ON THE MECHANISM OF POST-REPLICATION REPAIR IN *ESCHERICHIA COLI* CELLS: THE ROLE OF DNA POLYMERASE III

N. V. TOMILIN and M. P. SVETLOVA
Institute of Cytology of the Academy of Sciences, 190121, Leningrad, USSR

Received 14 March 1974
Revised version received 22 April 1974

1. Introduction

The newly synthesized DNA after UV-irradiation of *uvr*$^+$ or *uvr*$^-$ *E. coli* cells has a low single-stranded molecular weight equal to the average spacing between pyrimidine dimers in parental strands of DNA [1,2]. The decrease of DNA molecular weight in alkaline sucrose gradients is caused by the gaps (~ 1000 nucleotides long) in daughter strands, opposite to the pyrimidine dimers, arising at the replication fork [1,3]. In the course of subsequent incubation in growth medium these gaps are repaired. The finding of DNA strands with intermediate density in alkaline CsCl in UV-irradiated *E. coli uvrA6* cells allowed the authors to suggest that post-replication gaps are repaired by a recombination mechanism involving actual breakage and reunion of DNA molecules [4].

However, many facts do not accord with this hypothesis [5,6]. For example, *E. coli* AB 2487 *recA13* cells show no deficiency in post-replication repair after 15 erg/mm^2 of 254 nm UV-irradiation although the relative recombination frequency in these cells is about 10^{-4} compared to a value of 1 for *rec*$^+$ cells [7]; on the other hand, there is no breakage of parental DNA strands in UV-irradiated *uvrA6* or *uvrB5* cells at all [7,8]. It is possible therefore that UV-stimulated recombination between sister DNA duplexes, if it occurs, does not causally relate to the filling of gaps and the latter are repaired by de novo DNA synthesis [5].

In this paper data are presented indicating that inactivation at the non-permissive temperature (45°C0 of thermolabile DNA polymerase III in a double *polA1 polC26*^{ts} mutant of *E. coli* results in the failure to repair post-replication gaps after UV-irradiation.

2. Materials and methods

The strain *E. coli* BT1026 *polA1 polC26*^{ts} *(dnaE) endI*$^-$ *thy*$^-$ kindly supplied to us by Dr. Y. Hirota was used in this study. The cells were grown at 30°C in M9 medium supplemented with 2.5 mg/ml of casamino acids, 10 μg/ml of thymine and 20 μg/ml of L-arginine. After washing the suspension was irradiated in salt M9 a medium in a Petri dish at the density of about 5 X 10^8 cells/ml under a germicidal UV lamp BUV-60 (254 nm). The UV-light exposure dose rate (1.8 ergs/mm^2/sec) was measured using chemical dosimetry [9].

Immediately after irradiation the cells were pulse labeled for 10 min at 30°C in 1 ml of M9 with usual supplements (omitting thymine), with 50 μCi/ml of [^{3}H]thymidine (11 Ci/mmole) and 250 μg/ml of deoxyadenosine. Then the cells were centrifuged and incubated at 30° or 45°C for the desired time in M9 with usual supplements and 4 μg/ml of thymine. Control variants were stored in ice after pulse labeling and washing. Approximately 10^7 cells in 0.1 ml of salt M9 were placed on the 0.1 ml layer of 0.5% Sarkosyl–0.5 N NaOH on the top of the alkaline sucrose gradient and stored for 20 min in the dark. The lysates were centrifuged at 33 000 rpm at 20°C in the SKR–40 rotor of a UPR–8 ultracentrifuge (USSR). The gradients were fractionated from the meniscus under the pressure of chloroform deposited gradually on the

bottom of the centrifugal tube. The fractions were collected on filter paper discs, washed in 5% trichloroacetic acid, ethanol and dried. Radioactivity was measured in a toluene solution containing 0.1 g of POPOP and 4 g of PPO per liter in a Nuclear Chicago Mark II System.

We used an isokinetic sucrose gradient in 0.9 M NaCl–0.1 M NaOH–10^{-2} M EDTA with an initial concentrations of 5%. The parameters of the isokinetic gradient and $S^{\circ}_{20,w}$ for our rotor were calculated according to Noll [10] and molecular weights by the formula of Studier [11]. Calibration of the gradient with ^{3}H labelled λ DNA (17×10^{6} daltons) gave the correct molecular weight. Number-average molecular weight, from the distribution in the sucrose gradient, was calculated by the method of Rupp and Howard-Flanders [1].

