DNA Repair 10 (2011) 16–23

Contents lists available at ScienceDirect

DNA Repair

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

On the mutagenicity of homologous recombination and double-strand break repair in bacteriophage

Victor P. Shcherbakov*, Lidiya Plugina, Tamara Shcherbakova, Svetlana Sizova, Elena Kudryashova

Institute of Problems of Chemical Physics RAS, Chernogolovka, Moscow Region, 142432, Russia

ARTICLE INFO

Article history:
Received 2 July 2010
Received in revised form
15 September 2010
Accepted 15 September 2010
Available online 15 October 2010

Key words:
Genetic recombination
Bacteriophage T4
Double-strand break repair
Fidelity
DNA replication
Gene *uvsX*
46/47 protein

ABSTRACT

The double-strand break (DSB) repair via homologous recombination is generally construed as a high-fidelity process. However, some molecular genetic observations show that the recombination and the recombinational DSB repair may be mutagenic and even highly mutagenic. Here we developed an effective and precise method for studying the fidelity of DSB repair *in vivo* by combining DSBs produced site-specifically by the SegC endonuclease with the famous advantages of the recombination analysis of bacteriophage T4 *rII* mutants. The method is based on the comparison of the rate of reversion of *rII* mutation in the presence and in the absence of a DSB repair event initiated in the proximity of the mutation. We observed that DSB repair may moderately (up to 6-fold) increase the apparent reversion frequency, the effect of being dependent on the mutation structure. We also studied the effect of the T4 recombinase deficiency (amber mutation in the *uvsX* gene) on the fidelity of DSB repair. We observed that DSBs are still repaired via homologous recombination in the *uvsX* mutants, and the apparent fidelity of this repair is higher than that seen in the wild-type background. The mutator effect of the DSB repair may look unexpected given that most of the normal DNA synthesis in bacteriophage T4 is performed via a recombination-dependent replication (RDR) pathway, which is thought to be indistinguishable from DSB repair. There are three possible explanations for the observed mutagenicity of DSB repair: (1) the origin-dependent (early) DNA replication may be more accurate than the RDR; (2) the step of replication initiation may be more mutagenic than the process of elongation; and (3) the apparent mutagenicity may just reflect some non-randomness in the pool of replicating DNA, *i.e.*, preferential replication of the sequences already involved in replication. We discuss the DSB repair pathway in the absence of UvsX recombinase.

1. Introduction

Double-strand break (DSB) repair is an extensive and quickly growing field of exploration, and it has many aspects. This is determined by the special position that DSBs occupy in the basal metabolism of cells and organisms. DSB is the most dangerous damage in the cell. Appearance of a DSB serves as a primary signal for the activation of a complex cascade of events leading to repair of the damage or, in case of failure to repair, to apoptosis or cancer [1]. On the other hand, DSBs and their repair are positively involved in some important molecular genetic processes such as mating type switching in yeast, antibody diversity generation, and meiotic recombination. At least three different ways of DSB repair are known (see [2] for a comprehensive review). Two of them, homologous recombination repair (HRR) and single-strand annealing (SSA), depend on large regions of sequence homology, whereas the third one, nonhomologous end joining (NHEJ), does not require extensive homology [3]. HRR, which is shown to be the major DSB repair pathway in mammals [4,5], is considered to be an error-free pathway leading to accurate restoration of the initial sequence at the DSB. SSA must lead to a deletion of some segment of the sequence. NHEJ operates via direct linking of the ends of broken chromosome and is considered to be a mutagenic repair pathway.

The attempts to estimate the real fidelity of DSB repair gave unexpected results. On one hand, there probably exist highly precise variants of NHEJ [6]. On the other hand, HRR may be accompanied by a very high mutagenesis (see [7] for the review). The very opportune experimental model for studying fidelity of DSB repair was created in *Saccharomyces cerevisiae* [8]. In this model, site-specific DSBs are introduced into the genome by the HO endonuclease. Expression of the *HO* gene from a galactose-inducible promoter allowed efficient DNA cleavage at a single site in a large population of cells. The efficiency of recombination is estimated by measuring of the frequency of *His*$^+$ prototrophs that

* Corresponding author. Tel.: +7 496 522 3721/926 810 1202.
E-mail address: svp@icp.ac.ru (V.P. Shcherbakov).

