INTRODUCTION

J. Weigle described W reactivation and W mutagenesis in his seminal paper in 1953 [1]. He found that the survival of λ phage inoculated to UV-irradiated bacteria increased, and a great number of mutations were recorded in the phage. It was the discovery of SOS-induced repair and mutagenesis. Presently, the terms W reactivation and W mutagenesis are applied to processes observed in bacteriophages and plasmids. In a more general sense, the processes are named with regard to bacterial and eukaryotic cells as well, i.e., SOS repair and SOS mutagenesis. These terms were coined by M. Radman [2].

The molecular base of the phenomenon is so-called “translesion synthesis.” The gap in DNA opposite a damaged nucleotide is filled by special error-prone DNA polymerase V (PolV) assembled with RecA protein [3, 4]. The umuC and umuD genes, which encode PolV subunits (UmuD2C), belong to the SOS regulon; therefore, their expression is controlled by RecA–LexA proteins [5]. The action of PolV, which replaces replicative DNA polymerase III and, as a rule, incorporates a noncomplementary nucleotide opposite the damaged one, unblocks replication. The results are the better survival of the organism and an elevated frequency of mutations.

The MucA and MucB proteins, the genes of which are present in pKM101 plasmid (incompatibility group N), are closely similar to UmuD and UmuC, respectively. They also increase the survival and mutagenesis rates in UV-irradiated phages [5, 6]. It has been found that the lysis of MucA protein (RecA*-induced cleavage of 24 N-terminal amino acids) occurs much more efficiently than the corresponding RecA*-induced lysis of UmuD, which gives rise to the active UmuD* species [7].

We compared the effects of UmuD2C and MucA2B on SOS repair in λ phage and plasmids. Damaged nucleotides, which block replication, were formed in the phage and plasmids by the light-induced reaction of furanocoumarin derivatives with DNA, i.e., 8-methoxypsoralen (8-MOP), which yields monoadducts and interstrand crosslinks, and the angular derivative angelicin, which forms only monoadducts [11, 12]. Earlier studies of λ phage treated with 8-MOP under UV light (wavelength >320 nm) and inoculated to UV-irradiated (254 nm) bacteria of wild-type E. coli strain K12, which possess an active excision repair system Uvr, indicated that W mutagenesis was determined mainly by diadducts (interstrand crosslinks), whereas 8-MOP monoadducts were successfully repaired by the error-free UvrABC system [13]. Here, we report our results on W reactivation and W mutagenesis in λ phage and plasmids hosted by an uvr– mutant E. coli strain with an excision repair deficiency. The effect of plasmid pKM101 mucAB* on the SOS repair of 8-MOP and angelicin monoadducts was investigated. It was found that the replication block

SOS Repair of 8-Methoxypsoralene Monoadducts in DNA of Lambda Bacteriophage and Plasmids Is Mediated by MucA2B, but Not UmuD2C (PolV) Polymerase

G. B. Zavil’gel’skii and V. Yu. Kotova
Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, 117545 Russia
e-mail: zavilgel@genetika.ru
Received June 6, 2013

Abstract—The light-induced action of 8-methoxypsoralen (8-MOP) on λ phage and plasmids yields monoadducts and interstrand crosslinks. The survival and clear plaque mutation frequency in the phage photosensitized with 8-MOP and irradiated with UV at wavelength >320 nm are increased when the wild-type host (Escherichia coli uvr+) is subjected to UV irradiation (wavelength = 254 nm) prior to phage inoculation. These phenomena are known as “W reactivation” and “W mutagenesis.” It is shown that 8-MOP monoadducts in λ DNA induce clear mutations in the phage inoculated to UV-irradiated excision repair mutants of E. coli only when the error-prone repair is performed by MucA2B, but not PolV (UmuD2C) polymerase. The efficiency of the SOS repair (W reactivation) of 8-MOP monoadducts in plasmid and λ phage DNA also only increases with the presence of pKM101 plasmid muc+ in E. coli uvr–.

DOI: 10.1134/S1022795413120144
caused by 8-MOP monoadducts could be overridden by MucA2B, but not by UmuD2C.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. Bacterial strains: *Escherichia coli* K12 AB1157 F*- thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara14 xyl-5 metL-1 tpx-33 rpsL31 supE44; AB1886 uvrA6, AB2480 uvrA6 recA13; other markers as in AB1157. The strains were kindly provided by Prof. Howard-Flanders, United States. *E. coli* K12 TK603 arg+ ilv uvrA6; TK610 arg+ ilv uvrA6 mucA36 other markers as in AB1157; GW514 = TK603 (pKM101 mucAB+), GW516 = TK610 (pKM101 mucAB+). The strains were kindly provided by Prof. Walker, United States. Phage *λ*11 was obtained from Prof. Devoret, France. Plasmid pBR322.