3. Results

The sedimentation characteristics of the pulse-labeled DNA of unirradiated *E. coli* BT1026 *polA1* *polC*^{ts} are shown in fig. 1. The DNA synthesized during the 10 min pulse has a lowered molecular weight (A) but after an additional 10 min incubation in growth medium without radioactive precursor at 30°C or after 20 min at 45°C the normal molecular weight is restored (B). The appearance of pulse-labeled DNA in the *polA1* mutant in the low molecular weight fractions has been reported previously [12,13]. Our data indicate that the slow covalent ligation of nascent DNA fragments in discontinuous synthesis in *polA1* cells is not needed for DNA polymerase III action.

UV-irradiation (30 erg/mm^2) prior to the incubation with [^{3}H]thymidine induces the appearance of single-stranded breaks in daughter strands caused by unexcised dimers in template DNA (fig. 2A) in addition to the discontinuities produced by *polA1* mutation.

Slow sedimenting DNA in UV-irradiated cells converts to the fast sedimenting form only after 60 min of additional incubation at 30°C (fig. 2A). This result supports the conclusion [14] that *polA1* mutation alone not reduces the efficiency of post-replication repair.

The incubation of UV-irradiated (30 ergs/mm^2) BT1026 cells at 45°C does not result in the repair of gaps in daughter strands caused by dimers: after 60, 120 or 180 min at 45°C the label appears in the slow sedimenting molecules (fig. 2B) indicating the involvement of DNA polymerase III in post-replication repair. The number average molecular weight (M_n) calculated for the 60 min variant is approximately 18×10^{6}. This value is in good accord with the value of average

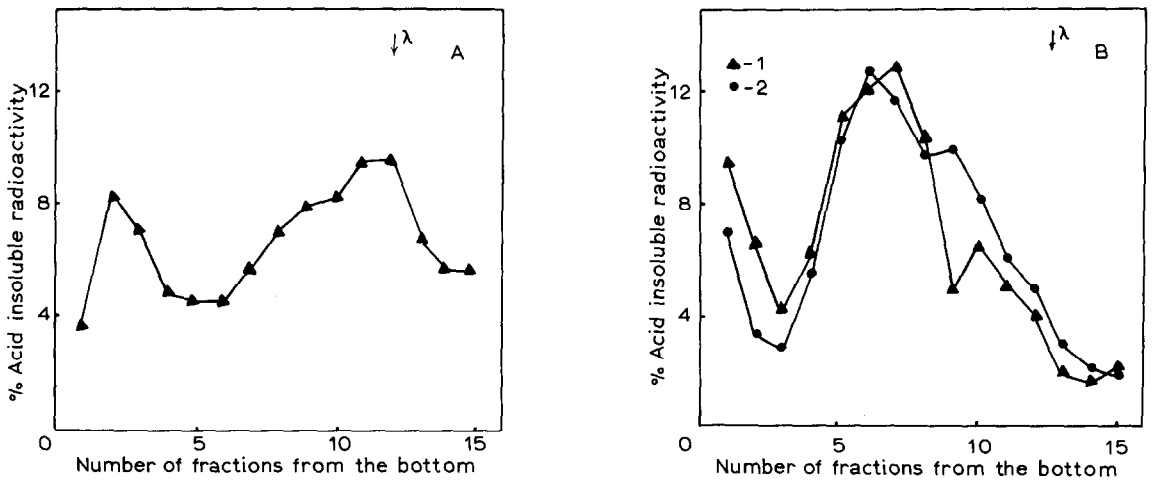

Fig. 1. The sedimentation pattern of pulse-labeled DNA in unirradiated *E. coli.* BT1026 cells. (A) analysed without additional incubation after pulse label. Centrifugation time was 85 min, (B) analysed after 10 min-incubation at 30°C [1] or 20 min-incubation at 45°C [2]. Centrifugation time was 65 min.

