In addition, for a given number of guanine residues flanking the G^{AAF} adduct on its 5′-side, differences (up to 5-fold) in the induced mutation frequency were observed as a function of the base 3′ to the adduct ($CGGG^{AAF}AT \approx CGGG^{AAF}GT > CGGG^{AAF}CT > CGGG^{AAF}TT$). These results are discussed, within the frame of an incorporation slippage model, in terms of differences in stability and occurrence of the slipped mutagenic intermediates.

Repetitive sequences are known to be hot spots for frameshift mutations which occur spontaneously or are induced by a wide variety of structurally diverse mutagenic agents (Koffel-Schwartz et al., 1984; Marnett et al., 1985; Streisinger & Owen, 1985; Refolo et al., 1988; Ferguson & Denny, 1990; Benamira et al., 1992; Lambert et al., 1992a). The classical "strand slippage" model for frameshift mutagenesis within repetitive sequences was proposed by Streisinger et al. (1966), who postulated that the insertion or deletion of a repeating unit of the sequence occurs as a consequence of slippage of the template strand relative to the primer strand during DNA synthesis. In this model, the intermediates derived from strand slippage contain extrahelical bases: if the extrahelical base is situated on the template strand, a deletion will occur; conversely, if the extrahelical base resides on the primer strand, an insertion will occur.

We have used the carcinogen *N*-2-acetylaminofluorene (AAF)[1] as a model compound with which to examine frameshift mechanisms. AAF reacts primarily at the C(8) position of guanine residues to form *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene [dG-C(8)–AAF] adducts (Kriek et al., 1967). Although AAF binds about equally to all guanine residues (Fuchs, 1984), frameshift mutations are induced at two types of hot spot sequences (Koffel-Schwartz et al., 1984): −1 frameshift mutations occur at runs of guanine residues (i.e., GGG → GG), and −2 frameshift mutations are observed at high frequency within sequences containing short repetitions of alternating GpC dinucleotides such as the *Nar*I site (GGCGCC → GGCC). Genetic studies have revealed that −1 and −2 frameshift mutations involve two distinct SOS-inducible pathways. AAF-induced −1 frameshift mutations are *rec*A and *umu*C/D-dependent while AAF-induced −2 mutations are occur independently of *Umu*C/D and are also *Rec*A-independent in a *lexA*(Def) host (Maenhaut-Michel et al., 1992).

In the current study, we focus on mechanisms of −1 frameshift mutations. Using site-specific modified plasmids containing a single AAF adduct, we have previously shown that the induction of −1 frameshift mutations within a sequence of three contiguous guanine residues (5′-$G_1G_2G_3$-3′) is strongly dependent on the position of the adduct within the run: when AAF is bound to G_3, −1 frameshift mutations are induced about 100-fold and 10-fold more frequently than dG-C(8)–AAF adducts at G_1 and G_2, respectively (Lambert et al., 1992b). The observed positional effect of the lesion suggests that correct insertion of cytosine residues opposite the AAF adduct necessarily precedes strand slippage; moreover, the extent of the observed mutagenic effect appears to be influenced by the length of the homopolymeric run 5′ from the adduct. These results are consistent with an "incorporation slippage" model in which the AAF adduct is replicated as a guanine by insertion of a cytosine residue; however, the presence of the lesion at the primer/template terminus impedes elongation and, thus, provides an increased opportunity for strand slippage to occur. Within repetitive sequences, slippage of the nascent cytosine residues onto the guanine residues located immediately 5′ from the adducted guanine on the template strand forms slipped mutagenic intermediates (SMI) containing an extrahelical AAF adduct and normal C:G base pairs at the primer/template terminus. Extensions from such termini fix the −1 frameshift mutation.

Recently we have shown that AAF adducts may play a further role in frameshift mutagenesis by stabilizing the slipped mutagenic intermediates (Garcia et al., 1993). It is likely that stabilization of the SMI is mediated via stacking interactions of the AAF-modified guanine residue between the neighboring base pairs; therefore, the nature of the base pairs between which the AAF-modified guanine residue is stacked could influence the stability of the putative intermediate and the resultant induced mutation frequency. In order to test this hypothesis, we have constructed a series of site-specific modified plasmids containing a single AAF adduct within the sequence 5′-$G_1G_2G_3$Y-3′ [Y = A, G, C, or T] and

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[1] Abbreviations: AAF, *N*-2-acetylaminofluorene; dG-C(8)–AAF, *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene; SMI, slipped mutagenic intermediates.

