Volume 16 Number 16 1988 Nucleic Acids Research

^{60}Co γ-rays induce predominantly C/G to G/C transversions in double-stranded M13 DNA

Barbara Hoebee[1], Jaap Brouwer[2], Pieter van de Putte[2], Henk Loman[1] and Jan Retèl[1,3]

[1]Department of Biophysics, Physics Laboratory of the Free University, De Boelelaan 1081, 1081 HV Amsterdam, [2]Laboratory of Molecular Genetics, State University of Leiden, 2333 AL Leiden and [3]Department of Oncology, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Received April 13, 1988; Revised June 21, 1988; Accepted July 20, 1988

ABSTRACT

Upon irradiation with gamma rays of an oxygenated aqueous solution of double-stranded M13 DNA, a very specific mutation spectrum was found with respect to both the type and the positions in the DNA sequence. Of the 23 mutations, which were sequenced, 16 represent a C/G to G/C transversion. A C/G to T/A transition was found once and a G/C to T/A transversion twice. The remaining 4 mutations are frameshifts, 2 are identical and formed by the insertion of a G/C basepair; the other 2 mutations are due to a duplication of 10 basepairs situated at different positions but with a remarkable homology in base sequence. Fourteen mutations, including the 2 duplications are found in the neighbourhood of a TGCT/ACGA sequence.

INTRODUCTION

When living cells are exposed to ionizing radiation such as gamma-rays, a broad variety of biological effects can be observed. The cells can loose their capacity to divide or to proliferate or are mutated or transformed into tumour cells. These effects can be largely explained by the fact that the cellular DNA is damaged.

A wide range of different types of radiation-induced DNA damages has been identified, viz. base damages, single-strand (ss) breaks, apurinic or apyrimidinic (AP) sites, alkali-labile sites, double-strand (ds) breaks and cross-links within the DNA or between DNA and protein (1,2). These chemical modifications in the DNA can be partly ascribed to direct energy disposition in the DNA molecule (direct effect) and partly to reactive waterspecies and (secondary) organic radicals which can diffuse to the DNA and may react with it (indirect effect). Most cells have the capacity to recognize and repair damages in DNA, thereby preventing most of the lethal consequences of the irradiation.

Damaged DNA may lead to mutations as a result of specific enzymatic processing of DNA lesions, or to post-radiation replication. So far little is known about the types of radiation-induced DNA lesions, responsible for

the induction of mutations and the underlying mechanisms by which they arise, in particular in the case of ds DNA. To get deeper insight into radiation-induced mutagenesis we investigated the mutations, introduced by gamma-irradiation into ds DNA by sequence analysis under conditions where virtually only the indirect radiation effect plays a role.

We used ds M13mp10 DNA (3) with an in frame insert of 144 basepairs (bp) in the region of the lacZα gene (see figure 1). This insert has no influence on the function of the LacZα product. Therefore cells carrying a lacZΔM15 transformed with the phage DNA give rise to blue plaques on X-gal indicator plates. Mutations like the introduction of stopcodons and frameshifts in lacZ or in the 144 bp insert or disturbance of an essential lacZ promotor or operator signal give colourless plaques after transformation. These mutations in the 144 bp insert and in the lacZ promotor/operator can be analyzed by DNA sequencing using the commercial available 17-mer primer. The 144 bp insert as mutation target sequence has further the advantage that all kinds of changes in this sequence which do not disturb the reading frame of lacZ can be introduced without affecting the lacZα gene function. This gives the opportunity to study the influence of the surrounding sequence on mutation induction at a given site.

MATERIALS AND METHODS

DNA isolation

Ds DNA was isolated according to Maniatas et al. (4) using CsCl gradients. After ethanol precipitation the DNA was dialyzed against 10^{-3} M sodiumphosphate buffer (pH 7.3) and 10^{-3} M EDTA to remove doubly and triply charged cations from the DNA as much as possible. This was followed by dialysis of the DNA solution against phosphate buffer alone to remove the EDTA.

