DNA Repair 10 (2011) 356–362

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Endonuclease VII is a key component of the mismatch repair mechanism in bacteriophage T4

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ARTICLE INFO

Article history:
Received 2 November 2010
Received in revised form
14 December 2010
Accepted 16 December 2010
Available online 14 January 2011

Keywords:
Mismatch repair
Endonuclease VII
Genetic recombination
Bacteriophage T4
Double-strand break repair
Gene uvsX

ABSTRACT

In previous papers we described an extra recombination mechanism in T4 phage, which contributed to general recombination only when particular mutations were used as geneticmarkers (high recombination or HR markers), whereas it was practically inactive towards other rIIB mutations (low recombination or LR markers). This marker-dependent recombination pathway was identified as a repair of mismatches in recombination heteroduplexes. We suggested that the first step in this pathway, recognition and incision of the mismatch, is performed by endonuclease VII (endo VII) encoded by the T4 gene 49. In the present paper, we tested this hypothesis in vivo. We used an experimental model system that combines site-specific double-strand breaks with the famous advantages of the recombination analysis of bacteriophage T4 rII mutants. We compared recombination of homoallelic HR and LR markers in the S17 and S17 E727 background (amber mutations in the uvsX and in the uvsX and 49 genes, respectively). In S17-crosses, the HR and LR markers retain their respective high-recombination and low-recombination behavior. In S17 E727-crosses, however, the HR and LR markers show no difference in the recombination frequency and both behave as LR markers. We conclude that endo VII is the enzyme that recognizes mismatches in recombinational heteroduplexes and performs their incision. This role for endo VII was suggested previously from biochemical studies, but this is its first in vivo demonstration.

1. Introduction

In previous papers [1–4], we described the properties of a marker-dependent mechanism operating in T4 phage recombination and have identified it as the mismatch repair (MMR). The MMR contributes to recombination frequency only when particular mutations are used as genetic markers (high-recombination or HR markers), whereas in crosses with other mutations (low-recombination or LR markers), MMR makes little contribution if any. We studied a correlation between the type of mismatches formed by various genetic markers (rIIB mutations) and the efficiency of their repair [3]. We observed that single-base substitutions and single-base indel mutations are not repaired, whereas all the repaired mismatches have more extended non-complementary sequences. Among the repaired markers, those with two or more contiguous mismatched or non-matched bases are most effectively repaired. Mismatches with unequal strands are repaired asymmetrically, such that the bulge is preferentially removed. The nearest environment is important: the presence of several A:T pairs near the mismatch enhances its repairability drastically. Examples of the LR/HR pairs of rIIB mutations in the proximal part of the rIIB gene are shown in Fig. 1.

Drake [5] found an inverse correlation between the frequency of marker recovery from heteroduplex heterozygotes and its apparent dimensions. Some of the mutations have been characterized both by Drake and by us. A general congruence between the data is apparent: the mutations that we found to be nonrepairable exhibited a high frequency of recovery, whereas the readily repairable marker (FC1) was recovered less frequently. Deletions larger than 10–20 bp were not found in heteroduplexes. There is evidence for efficient repair of heteroduplexes formed by large deletions in T4 phage [6,7], most probably via removal of the single-stranded loop.

The observed absence of repair of single-base substitutions and the features of the most readily repairable mismatches suggest a rather simple principle of marker discrimination by the MMR mechanism. Apparently, local single-strandedness of DNA plays the main role in the mismatch recognition. In this case, a single-strand-specific endonuclease should be the key enzyme in this process. Among known T4 endonucleases, endonuclease VII (endo VII) encoded by gene 49 was suggested as a plausible nickase that recognizes and incises the mismatches [4].

Endo VII is a resolvase responsible for clearing branched replicative DNA prior to packaging. It has been well characterized in vitro. The purified enzyme cleaves specifically at secondary struc-

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doi:10.1016/j.dnarep.2010.12.006

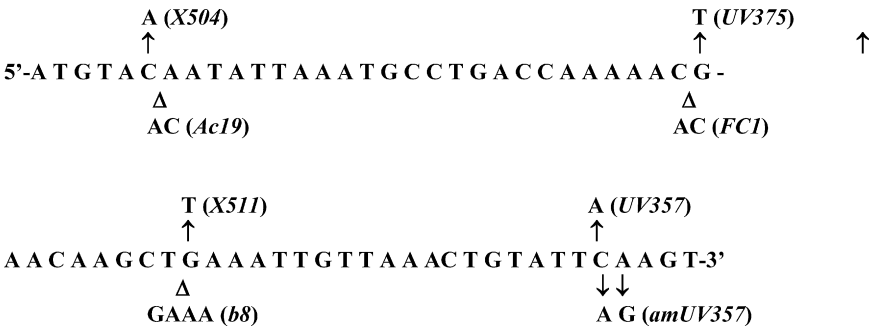

Fig. 1. Proximal part of the rIIB gene. The arrows show substitutions of bases at the mutation sites; the triangles indicate the points of base insertions. LR and HR markers are above and below the gene sequence, respectively.

tures in double-stranded DNA. These structures include branched DNAs, such as Holliday junction (HJ) [8,9], Y-structures [10], single-strand overhangs [11,12], base mispairings and heteroduplex loops [13–15], and bulky adducts [16]. The enzyme cleaves two nucleotides 3′ from the loop or the mismatch. Similar to its reactions with mismatches [14], the cleavage of heteroduplex loops by endo VII can also initiate restoration of perfect double-strandedness by T4 DNA polymerase and T4 DNA-ligase in vitro [15]. Loops of eight and 20 nucleotides were repaired efficiently. Generally, the enzyme introduces double-strand breaks by placing delayed staggered nicks in the 3′ flanking area of the impediment. The ability of endonuclease VII to cleave single-base mismatches in double-stranded oligonucleotides was demonstrated in vitro [17].