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Table 1: Names and Sequences of Oligonucleotides and Plasmids

plasmids	oligonucleotides	sequences
pUC-G_3A	14-G3A	ATCACGGGATCACA
pUC-G_4	14-G4	ATCACGGGGTCACA
pUC-G_3C	14-G3C	ATCACGGGCTCACA
pUC-G_3T	14-G3T	ATCACGGGTTCACA
pUC-G_2T	14-G2T	ATCACCGGTTCACA

have measured the frequency of −1 frameshift events induced by the AAF lesions within the different sequence contexts. In addition, we have systematically studied the influence of the length of the repetitive sequence on frameshift mutagenesis using AAF-modified plasmids in which contiguous guanine residues in the run G_n (n = 2, 3, or 4) have identical flanking bases.

EXPERIMENTAL PROCEDURES

Materials. T4 DNA polymerase, T4 polynucleotide kinase, and all restriction enzymes were purchased from New England Biolabs. Sequenase version 2.0 was obtained from United States Biochemical Corp. All oligonucleotides were synthesized by standard phosphoramidate chemistry and were HPLC-purified prior to being subjected to any of the experimental manipulations described in this paper.

Oligonucleotides Containing a Single AAF Adduct. Five different 14-mers containing 2, 3, or 4 guanine residues in a run (see Table 1) were reacted wtih 4 molar equiv of *N*-acetoxy-AAF by incubation at 37 °C, for 6 min, in 2 mM sodium citrate (pH 7) containing 5% ethanol as described (Koehl et al., 1989).

The AAF-modified oligonucleotides were fractionated on a C18 nucleosil column by reverse-phase HPLC. The solvent program consisted of a 5-min isochratic elution with 29.4% (vol/vol) methanol in 0.05 M triethylamine acetate (pH 6.0) followed by a 15-min linear gradient to 46.2% methanol. In all cases, monomodified oligonucleotides were completely resolved under these conditions, except for two of the monomodified derivatives of the 14-G4 oligonucleotide which contained four guanines (-$G_1G_2G_3G_4$-): 14-G4-G_2^{AAF} and 14-G4-$G3^{AAF}$ co-migrated under all tested conditions.

After HPLC purification, the position of AAF modification within the sequence and the purity of each oligonucleotide was analyzed using an assay that involves the 3′→5′ exonuclease of T_4 DNA polymerase as described previously (Fuchs, 1984). All monomodified oligonucleotides were found to be over 95% pure, except for the fraction of 14-G4 containing both 14-G4-G_2^{AAF} and 14-G4-G_3^{AAF} which contained equal amounts of the two monomodified oligonucleotides as determined by densitometric scanning of the autoradiograph following the T4 DNA polymerase assay.

Plasmids. Plasmids involved in the construction of the monomodified derivatives all were derived from plasmid pUC8. The nomenclature of the plasmids and the sequences of the mutation hot spots are shown in Table 1. These plasmids were constructed by replacement of the *Eco*RI/*Hind*III fragment of the polylinker region of pUC8 with synthetic DNA duplexes. The precise sequence of all plasmids was confirmed by DNA sequencing. Plasmids containing a single AAF adduct were constructed using the gapped-duplex strategy (Burnouf et al., 1989; Lambert et al., 1992). Monomodified derivatives were constructed by the hybridization of the oligonucleotides containing single AAF lesions into the gapped region of the heteroduplex, followed by ligation. The covalently closed, circular plasmids were isolated on CsCl density gradients. The sequence of the mutation target of all constructions is given in Table 1.