Irradiation conditions

Ds DNA (8 $\mu g.ml^{-1}$) dissolved in 10^{-3} M sodiumphosphate buffer (pH 7.3) containing 10^{-4} $MgCl_2$ was irradiated with ^{60}Co γ-rays at 0.5 $Gray.min^{-1}$. Before and during the irradiation oxygen was flushed through the solution. Under these conditions OH radicals are the main reacting species, inducing primary DNA damage, which may be modified by oxygen.

Transfection and plating

E.coli KMBL 5071 (F'(lacI^Q,ZΔM15,pro)/ara Δ(lac,pro),thi) was grown at 37°C in YT medium (5) to an OD_{660nm} of 0.25. The culture was cooled to 4°C and split up in portions of 1 ml. To each portion 125 µl 1 M $CaCl_2$ was

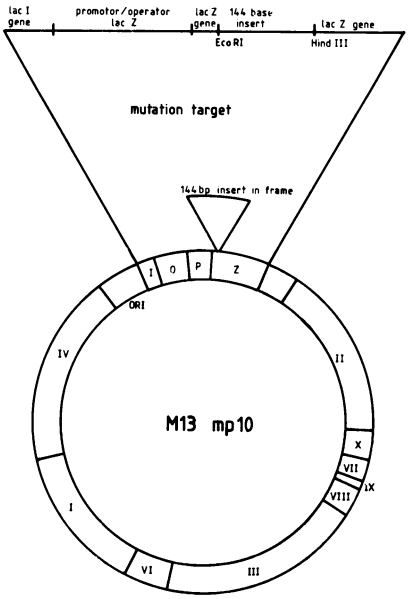


Figure 1: M13mp10 DNA with a 144 bp insert

added. After 5 min. 75 ng DNA per transformation mixture was incubated for another 30 min. on ice. The transformation mixtures were heat-shocked at 42 °C for 2-3 min. and subsequently cooled on ice for 15 min. The mixtures were poured out (total or in portions) on M9 plates (5) with 8 mg/l IPTG (isopropyl-β-D-thiagalactopyranoside, inducer of the lacZ operon) using topagar (9 g NaCl, 8 g agar per liter) containing 1.6 mg X-gal(5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, a lactose derivate that produces a blue colour after hydrolysis by β-galactosidase) and 0.2 ml E.coli KMBL 5071 with an OD_{660} of 1.0 per plate was added. The plates were incubated for 2 days at 37°C. Mutant phages (colourless plaques) were picked from the plate and diluted in 10 mM Tris, 1 mM EDTA, pH 7.5.

DNA sequencing

ss DNA from mutant M13 phages was isolated and sequenced using the chain termination method (6). A 17-base-long synthetic primer (Bio Labs, 5'-dGTTTTCCCAGTCACGAC-3') permitted sequencing from position 235 in the lacZ gene (see figure 2) to the 144 bp insert and the lacZ promotor/operator region.

Table 1: Induction of mutations in the lacZ gene, 144 bp insert and lacZ operator /promotor region in ds M13mp10 DNA.

Dose (Gray)	survival	number observed plaques	number mutants	mutation frequency a)	mutations determined by sequencing analysis
0	1.0	34.000	3	$0.9 \cdot 10^{-4}$	-
0.75	0.39	14.000	4	$2.9 \cdot 10^{-4}$	2
1.50	0.11	100.000	66	$6.6 \cdot 10^{-4}$	19
2.0 and 3.0					2 b)

a) Number of white plaques / number of blue plaques.
b) Results from a separate experiment.

RESULTS AND DISCUSSION

When oxygenated solutions of ds M13 DNA are irradiated with gamma-rays the mutation frequency increased with increasing doses. At 10% survival the mutation frequency exceeds the spontaneous level by a factor of approximately 7 (see table 1). A comparable value is found for irradiation in air of ss M13mp10 DNA in 10^{-2} M Tris-HCl/ 10^{-3} M EDTA (7) and M13 phages in broth in air (8).

