DNA Repair 21 (2014) 120–130

Contents lists available at ScienceDirect

DNA Repair

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

Double-strand break repair and genetic recombination in topoisomerase and primase mutants of bacteriophage T4

Victor P. Shcherbakov*, Elena Kudryashova

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

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 13 April 2014
Accepted 17 April 2014
Available online 5 May 2014

Keywords:
Bacteriophage T4
Double-strand break repair
RDR
Recombinational analysis
Genetic recombination
Gene 39
Gene 61
Topoisomerase II
Primase

ABSTRACT

The effects of primase and topoisomerase II deficiency on the double-strand break (DSB) repair and genetic recombination in bacteriophage T4 were studied in vivo using focused recombination. Site-specific DSBs were induced by SegC endonuclease in the rIIB gene of one of the parents. The frequency/distance relationship was determined in crosses of the wild-type phage, topoisomerase II mutant amN116 (gene 39), and primase mutant E219 (gene 61). Ordinary two-factor ($i \times j$) and three-factor (i $k \times j$) crosses between point rII mutations were also performed. These data provide information about the frequency and distance distribution of the single-exchange (splice) and double-exchange (patch) events. In two-factor crosses ets1 $\times$ i, the topoisomerase and primase mutants had similar recombinant frequencies in crosses at ets1–i distances longer than 1000 bp, comprising about 80% of the corresponding wild-type values. They, however, differ remarkably in crosses at shorter distances. In the primase mutant, the recombinant frequencies are similar to those in the wild-type crosses at distances less than 100 bp, being a bit diminished at longer distances. In two-factor crosses ets1 $\times$ i of the topoisomerase mutant, the recombinant frequencies were reduced ten-fold at the shortest distances. In three-factor crosses a6 ets1 $\times$ i, where we measure patch-related recombination, the primase mutant was quite proficient across the entire range of distances. The topoisomerase mutant crosses demonstrated virtually complete absence of rII$^+$ recombinants at distances up to 33 bp, with the frequencies increasing steadily at longer distances. The data were interpreted as follows. The primase mutant is fully recombination-proficient. An obvious difference from the wild-type state is some shortage of EndoVII function leading to prolonged existence of HJs and thus stretched out ds-branch migration. This is also true for the topoisomerase mutant. However, the latter is deficient in the ss-branch migration step of the DSB repair pathway and partially deficient in HJ initiation. In apparent contradiction to their effects on the DSB-induced site-specific recombination, the topoisomerase and primase mutants demonstrated about 3–8-fold increase in the recombinant frequencies in the ordinary crosses, with the recombination running exclusively via patches. This implies that most of the spontaneous recombination events are not initiated by dsDNA ends in these mutants.

1. Introduction

Double-strand breaks (DSBs) are the most dangerous lesions in DNA. Their efficient repair is crucial for maintaining the genome integrity [1–3]. This notwithstanding, DSBs are positively involved in a great variety of cell metabolic pathways including mitotic and meiotic recombination in eukaryotes, genetic recombination in prokaryotes and viruses, mating-type switching in yeast [4–6], repair of collapsed replication forks, and regulation of the cell cycle [7–9], immunoglobulin gene rearrangement [10] and intron homing [11]. In bacteriophage T4, the chromosome ends, similar to those generated by DSBs, are used to initiate recombination-dependent genome replication (RDR) [12]. This process under the name of break-induced replication (BIR) is now recognized as a fundamentally important mechanism to repair double-strand chromosome breaks in eukaryotes [13].

The T4 phage, a classical object of molecular genetics, is one of the most suitable model systems to study the interconnections of genetic recombination with the other fundamental genetic processes. There are two ways of DNA replication in phage T4: origin-dependent replication (ODR) and RDR [12,14]. ODR operates early in the infection. It is soon halted, and further replication is initiated from the recombinational intermediates. Most of the phage progeny DNA is produced via this recombination-driven pathway.

* Corresponding author. Tel.: +7 496 522 3721; fax: +7 926 810 1202.
E-mail addresses: svp@icp.ac.ru, shcherbakovvp@mail.ru (V.P. Shcherbakov).

http://dx.doi.org/10.1016/j.dnarep.2014.04.009
1568-7864/

Consequently, T4 experiences an extremely high level of genetic exchanges that enables one to register recombination exchanges between two contiguous nucleotides.

The DSB repair in T4 is similar to the meiotic recombination in eukaryotes, which is in fact a DSB repair process [15]. At the same time, it is RDR, the major way of phage DNA replication [16]. The close interconnection between repair, recombination and replication is not unique to T4. The same is typical of systems implicated in the maintenance of genomic stability in eukaryotes [1–5]. Pro tem, bacteriophage T4 remains one of the best-developed and convenient systems for analyzing the details of these vitally important mechanisms. Many T4 proteins and the relevant molecular transactions are conserved in evolution, so the data obtained in T4 help better understand similar processes in prokaryotic and eukaryotic cells.