Bacterial Strains and Mutation Analysis. The monomodified plasmids were introduced into an excision repair deficient strain (JM103 *uvrA6*) by electroporation (Dower et al., 1988). The SOS functions of the *E. coli* host were induced by UV-irradiation at 6 J/m^2 in 10 mM $MgSO_4$, and competent cells were prepared as previously described. The transformation mixture was plated on agar plates containing M9 medium, glycerol (0.2%), ampicillin (100 mg/L), 5-bromo-4-chloro-3-indolyl β-D-pyranoside (XGal, 200 mg/L) and isopropyl β-D-thiogalactopyranoside (IPTG, 60 mg/L). −1 frameshift mutants were detected as blue colonies on this medium. The molecular nature of the mutations was determined by direct sequencing of randomly picked blue colonies using Sequenase version 2.0.

RESULTS

Aims, Strategy, and Nomenclature. We previously demonstrated that the ability of AAF lesions to induce −1 frameshift mutations within homopolymeric runs was strongly dependent on the position of the AAF lesion; adducts at the 3′-end of a sequence of three contiguous guanine residues are approximately 100-fold more mutagenic than lesions at the 5′-end of such sequences.

In the present study, we wanted to investigate the effect of the nucleotide flanking the G_3 position on its 3′-side on the induced mutation frequency. For this purpose, we have constructed plasmids containing single AAF adducts within the sequence 5′-$G_1G_2G_3$Y-3′, where Y is A, G, C, or T, respectively. These plasmids have been named pUC-G_3A, pUC-G_4, pUC-G_3C, and pUC-G_3T, respectively. The surrounding sequence of the oligonucleotides that has been used in the construction of these plasmids is shown in Table 1.

In addition, the effect of the number of bases within the run was also investigated by comparing the mutation frequency induced when the adduct was located on the 3′-end of a run of two, three, or four guanines, respectively (plasmids pUC-G_2T, pUC-G_3T, and pUC-G_4). In each of these plasmids, the AAF lesion at the 3′-end of the run is in same local DNA sequence context, being flanked on the 3′-side by a thymine residue and on the 5′-side by another guanine residue.

All plasmid constructions were designed so that −1 frameshift mutations could be scored phenotypically by a change in colony color from white to blue. Indeed, −1 events within the polylinker region restore the reading frame of the plasmid-encoded 5′-terminal fragment of the *lacZ* gene allowing expression of functional β-galactosidase in an α-complementation host and consequently yielding blue transformants on XGal/IPTG medium.

Plasmids were introduced by electroporation into an excision repair deficient strain (JM 103*uvrA*.6) whose SOS functions had been induced by UV irradiation (6 J/m^2) in order to maximize the mutagenic response (Lambert et al., 1992b). Blue colonies derived from the different constructions were picked at random and sequenced in order to determine the molecular nature of the mutation. The sequence of a total of about 120 mutants revealed that ≃96% contained the loss of a single G residue within the target sequence. The remaining 4% of nontargeted mutants (five mutants) were found to be cloning artifacts showing small deletions within the cloning site.

Effect of Nucleotide Flanking the Run on Its 3′-Side. The frequency of frameshift mutations was measured following transformation of *JM103uvrA6* bacteria by plasmids pUC-G_3A, pUC-G_4, pUC-G_3C, and pUC-G_3T, which contained a single AAF adduct located at G_1, G_2, or G_3 (Table 2, Figure 1). Background mutation frequencies were determined using