Of the total number of 73 mutants scored in this experiment, 21 contained mutations in the 144 bp insert or in the lacZ promotor/operator region (see figure 2). Basepair (bp) substitutions were the predominant type of mutation: nineteen mutants contained a single bp substitution, whereas two contained a frameshift and two a duplication of 10 bp (table 2). All but one bp substitutions were transversions; all the frameshifts were insertions.

Unexpectedly the base substitutions are mostly C/G to G/C transversions (16 out of 19, table 2). This type of transversion is only rarely found after irradiation of ss M13mp10 DNA (7), M13 phages (8), lambda phages (9) or lambda prophages integrated into the genome of a lysogen (9). C/G to G/C transversions are also rarely induced as a result of other treatments known to cause mutations, viz. depurination of ss M13mp2 DNA under conditions of SOS repair (10), UV irradiation of M13mp2 phages (11), photoreaction of ds M13mp10 DNA treated with psoralens (12), reaction of aflatoxin derivative with ds M13mp8 (13) or N-acetoxy-N-2-acetylamino-fluorene reacted with plasmid pBR 322 DNA (14). Finally hardly any C/G to G/C transversions are found among the mutations formed spontaneously (15) or after SOS-repair induction (16) in the E.coli lacI gene.

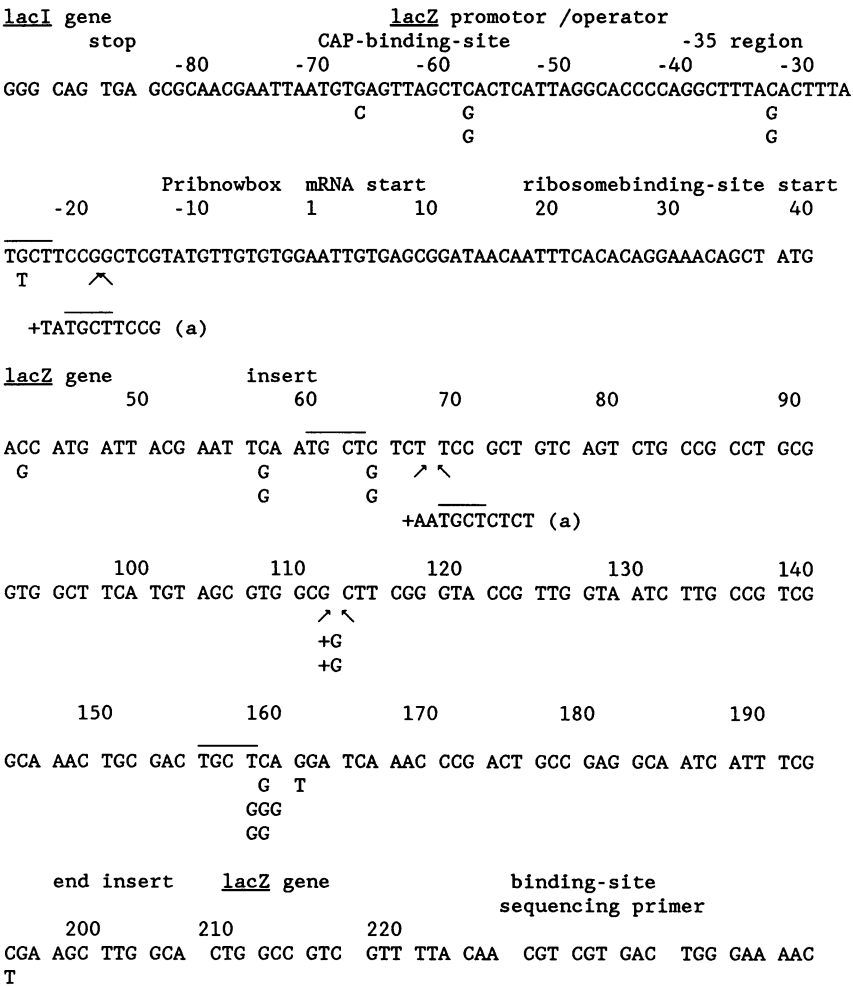


Figure 2: Mutation spectrum induced by γ-irradiation of ds M13mp10 DNA with a 144 bp in frame insert. (———: sequence homology TGCT, see text) (a) The position of the two 10 bp duplications is arbitrarily chosen.

