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Cluster of point mutations predetermined by a quasipalindromic nucleotide sequence in plasmid pBR322 DNA

R.I. Salganik, G.L. Dianov and O.A. Medvedev

Institute of Cytology and Genetics, Siberian Branch of the USSR Academy of Sciences, Novosibirsk 630090, USSR

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Development of a cluster of point mutations due to the correction of an imperfect hairpin in plasmid DNA was investigated. Plasmid pBR322 DNA containing opposite double-strand DNA lesions in the region of a quasipalindrome was constructed. For this aim plasmid DNA was cleaved at the *Bam*HI site, and cytosine residues of the sticky ends were modified by *O*-methylhydroxylamine. Modified linearized plasmid DNA was ligated and used for transformation of *E.coli* cells. Tetracycline-sensitive transformants were selected, and the mutants were characterized by restriction and sequencing analysis. One mutant contained a cluster of point mutations. The distribution of mutations was consistent with the cluster having arisen through correction of the imperfect hairpin formed by the quasipalindrome.

O-Methylhydroxyamine; Base-directed DNA modification; Quasipalindrome repair; Clustered mutation

1. INTRODUCTION

When palindromes or quasipalindromes occur in supercoiled DNA molecules or in single-stranded DNA regions, they form hairpin structures [1–3]. The hairpins formed by quasipalindromes are imperfect due to the violation of base-pairing. It was proposed by Ripley and Glickman [4] that hairpins arise during replication when the palindromes occur in the single-stranded regions of the replication forks. The authors supposed that when imperfect hairpins appear, they are corrected by the repair enzymes and thereby point mutations predetermined by the local hairpin mispairings are generated [5–6]. It was also demonstrated that spontaneous frameshift mutations of T4 rII gene arose due to correction of quasipalindrome structure [7].

If such events take place spontaneously and systematically, the quasipalindromes would be very rare in genomes. However, imperfect complementary DNA sequences persist in pro- and eukaryotic genomes in substantial amounts [8–10].

We supposed that point mutations predetermined by the primary DNA structure might appear due to the premutational lesions of quasipalindromes. Modification of nucleotides in one strand of palindromic DNA could give rise to gapped DNA, made by the excision repair enzymes and, as a consequence, to the formation of an imperfect hairpin. Modification of nucleotides in the other DNA strand of the palindromic region could lead to correction of the imperfect hairpin by the repair enzymes, and to generation of a cluster of predetermined point mutations (fig.1).

The aim of the work presented here was to check these considerations. Earlier we developed methods for selective chemical modification of particular nucleotide residues at predetermined sequence positions [11–13]. One of these methods was used here to produce premutational lesions in a preselected quasipalindromic regions of the plasmid pBR322 DNA.

In fact, targeted base-specific chemical modification of a quasipalindromic nucleotide sequence allowed us to identify a plasmid mutant with a cluster of base substitutions and base insertion within a premutationally modified quasipalindrome. The origin of nucleotide changes observed is readily explained as a result of the correction of non-complementary base pairs in the imperfect hairpin formed by the deliberately modified quasipalindrome.

2. MATERIALS AND METHODS

2.1. *Construction of modified plasmid DNA, transformation of* E. coli, *screening of mutants and DNA analysis procedures*

The construction of plasmid DNA molecules containing two opposite cytosine residues modified with *O*-methylhydroxylamine (OMHA) at the restriction sites have been described in detail earlier [12].

Briefly, plasmid pBR322 DNA was cleaved with restriction endonuclease *Bam*HI and after phenol extraction and ethanol precipitation was dissolved in 10 mM Tris-HCl/1 mM EDTA (pH 8.0). The cytosine residues in the sticky ends were modified by OMHA. For this purpose, an equal volume of *Bam*HI-cleaved DNA (0.1–1 μg/ml), 0.6 M sodium cacodilate (pH 5.0) and 3 M OMHA (pH 5.0) were mixed and incubated at 37°C for 2 h. OMHA was removed by passage through a Sephadex-50 column. The linear DNA (1–5 μg) was converted into circular form with 4–10 units of T4 DNA ligase in 200