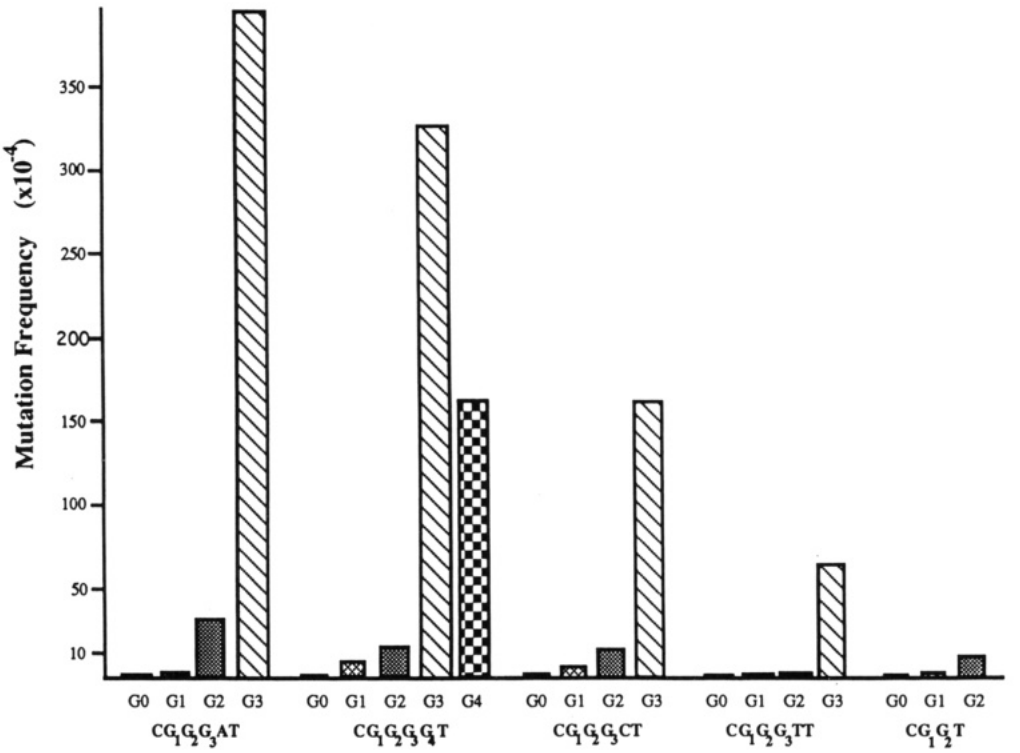

FIGURE 1: Effect of the position of AAF adducts within contiguous guanine sequences and effect of the 3′-flanking base on the −1 frameshift mutation frequency.

Table 2: −1 Frameshift Mutation Frequencies Induced by Single AAF Adducts in Contiguous Guanine Sequences

	position of AAF adduct				
plasmid	G0	G1	G2	G3	G4
pUC-G3A	<1.4[a]	2.3	32	398	
pUC-G4	0.58	9.7	17[b]	335[b]	167
pUC-G3T	<0.24	2.9	2.2	76	
pUC-G3C	1.5	7	17	167	
pUC-G2T	0.3	2.5	14		

[a] Mutation frequencies are expressed as mutants per 10^4 transformants. [b] Mutation frequencies were estimated from the frequency measured with an equimolar mixture of pUC-G4-G2^{AAF} and pUC-G4-G3^{AAF} as these two adducted oligonucleotides could not be separated by HPLC (see Materials and Methods). The mutation frequency measured for the mixture was equal to 176×10^{-4}. The frequency attributable to the AAF adduct on G_2 was estimated as the average of the frequencies obtained with the different G_2^{AAF} constructions [pUC-G_3A, pUC-G_3C, and pUC-G_3T (average frequency = 17×10^{-4})]. The mutation frequency attributable to pUC-G_4-G_3^{AAF} can thus be calculated as 335×10^{-4}.

unmodified derivatives of each of these plasmids that were constructed by building an unmodified oligonucleotide into the gapped-duplex plasmid. Consistent with our previous study (Lambert et al., 1992b), the background mutation frequency was in the range of 10^{-4}.

With all plasmids, a marked positional effect of the AAF adduct within the run of guanine residues was observed: the mutation frequency induced by an adduct at G_3 was 20–200-fold higher than that induced by an adduct at G_1. The observation that adducts at the 3′-end of homopolymeric sequences are much more mutagenic than adducts at the 5′-end of such sequences is entirely consistent with our previous observations (Lambert et al., 1992b). An effect of the base 3′ to the adduct at G3 on the observed frequency of frameshift mutation is apparent (Figure 1). The G_3^{AAF} adducts with a 3′-flanking adenine was the most mutagenic, inducing −1 frameshift mutations at a frequency of 398×10^{-4}. The estimated frequency (see Table 2) for the G_3^{AAF} adduct flanked by a guanine residue was 335×10^{-4}. AAF adducts flanked by pyrimidines on their 3′-side were much less mutagenic: when the 3′-flanking base was cytosine or thymine, the mutation frequency was equal to 167×10^{-4} and 76×10^{-4}, respectively.