1568-7864/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2010.09.006

resulted from recombination between *his* heteroalleles on one side of the DSB; measurement of the reversion frequency of *trp1* alleles located on the other side of DSB provided an estimate of the fidelity of DNA synthesis associated with the DSB repair. Reversion of *trp1* was found to be up to 1000-fold higher among the recombinants than in cultures that had not been induced to express HO endonuclease [8,9]. This low fidelity of DNA synthesis during DSB repair was explained by the involvement of the translesion synthesis (TLS) polymerase ζ, which is devoid of 3′–5′ exonuclease activity and also has low nucleotide selectivity, in the recombination induced by the DSB [7]. One more TLS polymerase, human polη, was shown to perform DNA synthesis in a D-loop recombination intermediate, in which the invading strand serves as a primer [10]. In addition, polη was shown to participate in homologous recombination that mediates immunoglobulin gene diversification [11,12]. Expression of TLS polymerases, including polη, is elevated during meiosis in yeast and in mammalian germ-line tissues [13,14] suggesting their possible involvement in DSB repair during meiotic recombination. This is in line with the old observations in *S. cerevisiae* that the mutation rate during meiosis is higher than the spontaneous mutation rate in mitotic cells [15,16]. More recent results of the genome scale statistical analysis of the relationship between recombination, intraspecies nucleotide diversity and interspecies divergence are interpreted as evidence for the mutagenicity of recombination [17–22]. It is reasonable to think that it is not recombination *per se* that is mutagenic, but rather the repair of DSBs serving as points of initiation of meiotic recombination [23]. All these observations deserve most intent attention and comprehension. A crucial role of recombination in reducing the mutational load and maintaining genetic stability was substantiated in many works [24–29], so the mutagenicity of DSB repair may look puzzling.

In the present work, we used the experimental model system that combines site-specific DSBs with the advantages of the recombination analysis of *rII* mutants of phage T4 [30]. The system is based on the T4 *ets1 segC*Δ strain (see Fig. 1). The *ets1*, a 66-bp fragment of phage T2L containing the cleavage site for SegC endonuclease, was inserted into the proximal part of the *rIIB* gene in a T4 strain carrying a deletion in the *segC* gene. In crosses of the *ets1 segC*Δ phage with a *segC*$^+$ partner, SegC endonuclease makes a DSB in the middle of *ets1* insertion, thus promoting recombination on both sides of *ets1*. This focused recombination was comprehensively studied in a series of crosses of *ets1* against other *rIIB* and *rIIA* mutants separated from *ets1* by 12–2040 bp. The frequency/distance relationship observed was in good agreement with the splice/patch-coupling (SPC) model. The SPC pathway belongs to a class of DSBR models [31–33]. In Fig. 1, a variant of SPC is depicted in which both ends of the broken chromosome invade the same unbroken chromosome in a coordinated manner [34]. The progeny chromosomes are recombinant: they contain sequences originating from the broken (empty lines) and unbroken (filled lines) parents.

The *rII* region of T4, in which the processes of homologous recombination and DNA replication are initiated, is ideal for monitoring these processes *in vivo*. Our general approach here is similar to that used by Strathern et al. [8]. We compared the reversion frequencies of several *rII* mutations in the presence and in the absence of the nearby DSB. It is most probably that in bacteriophage T4 the same DNA polymerase operates in normal DNA replication and in DSB repair. Moreover, it is generally accepted that most of the phage DNA is replicated via so called recombination-depended replication (RDR), which is identical to the proposed model of DSB repair, including a single-stranded DNA end invasion with the formation of a D-loop [35,36]. Nevertheless, we observed moderate but statistically significant mutator effects of DSB repair both in the wild-type T4 strains and in the *uvsX* mutant.