Correspondence address: R.I. Salganik, Institute of Cytology and Genetics, Siberian Branch of the USSR Academy of Sciences, Novosibirsk-90, USSR

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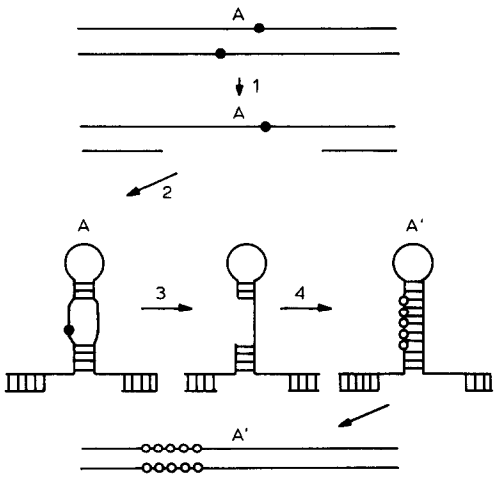

Fig.1. Production of templated mutation cluster after repair of *trans*-lesion at quasipalindromic sequence. A, original sequence; A', mutated sequence; 1, first damage excision; 2, hairpin formation; 3, excision of second damage; 4, hairpin templated repair; 5, gap filling. (●)-OMHA-modified cytosine residue.

μl of buffer containing 6.6 mM Tris-HCl (pH 7.6), 1 mM dithiotreitol, 0.5 mM ATP, and 6.6 mM $MgCl_2$ overnight at 0°C. Circular DNA molecules were purified by electrophoresis through agarose gel and used for transformation. Transformation of E. coli HB101 cells was carried out according to Mandel and Higa [14]. The transformants were selected on broth plates containing ampicillin (50 μg/ml) and then replated on ampicillin and tetracycline (20 μg/ml) plates to screen *amp*-resistant and *tet*-sensitive colonies.

Plasmid isolation, purification, and electrophoresis were performed by standard methods [15]. The *Bam*HI region of plasmids DNA was sequenced by the Maxam and Gilbert method [16].

2.2. *Enzymes and chemicals*

T4 DNA ligase and restriction nucleases *Bam*HI and *Bsp*RI were manufactured by the Novosibirsk Research Institute of Biologically Active Compounds. OMHA was a kind gift from Prof. E. Budowsky.

RESULTS

3.1. *Construction of the plasmid pBR322 with a damaged quasipalindrome sequence*

3.1.1. Selection of quasipalindrome

The nucleotide sequence of pBR322 *tet* gene was computer-searched for inverted repeats. The result (not shown) demonstrated that the 300–400 bp close to *Bam*HI restriction site contain the highest number of inverted repeats. The probability of occurrence of hairpin loops would be high in this region in the case of long stretches of single-stranded DNA appearing after excision of the lesioned DNA. We therefore chose this region for the premutational DNA modification.

3.1.2. Base-specific modification of the quasipalindrome

The scheme of construction of base-specific modified plasmid DNA is presented in fig.2. To produce a premutational lesion of the quasipalindrome, plasmid

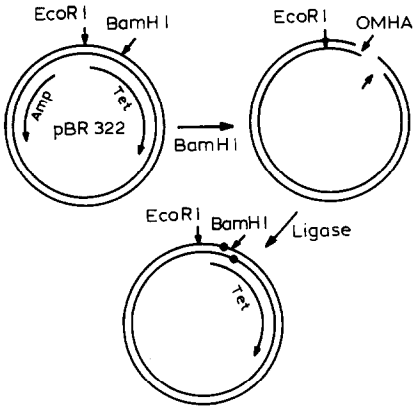

Fig.2. Construction of plasmid pBR322 containing *trans*-lesion of two cytosine residues at the *Bam*HI site.

DNA was first cleaved with the restriction endonuclease *Bam*HI. OMHA was then used to modify the cytosine residues of the sticky ends (5'-GATC-3') of the linearized plasmid DNA. The OMHA is known to interact with cytosine residues in the single-stranded producing *N*-methoxycytosine, but does not react with cytosine in the double-stranded DNA [17]. The OMHA-modified linear plasmid DNA was converted to covalently closed molecules by ligation [12]. It should be noted that modification with OMHA inhibited but did not restrict completely the ligation. Ligated plasmid DNA was used for transformation of *E. coli* strain HB101.