The bacteriophage T4 genes 39, 52 and 60 encode subunits of type II DNA topoisomerase. This enzyme interconverts topological DNA isomers by passing a segment of the duplex DNA through a transient double-strand break [17]. The phenotype of mutants defective in the topoisomerase was first classified as DNA-delay (DD) [18], i.e. defective in ODR. It was later shown [19] that DNA replication initiates at the normal time but at a reduced rate in these mutants. This synthesis is unidirectional (counterclockwise), i.e. the phage topoisomerase is dispensable for the counterclockwise replication but necessary for bidirectional replication [20]. The rate of DNA synthesis increases in the topoisomerase mutants after 10–15 min of replication. This late DNA synthesis is dependent on the phage-encoded recombination proteins [21]. The late synthesis is not quite normal, however. It produces ssDNA fragments, and the mutants are defective in concatemer formation [22]. Therefore, the T4 topoisomerase must be also involved (directly or indirectly) in the late DNA synthesis.

The specific molecular and metabolic roles of the T4 topoisomerase are not quite clear. In the plasmid replication assay, it was shown that in the absence of topoisomerase synthesis stops when most of the DNA strands are less than 1.5 kb [23]. It was suggested that the positive supercoils accumulating in the absence of topoisomerase inhibit the progression of the replication fork. Perhaps the superhelical tension leads to displacement of the newly replicated leading strand in the topoisomerase mutants. This may explain the production of ssDNA. These data (see also [24]) suggest that the T4 topoisomerase must execute relaxation of positive superhelical tension to facilitate the progression of replication fork.

The T4 enzyme needs ATP for its activity [25], which is surprising given that the DNA relaxation is a thermodynamically favorable reaction. As was observed by D. McCarthy [26], T4 topoisomerase and primase mutants cannot initiate DNA synthesis in the host gyrase mutant. Models have been proposed in which the DNA-delay gene products either form an autonomous phage gyrase or interact with the host gyrase and adapt it for proper initiation of the phage DNA replication. This suggests at least a dual function for the T4 topoisomerase in DNA replication.

One more aspect, which seems to be relevant to the problem, is interaction between recombination and transcription occurring in the same DNA segment. The advancing RNA polymerase complex may generate positive supercoils in the DNA ahead of it and negative supercoils behind it [27]. The removal of the supercoils generated by transcription may be an important function of the DNA topoisomerases in prokaryotes and eukaryotes [27,28].

Shcherbakov et al. [29] observed a "left–right" bias in the recombinogenic action of two DNA ends of the broken chromosome, which was particularly prominent in the 59 (41-helicase loader) and 39 (topoisomerase) mutants. They inferred that the cause, underlying the bias, might be interference between strand exchange and transcription. Topoisomerase and helicase functions are necessary to turn the process in favor of strand exchange. The left/right bias in the recombinant frequencies was explained as follows. Both ends of the broken chromosome undergo 5′-resection that results in ss-DNA strands. The left single strand is transferred to the transcribed strand while the right single strand is transferred to the non-transcribed strand of the intact chromosome. Therefore, topoisomerase was necessary to relax the positive supercoiling on the left and was dispensable on the right of DSB.

The phenotypes of the topoisomerase and primase mutants are similar in several respects including the enhanced level of genetic recombination [21,30,31] and the inability to grow at 25 °C [32]. This similarity may look puzzling given rather different catalytic activity of the enzymes. In vitro, the T4 primase is required only to synthesize RNA primers for lagging DNA strand [33]. However, the primase mutant shows very little ODR in vivo as if the leading strand synthesis were also jammed [34]. Kreuzer and Brister [14] proposed that either leading strand synthesis at some T4 origins is primed by the primase, or normal T4 replication requires obligatory coupling of leading strand synthesis with primase-dependent lagging strand synthesis. The necessity for the coupling could help to explain partially the similarity of the primase and topoisomerase mutant phenotypes: the need for gyrase function and the necessity to couple the leading and lagging strand synthesis must both result in inability of the topoisomerase and primase mutants, respectively, to perform ODR. The enhanced level of recombination may reflect an extra production of recombinogenic ssDNA because of the superhelical tension in the topoisomerase mutant and the inability to replicate lagging strand in the primase mutant during the D-loop-initiated replication.

While ODR in the topoisomerase and primase mutants crucially depends on the gyrase and probably on some other host functions, the late replication (RDR) seems to be more autonomous, including a capability of uncoupled synthesis of the two DNA strands. When studying genetic recombination, we deal with the RDR products. Initiation via the D-loop needs single-stranded DNA ends that may first appear as abortive products of jammed ODR. The process initiated in this manner must produce a great surplus of recombinogenic ssDNA because of the inability to replicate the lagging strand in the primase mutant and the displacement of the leading strand (bubble migration) in the topoisomerase mutant. Finally, these multiple D-loops may provide sufficient quantity of mature phage DNA.

Here we used an experimental model system combining site-specific DSBs with the famous advantages of the recombination analysis of rII mutants [16] to study the impacts of the topoisomerase II and primase deficiency on DSB-induced genetic recombination. The classical recombination analysis based on ordinary standard crosses between point rII mutants was also used.

2. Materials and methods

2.1. Strains

The bacteriophage T4 rII markers used in this study were described earlier [16]. F.W. Stahl kindly supplied the topoisomerase amber mutant amN116 and the primase amber mutant E219. The *Escherichia coli* amber-suppressor strain CR63 was used as a host for preparing phage stocks, phage titration and measuring the total phage yield. *E. coli* BB strain not suppressing amber mutations and permissive for the rII mutants was used as a host in phage crosses. *E. coli* CR63(λ_h) was used to titrate recombinants with 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