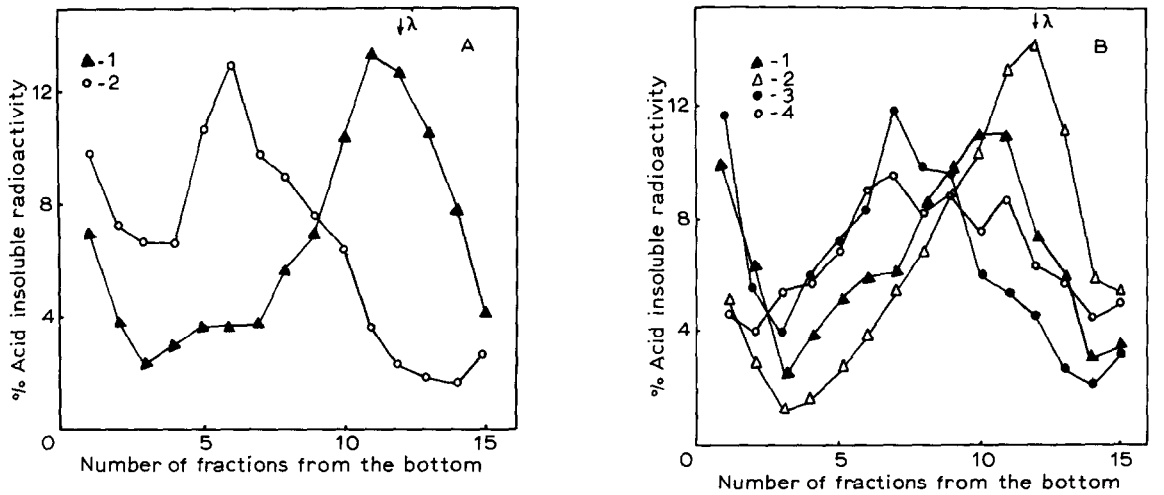

Fig. 2. Post-replication DNA repair at 30°C (A) or 45°C (B) after UV-irradiation of BT1026 cells. Centrifugation time was 85 min (A and B). (A) 1) 30 ergs/mm², 10 min pulse label at 30°C; 2) as 1) with additional 60 min-incubation at 30°C in growth medium without [³H]thymidine. (B) 1) as A,1) with additional incubation for 60 min at 45°C, or 2) 120 min in growth medium at 45°C; 3) 15 ergs/mm², 10 min-incubation with [³H]thymidine at 30°C, then for 60 min at 45°C in non-radioactive medium, 4) prestarved for 10 min at 45°C, irradiated (15 ergs/mm²) pulse labeled for 10 min at 45°C and incubated at this temperature for 60 min in non-radioactive medium.

spacing between dimers in parental DNA strands at this dose – about 20×10^6 daltons [10].

After UV-irradiation with 15 ergs/mm² almost complete repair of dimer-induced gaps in daughter DNA strands is observed after 60 min at 45°C (fig. 2B). This seems to be due to residual DNA polymerase III activity: it was shown previously that after transfer to 45°C of unirradiated BT1026 cells residual DNA synthesis occurs which corresponds to about 5% of synthesis at 30°C [15].

The defect in post-replication repair after 15 ergs/mm² in BT 1026 may be demonstrated in modified experiments. If unlabeled cells are pre-starved for 10 min at 45°C, then irradiated, labeled for 10 min with [³H]thymidine at 45°C (very low incorporation) and incubated fro 60 min at 45°C, the dimer-induced-gap-repair occurs only partially (fig. 2B).

Therefore, we suggest that DNA polymerase III is necessary for the post-replication DNA repair in UV-irradiated *polA1* cells.

4. Discussion

The simple interpretation of results presented in our paper is that the gaps opposite to the pyrimidine dimers arising at the first round of replication of damaged portion of DNA are repaired by synthesis de novo with DNA polymerase III. The alternative interpretation (see Introduction) is that DNA polymerase III and/or replication is needed for recombination between sister DNA duplexes in UV-irradiated cells: it is known that the frequency of recombination is reduced at the non-permissive temperature in *dnaB* mutant [16]. However, the dependence of recombination repair on replication suggests the copy-choice (de novo synthesis) mechanism of recombination but not breakage-reunion.

Several phenomena are observed under conditions of thermoinactivation of DNA polymerase III in the double mutant *polA1 polC26*^{ts} stopping of normal semiconservative DNA replication, sensitization to the lethal action of gamma-rays [15] and UV-light [17], and inability to rejoin the incision-induced DNA breaks [17].