3.2. *Analysis of mutants.*

Sixty-nine bacterial colonies with plasmids containing a *tet* gene inactivated by mutation were identified among 380 transformants. In a control experiment, plasmid DNA was cleaved with *Bam*HI, incubated in a buffer without OMHA, and ligated. No transformants with mutations in the *tet* gene were detected among 5000 colonies analysed.

*Bsp*RI restriction endonuclease, which is isoschisomer of *Hae*III, was used for analysis of plasmid DNA. The numerous restriction sites for *Bsp*RI within the *tet* gene is an advantage, making deletions or insertions as small as 5–10 bp detectible by the gel-electrophoresis of the DNA digest.

A survey of the electrophoregrams demonstrated that insertions (11), large deletions (29), and small deletions (28) lead to inactivation of the *tet* gene in the majority of the mutants.

However, there was an exceptional mutant without rearrangements. Its *Bsp*RI digests gave electrophoretic patterns indistinguishable from those of the wild-type plasmid DNA. The DNA sequence around the *Bam*HI site (350–425 bp) was determined for this mutant. A comparison of the mutant with the original sequences demonstrated the presence of 5 clustered base substitu-

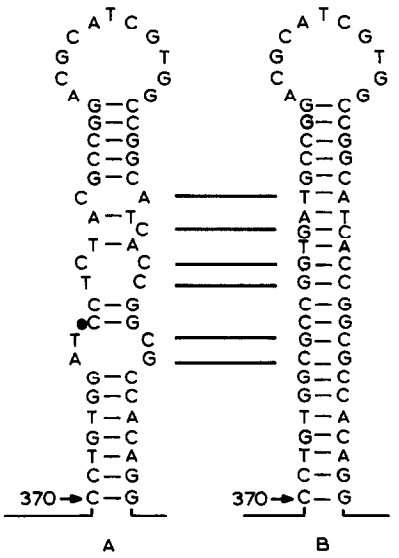

Fig.3. DNA secondary structure formed by quasipalindromic sequence of pBR322 (A) and palindromic sequence of pMC36 (B). (●)-OMHA-modified cytosine residue. Open boxes mark changed nucleotides.

tions and a base insertion (fig.3) As expected, all base changes induced were within the initially modified quasipalindrome sequence.

4. DISCUSSION

We have shown earlier that repair of mutagen-induced *trans*-lesions of DNA lead to the induction of DNA rearrangements, while OMHA modification of one cytosine of a *Bam*HI site results mainly in point mutations [12]. A molecular mechanism for the repair of a DNA *trans*-lesion, resulting in deletions of DNA sequences, was recently suggested [18–19].

The mutant described in this paper contains a cluster of 5 base substitutions and one base insertion. We assume that these base changes were due to the correction of original quasipalindrome mismatches and appearance of a perfect palindrome (fig.3B).

The consequence of mutational events is schematically represented in fig.1. It can be suggested that at first, DNA repair enzymes recognize and excise one of the OMHA-modified cytosine residues. The proposed scheme implies that excision is followed by the appearance of gap (fig.1, step 1). The nascent single-stranded DNA containing self-complementary nucleotide sequences acquires the capacity to form an imperfect hairpin (fig.1, step 2). The repair of the other damaged cytosine produces a gap in the imperfect hairpin, and filling of this gap results in the templated base substitutions observed in the mutant (fig.1, steps 3 and 4).

Just one mutant containing a cluster of point mutations, arising as a result of the repair of damage in a quasipalindrome sequence, was recovered among the 69 analysed. Although real, the molecular events giving rise to mutations of this kind seem to be infrequent. Other ways of repairing the damaged bases in the opposite sites of the DNA strands appear to be more common.

Spontaneous and induced mutants explainable in terms of a quasipalindrome correction scheme were described earlier. The correction of quasipalindromes during reproductive replication was suggested to account for their appearance [4,7].

The result presented here suggests that *trans*-lesions of DNA are at least one of the real pathways for quasipalindrome-directed mutagenesis.

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