Effect of Length of the Run. On the basis of our previous results (Lambert et al., 1992b), we postulated that the number of guanine residues 5′ to the AAF adduct on the template strand was an important determinant of the frequency of induced −1 events. However, that interpretation was complicated by the fact that, in those experiments, the AAF adducts were flanked on the 3′-side by different bases. As shown above, the nature of base 3′ to the G_3^{AAF} adduct can alter the mutagenic response by approximately 5-fold. We therefore compared the mutagenicity of three plasmids: pUC-G_4-G_4^{AAF}; pUC-G_3T-G_3^{AAF}; and pUC-G_2T-G_2^{AAF}. In all of these plasmids the AAF adduct at the 3′-end of the contiguous guanine run is situated in an identical sequence context, being flanked by a T residue on the 3′-side and by a G residue on the 5′-side. However, the number of guanine residues 5′ to the adduct is decreased from three residues in pUC-G_4-G_4^{AAF} to one residue in pUC-G_2T-G_2^{AAF}. The length of the run does indeed influence the mutagenicity of the AAF lesion. The frequency of mutation induced by an AAF adduct with three 5′ guanines (167×10^{-4}) is twice that of the adduct with two 5′ guanines (76×10^{-4}), which in turn is five times greater than the adduct with only a single 5′ guanine residue (14×10^{-4}).

DISCUSSION

In this paper, we have systematically altered the sequence context of single AAF adducts in runs of contiguous guanine residues. Our results suggest that both the position of an

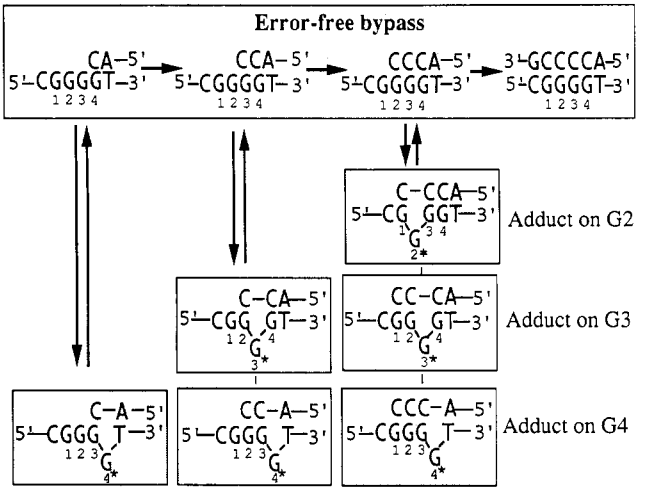

FIGURE 2: Model for slippage-mediated frameshift mutagenesis for a run of four guanine, $G_1G_2G_3G_4$. Depending on the position of the adduct (G_2^{AAF}, G_3^{AAF}, or G_4^{AAF}), the different slipped mutagenic intermediates (SMI) that can be formed are shown in grey boxes. The adduct at G_1 is not considered since it cannot form a slipped intermediate. The AAF adduct is designed by an asterisk (*). When AAF is bound to G_2, G_3, and G_4, one, two, and three SMI can be formed, respectively. Double arrows between the slipped and the nonslipped structures indicate the transient nature of the slipped intermediates.

adduct within the run and the nature of the base 3′ to the adduct are important parameters in determining the extent to which the lesion induces −1 frameshift mutations. These observations have strong inferences for the mechanisms by which induced frameshift mutagenesis can result from strand slippage during DNA synthesis within homopolymeric sequences.

The position of the adduct within the run is a key factor in the induction of −1 frameshift mutations. For a run of three guanines, 5′-$G_1G_2G_3$-3′, the mutation frequency induced when the adduct is located on G_3 is 20–200-fold higher than the frequency induced when the adduct is bound to G_1; adducts at G_2 generally give rise to intermediate mutation frequency values. This positional effect, previously shown for a single sequence context (Lambert et al., 1992b) and extended here to several different sequence contexts, suggests a mechanism for −1 frameshift mutagenesis that we have termed the incorporation slippage model (Lambert et al., 1992b). Several steps involved in this model can be distinguished (Figure 2):