We show in this paper that post-replication repair in UV-irradiated cells does not occur without functional DNA polymerase III. It seems possible that all these phenomena depend on one pathway of DNA metabolism in which operates a specific subcellular

structure containing DNA polymerase III. The real function of this structure may be the control of penetration (just prior to replication) of single-stranded DNA gaps (or breaks) into the replication machinery: if DNA polymerase III is active the gaps are filled and replication continues; in the absence of functional DNA polymerase III replication stops. In gamma-irradiated *polA1 polC26*^{ts} cells, with many long-lived breaks, replication stops immediately after transfer to 45°C [15].

Youngs and Smith [17] have postulated that DNA polymerase III acts in the *recA recB*-dependent branch of excision-repair which determines long patches (~ 1000 nucleotides) of repair synthesis observed in *polA1* cells [18]. The lengths of post-replication gaps have the same order of magnitude [3]. Recently, Wickner and Kornberg [19] have shown that DNA polymerase III* with copolymerase III* fills in very long (~ 5000 nucleotides) single-stranded templates ϕX174 DNA in vitro. These data indicate that the properties of DNA polymerase III satisfy the requirements for the enzyme which repair postreplication gaps after UV-irradiation by de novo synthesis.

The defects in many genes are known to decrease the efficiency of post-replication repair: *recA* [2], *exrA* [17]; slow and incomplete gap-repair was found in the *recB recC recF sbcB* multiple mutant [20]. Possibly the normal products of these genes act in cooperation with DNA polymerase III. This is supported by the finding that dinitrophenol known as an inhibitor of *recA recB*–dependent slow repair of X-ray induced breaks [21] produces also sensitization to gamma-rays of *polA1 polC26*^{ts} (BT 1026) cells at 30°C exactly like that produced by incubation at 45°C [22].

References

[1] Rupp, W. D. and Howard-Flanders, P. (1968) J. Mol. Biol. 31, 291–304.
[2] Smith, K. C. and Meun, D. H. C. (1970) J. Mol. Biol. 459–472.
[3] Iyer, V. N. and Rupp, W. D. (1971) Biochim. Biophys. Acta 228, 117–126.
[4] Rupp, W. D., Wilde, C., Reno, D. L. and Howard-Flanders, P. (1971) J. Mol. Biol. 61, 25–44.
[5] Lehmann, A. R. (1972) J. Mol. Biol. 66, 319–337.
[6] Setlow, R. B. and Setlow, J. K. (1972) in: Ann. Rev. Biophys. Bioeng. 1, 293–346.
[7] Smith, K. C. (1971) in: Photophysiology, Giese, A. C. ed. Acad. Press, N.Y. and London. Vol. 6, pp. 209–278.
[8] Kato, T. (1972) J. Bacteriol. 112, 1237–1246.
[9] Calvert, J. D. and Rechen, H. J. L. (1952) J. Amer. Chem. Soc. 74, 2101–2109.
[10] Noll, H. (1967) Nature, 215, 360–363.
[11] Studier, F. W. (1965) J. Mol. Biol. 11, 373–390.
[12] Kuempel, P. L. and Veomett, G. E. (1970) Biochem. Biophys. Res. Commun. 41, 973–980.
[13] Okazaki, R., Arisawa, M. and Sugino, A. (1971) Proc. Natl. Acad. Sci. U. S. 68, 2954–2957.
[14] Youngs, D. A. and Smith, K. C. (1973) J. Bacteriol. 116, 175–182.
[15] Tomilin, N. V. (1974) Mol. Gen. Genet. 129, 93–107.
[16] Stallions, D. R. and Curtiss, R. (1971) 105, 886–895.
[17] Youngs, D. A. and Smith, K. C. (1973) 244, 240–241.
[18] Cooper, P. K. and Hanawalt, P. C. (1972) Proc. Natl. Acad. Sci. U. S. 69, 1156–1160.
[19] Wickner, W. and Kornberg, A. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3679–3683.
[20] Clark, A. J. (1974) Ann. Rev. Genet., in press.
[21] Schufren, E., Smith, K. C. and Kaplan, H. S. (1973) Radiat. Res., 55, 346–355.
[22] Tomilin, N. V. and Andreev, O. A. (1974) in: Materials of the Conference of the Institute of Cytology AS USSR, in press.