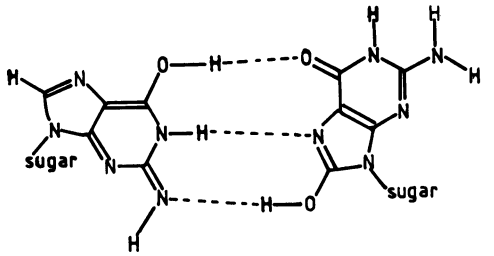
Therefore the conclusion seems justified, that the C/G to G/C transversions are likely due to a special kind of DNA lesion, that is introduced when ds DNA is irradiated under conditions that the OH radicals are the main reactive species. Such a lesion could be 8-hydroxyguanine (17), one of the radiation products of guanine.

Table 2: Mutations and their surrounding sequence. Only the sequence of the + strand is given. (Underlined: base which is substituted;—— : sequence homology TGCT, see text)

Base Substitutions	surrounding sequence	position (see fig.2)	number	
substitution				
C → G :	ACTGCTCAGGATC	+161	6	a)
	AATGCTCTCTTCC	+65	2	
	CGAATTCAATGCT	+58	2	
	GCTTTACACTTTA	-32	2	
	TTAGCTCACTCAT	-57	2	
	CTATGACCATGAT	+43	1	
G → C :	TAATGTGAGTTAG	-66	1	b)
			16	
C → T :	ATTTCGCGAAGCT	+196	1	
G → T :	TGCTCAGGATCAA	+163	1	
	CTTTATGCTTCCG	-24	1	
			19	
Frameshifts:				
insertion of a G :	GTGGCGCTTCGG +G	+113	2	
duplication of 10 bp:	ACGAATTCAATGCTCTCTTCCGCTGTC	+68	1	c)
	+AATGCTCTCT			d)
	TTACACTTTATGCTTCCGGCTCGTATG	-18	1	
	+TATGCTTCCG			d)
			23	

a) One of these mutations is induced by a dose of 0.75 Gray and one by a dose of 2 Gray.
b) This mutation is induced by a dose of 3 Gray.
c) This mutation is induced by a dose of 0.75 Gray.
d) The position of the duplication is arbitrarily chosen.

The syn form of this product can basepair with guanine in the enol/imino form in the same way as proposed by Topal and Fresco (18) for



guanine
enol/imino form

8-hydroxyguanine
syn form

Figure 3: Alternative base pairing between guanine and 8-hydroxyguanine

undamaged DNA (see figure 3). Even a third hydrogen bridge seems possible between the OH group of 8-hydroxyguanine and the imino group of the enol guanine. Stabilization of the basepairing by the third hydrogen bridge could explain why this basepairing is favoured by the hydroxyguanine compared to undamaged guanine. Since 8-hydroxyguanine is formed during radiation of DNA both in the presence of oxygen as well as in absence of oxygen (17) we would expect that C/G to G/C transversions are also induced under anoxic conditions.