(1) Correct insertion of cytosine occurs during DNA synthesis. Indeed, incorporation of cytosine opposite AAF lesions has been demonstrated *in vitro* (Rabkin & Strauss, 1984; Lindsley & Fuchs, 1994), and clearly occurs *in vivo* since the majority of translesion bypass in our experiments is error free. It is unlikely, however, that correct incorporation opposite dG-C(8)–AAF is a mechanistically simple event since *in vitro* DNA synthesis experiments utilizing single turnover kinetics have shown that such insertion is several orders of magnitude slower than incorporation opposite a normal guanine residue (Lindsley & Fuchs, 1994). Indeed, the AAF lesion exists primarily in a syn conformation (O'Handely et al., 1993) and as such is likely to represent a strong block to DNA synthesis (Belguise & Fuchs, 1994); correct insertion is thus likely to occur only when the adducted guanine residue transiently assumes the anti conformation and allows the three normal hydrogen-bonding positions of guanine to pair with cytosine.

(2) Even after correct insertion of cytosine, the presence of the AAF adduct at the replication fork impedes elongation of the primer/template termini (Lindsley & Fuchs, 1994) and provides increased opportunity for the formation of a replication intermediate where the cytosine incorporated across from the adduct becomes paired with the guanine immediately located 5′ from the adduct, forming the so-called slipped mutagenic intermediates (SMI) (Lambert et al., 1992). The resulting SMI should be viewed as a structure that can rapidly interchange with the non-slipped configuration (Figure 2). Elongation can occur from both the misaligned and the correctly aligned structure and, depending on which structure becomes elongated, will determine the mutation frequency.

(3) SMI formed as a consequence of strand slippage are stabilized by AAF adducts (Garcia et al., 1993). Within AAF-modified DNA duplexes, the stabilization of the SMI is characterized by (i) a significant increase in the melting temperature of the SMI duplex relative to either an AAF-modified homoduplex or an unmodified SMI duplex and (ii) a localization of the position of the extrahelical base to the position of the adduct. This is in contrast with the non-modified bulge where the extrahelical base is delocalized over the entire length of the repeat.

A prediction of the incorporation slippage mechanism is that adducts on G_1 should be essentially nonmutagenic. Our results show that, while the mutagenicity of G_1^{AAF} adducts is negligible in comparison to AAF adducts situated at the 3′-end of the homopolymeric runs, there is nevertheless a slight increase above the background frameshift frequency. This could be attributable to a minor mutational mechanism through which such lesions are processed. Alternatively, the oligonucleotides used to construct the G_1^{AAF} plasmids could have contained small quantities of oligonucleotides that were modified at the more mutagenic positions. Although normal exposures of the autoradiographs of the T4 polymerase assay used to assess oligonucleotide purity show that the oligonucleotides are essentially pure, overexposure of the autoradiographs do reveal faint bands corresponding to other sites of modification.

In initial formulations of incorporation slippage models, we considered only the slipped intermediate that could be formed by slippage of the cytosine residue situated opposite the adduct to a guanine residue located one nucleotide 5′ to the adduct on the template strand (Lambert et al., 1990; Schaaper et al., 1990). However, this simple model would predict G_2^{AAF} adducts to be about equally mutagenic as G_3^{AAF} and G_4^{AAF} adducts, a prediction clearly inconsistent with the experimental observation (Table 2, Figure 1). Indeed, the mutation frequency increased 5-fold between pUC-G_2T-G_2^{AAF} and pUC-G_3T-G_3^{AAF}. An additional 2-fold increase between pUC-G_3T-G_3^{AAF} and pUC-G_4-G_4^{AAF} is observed. We suggest that the difference in mutagenicity between adducts at G_2, G_3, and G_4 is related to the number and stability of the slipped mutagenic intermediates that can be formed. After successful incorporation of cytosine opposite the dG-C(8)–AAF adduct, the rate of elongation of the daughter strand is highly decreased not only for the incorporation of the next nucleotide but for several subsequent nucleotide addition steps (Lindsley & Fuchs, 1994). Similarly, *in vitro* studies have shown that elongation is hindered several bases 5′ from DNA duplexes containing mismatches (Reckman et al., 1983). As shown in Figure 2, when the adduct is present on G_3, two misaligned intermediates can form (i) one characterized by a single correct G:::C base pair at the terminus and (ii) another, potentially more stable intermediate, with two terminal G:::C base pairs. Only the former intermediate can form when the adduct is located at G_2. Similarly, when the adduct is at G_4, three