These properties of endo VII make it a suitable candidate for the role of the key enzyme in MMR operating in T4 recombination. It is important that it cuts a DNA strand on the 3′-side of the mismatch [14,15]. This is in perfect agreement with the fact that the next step in MMR, excision of the mismatched strand, is performed by the 3′–5′ exonuclease of the DNA polymerase [4]. Our observation that single-base mismatches are not repaired may just reflect the situation in vivo. For example, repair of single-base mismatches in vivo may proceed too slowly to make noticeable contribution to the recombinant frequency.

Although the role of endo VII in MMR looks plausible, this has not been confirmed in direct experiments. Mutations in the gene 49 are virtually lethal, which hampered studies of MMR in the absence of endo VII. Here we make use of the fact that mutations in the uvsX gene encoding the T4 recombinase protein suppress the 49⁻ lethality, so that the double uvsX⁻ 49⁻ strains multiply rather well under non-permissive conditions [18,19]. To study the mismatch repair in vivo, here we used an earlier developed model system of double-strand break (DSB)-induced recombination [20] for pair wise comparison of the HR and LR markers in the uvsX⁻ and uvsX⁻ 49⁻ background. We observed that the HR markers retain their high-recombination character in the uvsX⁻ background, whereas in the absence of endo VII (uvsX⁻ 49⁻ background), the HR markers do not differ from the corresponding homoallelic LR markers.

2. Materials and methods

2.1. Strains

The bacteriophage T4 rIIB markers used in this study are presented in Fig. 2. The rIIB mutants (LR and HR markers) were described earlier [1–3]; the uvsX (S17) and 49 (E727, deficient in endo VII) amber mutants were kindly supplied by Ebisuzaki. *Escherichia coli* amber-suppressor strain CR63 was used as a host for preparing phage stocks, for phage titration and for measuring total phage yield. *E. coli* BB strain not suppressing amber mutations and permissive for rII mutants was used as a host in phage crosses. *E. coli* CR63(λ_h) was used to titrate recombinants with the rII⁺ phenotype.

2.2. Phage crosses procedure

An aliquot of *E. coli* BB overnight culture was diluted 100-fold in L broth and aerated at 37 °C. At a cell concentration of 1×10^8 per ml, the suspension was cooled to 0 °C; the cells were pelleted by centrifugation and resuspended in cooled L broth at a density of 4×10^8 cells/ml (standard culture). A mixture of phage parents in a volume of 0.5 ml was added to 0.5 ml of the cooled BB suspension. The multiplicity of infection was five particles of each parent per cell. The infected cells were incubated for 10 min at 33 °C, diluted 1000-fold in pre-warmed L broth and incubated at the same temperature for another 80 min. Cell lysis was completed by adding 0.3 ml of chloroform to 5 ml of the diluted culture.

2.3. Determination of recombinant frequency and plating efficiency

The rII⁺ recombinant frequencies were calculated by dividing the titer determined on a λ-lysogenic host by the total lysate titer. The recombinants were measured after plating on *E. coli* CR63(λ_h) lawn. The resulting frequencies were corrected for plating efficiency (PE) that was determined as follows. Phage strains with the genotype of the expected recombinant (an equal mixture of segC⁺ and segCΔ variants) were used to infect the standard culture of *E. coli* BB with a total multiplicity of 10. The infected cells were incubated and processed the same way as those in the standard crosses. After proper dilutions, the lysates were plated on the *E. coli* CR63 and CR63(λ_h). The ratio of the titer on CR63 to that on CR63(λ) was used as a PE quotient to correct the titers of recombinants observed on the λ-lysogen: the apparent rII⁺ recombinant titers were multiplied by the PE quotient. The values of PE quotients were within 1 ± 0.04.

3. Results

To study the effect of endo VII deficiency on MMR in T4, we used a model system [20] developed for the studies of double-strand break (DSB) repair and based on the ets1 segCΔ strain of bacteriophage T4. A 66-bp fragment of phage T2L containing the cleavage site for SegC endonuclease (ets1) was inserted into the proximal

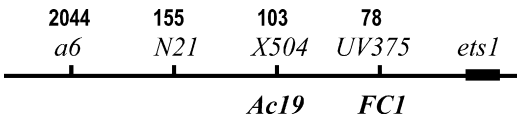

Fig. 2. The map of rII mutations used in this work. The numbers above the markers designate their distance from the ets1 in bp.