Growth media and conditions. LB medium: 1% tryptone, 0.5% yeast extract, 0.5% NaCl. For the upper and lower layers in Petri dishes, the medium was solidified with 1.8 and 0.7% agar. Bacteria were grown in LB with or without 100 μg/mL ampicillin with constant agitation at 30°C until the mid-log phase. Bacterial cells treated with Ca2+ ions were transformed as in [14].

Buffers and reagents. Tris buffer: 0.05 M Tris HCl, pH 7.5; TM buffer: 0.05 M Tris HCl, 0.01 M NaCl, 0.001 M MgSO4, pH 7.8; 8-MOP from Sigma. Angelicin was kindly provided by Prof. Rodighiero (Italy).

Irradiation of bacteria, phage, and plasmids. Solutions of DNA in Tris buffer and phage suspension in TM buffer with 40 μg/mL 8-MOP or 200 μg/mL angelicin were irradiated in Pyrex cells at 4°C. The source of light with a wavelength of >320 nm was a high-pressure mercury lamp SVD-120A with a light filter, UFS-6 or ZhS-4, placed 20 cm away from a sample. The lamp with ZhS-4 was used to produce light with wavelength >380 nm. Bacterial suspension in TM buffer was irradiated with UV (254 nm) produced by a BUV-15 low-pressure mercury lamp. Doses of UV irradiation were measured with a UFD-4 dosimeter.

W reactivation and W mutagenesis. The degrees of SOS repair in DNA of a plasmid or phage can be quantitated by comparing their survival rates in intact and UV-irradiated bacteria (W reactivation) [15–17]. The survival rates and frequencies of clear mutations in phage lambda were measured by phage inoculation to intact and UV-irradiated (254 nm) *E. coli* cells. Mutants were counted in phage *λ*11 and able to form two single-step mutations, i.e., clear and vir. Here, we recorded mutations with the clear phenotype after inoculation of the phage–bacterium mixture to a lawn of nonlysogenic bacteria; i.e., the integral clear + vir mutagenesis was evaluated. The spontaneous background of clear mutations in *λ*11 with various phage samples was within 5–8 × 10−4. For *λ*11 treated with photosensitizer + light (>320 nm), the frequency of clear mutations was determined with regard to surviving particles. The degree of W mutagenesis was determined as the ratio between the mutation frequency after phage inoculation to UV-irradiated cells and the frequency with unirradiated cells. The multiplicity of infection did not exceed 0.01. Bacteria were harvested in the log phase, washed twice in TM buffer, concentrated five times in the same buffer, and irradiated with various UV doses using a BUV-15 lamp (254 nm). The adsorption of the phage on intact and UV-irradiated cells was carried out in TM buffer at 37°C for 15 min. The resulting mixture was inoculated by the two-layer method with AB2480 as the indicator strain.

To measure W reactivation in pBR322 plasmid, bacteria were irradiated in two regimes, i.e., (1) cells were treated with Ca2+ ions prior to irradiation and (2) cells were first irradiated and then treated with Ca2+ to obtain competent cells. The results were identical in both cases [18]. The degree of W reactivation (α) for the phage and plasmid was calculated as follow:

$$\alpha = \frac{N_{ni} \times N_{uu}}{N_{mi} \times N_{iu}}$$

where $N_{mi}$ is the titer of unirradiated phage or plasmid in unirradiated bacteria, $N_{iu}$ is the titer of unirradiated phage or plasmid in UV-irradiated bacteria, $N_{mi}$ is the titer of irradiated phage or plasmid in unirradiated bacteria, and $N_{ii}$ is the titer of irradiated phage or plasmid in irradiated bacteria.

RESULTS

Figure 1 presents W reactivation and W mutagenesis curves for *λ*11 phage treated with 8-MOP or angelicin with irradiation (wavelength >320 nm) and inoculated to *E. coli* strain AB1886 uvrA6 irradiated with UV (254 nm). It appears from the curves that W reactivation and W mutagenesis occur only with angelicin as photosensitizer, but not with 8-MOP.

The influence of pKM101 mucAB+ on W reactivation and W mutagenesis in *λ*11 treated with 8-MOP + UV (>320 nm) is illustrated in Fig. 2. Neither W reactivation nor W mutagenesis are observed in *λ*11 grown on TK603 uvrA6 without pKM101; hence, UmuD2C polymerase, encoded by chromosomal *umuDC* genes, cannot override the replication block caused by 8-MOP monoadducts. The phage treated with 8-MOP + UV (>320 nm) shows W reactivation and W mutagenesis on cells harboring pKM101 mucAB+ (strain GW514).

Figure 3 illustrates W reactivation in pBR322 treated with 8-MOP + UV (>320 nm) in strains TK603 uvrA6 and TK610 uvrA6 mucC36 with or without pKM101. As in experiments with *λ*11, W reactivation only occurs with pKM101.

The degree of W reactivation of 8-MOP monoadducts in pBR322 (Fig. 3) is significantly greater than that in *λ*11 (Fig. 2). This fact is related to the different