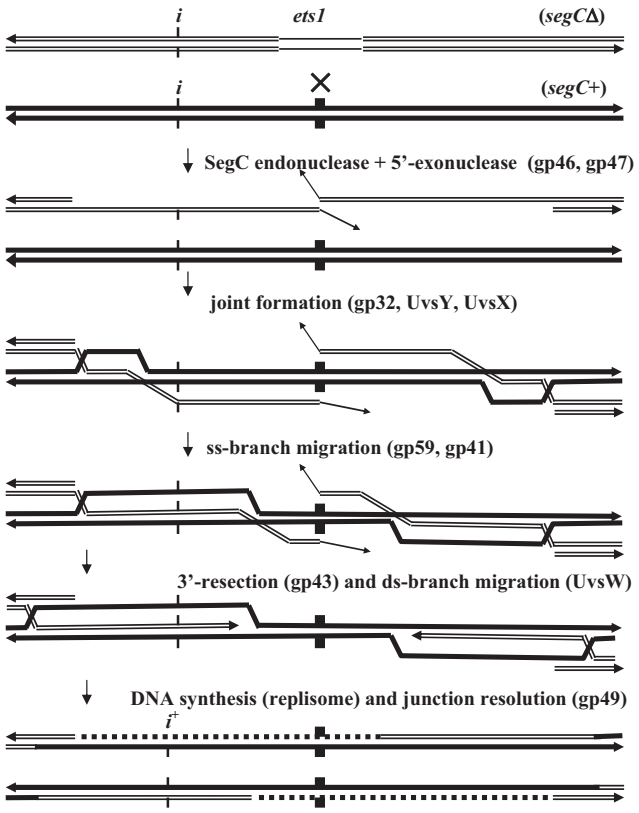

Fig. 1. The scheme illustrates interaction of two parental DNAs as if in cross *ets1 i segC*Δ × *i* [30]. The DNA molecule bearing SegC cleavage site (empty lines) is cleaved in the middle of the *ets1* insertion by T4 SegC endonuclease. 5′-Strands are resected to produce the extended recombinogenic 3′-single-stranded ends. Exonucleases involved might be gp46/47 or RNase H. The half-molecules interact with the unbroken molecule (solid lines), also bearing *i*-marker, via single-strand exchange to produce the D-loop structure and a protruding single-stranded "whisker". This step, the search for homology, is promoted by UvsX protein in collaboration with accessory protein UvsY and helix destabilizing protein gp32. The subsequent single-stranded branch migration is facilitated by the gene 41 protein, a DNA helicase bound to the displaced DNA strand by the gene *59* protein. The protruding single-stranded 3′-end (whisker) is removed by 3′ → 5′ exonuclease of T4 DNA polymerase (43Exo), the step followed by initiation of DNA replication (the loading of a whole replication machine is supposed); the formation of Holliday junctions and double-strand branch migration is catalyzed by UvsW protein. The Holliday junctions are resolved by endonuclease VII. The position corresponding to the site where the fragment *ets1* has been inserted (*ets*$^+$ allele) is marked in all the structures with the bar. Here, a variant of SPC is depicted in which both ends of the broken chromosome invade the same unbroken chromosome in a coordinated manner. In case of sequential action of two ends of the broken chromosome, replication of the phage chromosome is induced (RDR or BIR). Errors during the DNA synthesis may result in reversion of *i*-allele to the *rII*$^+$ state as is shown here.

2. Materials and methods

2.1. Strains

Bacteriophage T4 *rIIB* mutations, reversion of which to *rII*$^+$ (or *rII amber*) phenotype was studied, are presented in Fig. 2. Their origin was described in [37]. The *rIIB* mutant strains were either *segC*$^+$ or *segC*Δ, *i.e.*, producing or not producing endonuclease SegB. The strain with the amber mutation *S17* in the *uvsX* gene was a gift from K. Ebisusaki. *Escherichia coli* BB strain permissive for all *rII* mutants was used as a host in crosses. Amber-suppressing *E. coli* strain CR63 was used for preparing phage stocks. *E. coli* B and its amber-suppressing variant B*sup1* were used to distinguish *r* and *r*$^+$ plaque morphology; *E. coli* CR63(λh) non-permissive for *rII* mutants except those of amber-type was used to determine *rII*$^+$ revertant frequency.