slipped intermediates can form (Figure 2). The individual contribution of the different potential intermediates to the observed mutation frequency is likely to be determined by both kinetic and thermodynamic factors. For example, the slipped mutagenic intermediate containing three normal G:: :C base pairs at its terminus (as drawn in the case of the pUC-G_4-G_4^{AAF} construction, Figure 2) may be quite stable but may actually not form frequently enough to contribute significantly to mutagenesis since at this stage of translesion bypass misalignment may no longer be a frequent event. Indeed, from a kinetic poinit of view, when two additional bases have already been inserted past the adduct, the next nucleotide insertion step may no longer be slow enough to provide the requisite time for slippage to occur. In addition, from a thermodynamic point of view, the two correct G:::C base pairs at the primer terminus should stabilize the homoduplex structure and prevent its slippage. If this hypothesis is correct, one would predict that the increase in mutation frequency triggered by the adduct located at the 3′-end of a run G_n, as seen here for $n = 2$, 3, and 4 will plateau off for longer runs.

The observation that an AAF adduct bound at position G_1 or G_2 within the sequence 5′-CCC$G_1G_2G_3$-3′ induces −1 frameshift mutations within the adjacent run of three cytosines located on the 5′-side of the guanine run provided a demonstration *in vivo* that an AAF adduct can influence the fidelity of replication 5′ from its location on the template strand (Lambert et al., 1992b). These mutations, referred to as semi-targeted, are likely to originate from a slippage event occurring within the run of cytosines after correct bypass of the entire run of contiguous guanine residues containinig the AAF adduct.

Within runs of three guaninie residues, the mutation frequency induced by the adduct at G_3 when the nucleotide flanking the 3′-side of the repeat was a purine was up to 5-fold higher than when the 3′-flanking base was a pyrimidine. A similar conclusion was reached following analysis of forward mutation spectra induced by 1-nitroso-8-nitropyrene, which also binds to the C(8) position of guanine (Lambert et al., 1992a). We suggest that these observations reflect the stability of SMI formed in the different DNA sequence contexts. Chemical probing the thermal denaturation studies of the structure of AAF containing SMI show that the bulge is localized to the position of the adduct and that the presence of the AAF adduct stabilizes the SMI relative to bulged heteroduplexes, which do not contain an adduct (Garcia et al., 1993). Although the geometrical arrangement of the adducted guanine within the heteroduplex is not yet known, one can hypothesize that the observed stabilization results from stacking interactions of the fluorene nucleus with the neighboring base plates. The observed modulation of the induced mutation frequency by the base located on the 3′-side of the adducted guanine may reflect differential stacking between the adduct and the base pair on the 3′-side. The results would indicate a better stacking interaction with purines as compared to pyrimidines. Although there is a clear effect of the base located immediately 3′ from the mutagenic lesion, bases further removed from the adduct site may still influence the mutation frequency. In this respect it is interesting, for example, that in pUC-G3A, pUC-G3C, and pUC-G3T there is a significant variation in the mutation frequency induced by the G_2^{AAF} adducts (Table 2) despite the fact that in each of these cases the G-AAF adduct is located in an identical sequence with respect to the immediate 3′ and 5′-flanking bases.

The importance of considering the DNA sequence context of the AAF adduct, both with respect to the nature of the flanking bases and the length of the 5′-flanking guanine sequence is well illustrated by considering the mutagenicity of single AAF adducts in pUC-G_4. The frequency of frameshift mutation induced by the AAF adducts increases in the order pUC-G_4-G_1^{AAF} < pUC-G_4-G_2^{AAF} < pUC-G_4-G_3^{AAF} consistent with the notion that the number and the stability of the potential SMI increase with the number of guanine residues 5′ to the adduct. However, the frequency of mutation induced by pUC-G_4-G_4^{AAF} is significantly less than that of pUC-G_4-G_3^{AAF}. In this case, the fact that the G_4^{AAF} adduct is 3′ flanked by a T rather than a G renders that adduct less mutagenic than the G_3^{AAF} adduct, despite the fact that the latter adduct contains one less 5′-flanking guanine residue.

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