Using chemically synthesized oligonucleotides with a 8-hydroxyguanine at a specific position as templates for in vitro DNA synthesis with E.coli polymerase I (Klenow fragment) it was shown by Kuchino et al. (19) that 8-hydroxyguanine is able to cause mutations. However no preference for the incorporation of guanine opposite to 8-hydroxyguanine was found. The 8-hydroxyguanine residue directed the incorporation of A, T, G and C with an almost equal frequency. Moreover when the modified guanine is placed between two pyrimidines, these neighbouring pyrimidines lead also to misreading. If our assumption that 8-hydroxyguanine is involved in the formation of C/G to G/C transversions is correct, the results we obtained suggest that the induction of mutations by this product under conditions of in vivo replication may be considerably more specific than under conditions of in vitro DNA synthesis as used by Kuchino et al. On the other hand, the possibility must be considered that the C/G to G/C transversions are the result of specific repair processing of 8 hydroxyguanine or some other radiation-induced DNA lesion.

The other three base pair substitutions, a C/G to T/A transition and two G/C to T/A transversions are more commonly found. The C/G to T/A transition is the predominant base substitution in the mutation spectrum of

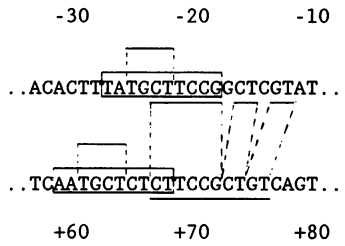


Figure 4: Sequence homology of the two duplications (□ duplicated sequence, duplication not shown; ___ sequence homology)

irradiated ss DNA in 10^{-2} M Tris/10^{-3} M EDTA (7). The induction of this mutation may be ascribed to a major cytosine radiation product, 5,6 - dihydroxy - 5,6 dihydrouracil (20), which can basepair with adenine leading to a C/G to T/A transition. The fact, that we have found a C/G to T/A transversion only once in ds DNA means, that uracil derivatives are introduced in relatively small amounts in ds DNA as compared to ss DNA or more likely that this type of damage can be repaired effectively in ds DNA.

Not all the observed mutations lead to the introduction of a stopcodon or a frameshift in the 144 bp insert or disturb an essential promotor/ operator signal in the lacZ promotor/operator region. Two neutral mutations are located at position +65 (see figure 2). This mutation leads to a change of a CTC codon in a CTG codon. However, both codons code for leucine. A third mutation located at position +43 leads to a triplet coding for serine (AGC) instead of threonine (ACC). However, the first aminoacids of LacZ are not essential for a functional β-galactosidase. The three mutants presumably contain a second mutation in the part of the lacZ gene that was not sequenced.

The frameshifts are insertions of a guanine at an identical position (position +113 in figure 2) leading to -GGC GGC- sequence. This insertion is hard to explain by the DNA 'slippage' model proposed by Streisinger et al. (21) because there are no streches of identical bases, repeated sequences or inverted repeats (22) in the surrounding sequences which can stabilize the intermediate necessary for DNA 'slippage'. We do not know yet what the basis is for this insertion.

The other two mutations are 10 bp duplications; one located in the 144 bp insert and one between the -30 region in the lacZ promoter and the Pribnowbox (see figure 2). Both duplications contain the sequence ATGCT/ TACGA and also in the surrounding sequences a remarkable homology is

present (see figure 4). Another intriguing aspect regarding the mutations, that we have identified so far, is their location. Most of the basepair substitutions (12 out of 19) are concentrated at three different positions, all in the neighbourhood of a TGCT/ACGA sequence (see figure 2). The same sequence is also found in the two 10 bp duplications, where the TGCT sequence is part of the homology. This suggests, that the TGCT/ACGA sequence is in some way involved in the induction of mutations by gamma-rays. Whether such a specific sequence can influence the formation of radiation lesions is not known; alternatively protein binding to TGCT/ACGA sites might interfere with repair of lesions in the neighbourhood of such sequences. The role of this sequence in radiation-induced mutagenesis will be one of the subjects of our further investigations.

ACKNOWLEDGEMENTS

We thank Robin Hoed for technical assistance and Jenny Hofman for preparing the typescript.

This investigation was supported by the J.A. Cohen Instituut, Institute for Radiopathology and Radiation-protection, Leiden, The Netherlands.

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