THE LATER FATE OF DIMERS TOLERATED AT REPLICATION IN *ESCHERICHIA COLI* B/r Hcr$^+$ thy$^-$ trp$^-$

Milena SEDLIAKOVÁ, Jela BROZMANOVÁ and František MAŠEK
Cancer Research Institute, Slovak Academy of Sciences, Department of Molecular Genetics, Mlynské Nivy 59, 88032 Bratislava, Czechoslovakia

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1. Introduction

Pyrimidine dimers, the main photoproducts in DNA in cells irradiated by UV light [1], can be removed from the DNA excision, a process which takes place in excision-proficient cells [2]. Excision-deficient cells, incapable of excision, can tolerate a certain number of dimers due to a mechanism which may be based on genetic recombination [3].

It has been shown that UV-specific endonuclease-sensitive sites occur in excision-deficient *E. coli* cells in both parental DNA synthetized before irradiation and daughter DNA synthetized after irradiation [4]. Starting from the supposition that this endonuclease acts specifically on dimers, it has been suggested that excision-deficient *E. coli* cells tolerate dimers through transferring about half of them into DNA daughter chains [5].

Recently we have reported that the excision of dimers can be depressed in various excision-proficient *E. coli* cells by pretreatment in thymine- and amino acid-less medium (AA$^-$T$^-$) pretreatment [6] and the unexcised dimers can be detected in DNA replicated after UV irradiation [7]. Since excision-proficient *E. coli* cells, preincubated without thymine and amino acid, can replicate templates containing a great number of dimers, it was possible to follow the later fate of unexcised dimers by two-dimensional paper radiochromatography of the hydrolysates of denatured DNA.

In this paper data are presented indicating that in excision-proficient cells, whose excision activity was depressed by AA$^-$T$^-$ pretreatment, few dimers (if any) are transferred into DNA daughter chains.

2. Materials and methods

2.1. *Bacterial strain and cultivation conditions*

Escherichia coli strain B/r Hcr$^+$ thy$^-$ trp$^-$ was used. Cells were density- and radioactively-labelled for several generations as described previously [7]. Density labelling was achieved by growing the cells in a heavy medium which contained 0.1% $^{15}NH_4Cl$ (99 atom % purity) instead of 0.2% $^{14}NH_4Cl$ and 0.1% [^{13}C]glucose (78 atom % purity) instead of 1% [^{12}C]glucose. Radioisotope labelling of the DNA was accomplished by growing the cells in the heavy medium supplemented with 20 μCi [6-^{3}H]thymine/ml.

Cells in the exponential phase of growth ($1-3 \times 10^8$ cells/ml) were chased for 20 min by the addition of 2% of ^{12}C-glucose and then transferred to the light medium without the essential compounds (thy$^-$, trp$^-$). In this medium the cells were incubated for 90 min and then irradiated with a fluence of 30 J/m^2. After exposure, the incubation was continued in a fully supplemented light medium (no radioisotope labelling) in which 2 μg thymine/ml was substituted for by 4 μg thymidine/ml for 180 min.

2.2. *Ultraviolet irradiation*

A Philips TUV 15W germicidal lamp, emitting predominantly 2537 Å light served as source of radiation. The incident dose rate was 6.3 ergs/mm^2 · sec^{-1}. Depth of the irradiated layer was 1–2 mm; the suspension was manually stirred during the irradiation.

2.3. *Isopycnic CsCl centrifugation of native and denatured DNA and thymine dimer estimation*

DNA was isolated by the lysozyme–pronase pro-

cedure [8]. Native DNA was analysed as follows: 0.5 ml of lysate was mixed with 3 ml of a solution of CsCl to reach a final density of 1.725 g/cm^3. Samples were homogenized in glass homogenizers, transferred to polyallomer tubes that were filled with mineral oil. Centrifugation was performed in a SPINCO angle 50 Ti rotor at 35 000 rpm, 20°C for 40 h. After centrifugation, fractions corresponding to the peaks of heavy-heavy (HH) and heavy-light (HL) double stranded DNAs (see fig.1) were pooled, dialysed and subjected to denaturation.

Denatured DNA was prepared by heating the native DNAs in NET buffer, pH 8 (2 mM Tris, 3 mM EDTA, 10 mM NaCl) in a boiling water bath for 15 min followed by rapid cooling. After denaturation CsCl was added to the samples up to a final density of 1.750 g/cm^3. Subsequently the samples were once more centrifuged in the above-described manner. Fractions corresponding to the heavy (H) regions (see fig.2) were pooled and thymine dimers were determined in H single strands originating from both HH and HL double stranded forms. Pooled fractions were dialysed to remove CsCl and dried. The dried samples were hydrolysed with 0.2 ml of 98% HCOOH in sealed thick-walled glass tubes at 175°C for 60 min. Thymine dimers were estimated by two-dimensional paper radiochromatography of the DNA hydrolysate according to Carrier and Setlow [9].

3. Results and discussion

In order to reveal the fate of dimers during DNA replication, cells were density- and radioactivity-prelabelled, UV irradiated and postincubated in a light medium. Three hours after UV irradiation, samples were taken and HH-DNA (unreplicated after UV treatment) was separated from HL-DNA (replicated after UV treatment) by CsCl gradient centrifugation (fig.1). Fractions were collected from the HH and HL regions, as shown in fig.1, pooled, and the DNAs were heat denatured in CsCl gradients (fig.2A). Then, the ratio of dimers to monomers ($\hat{TT}$:T) was determined in the heavy (H) single-strands, originating from both

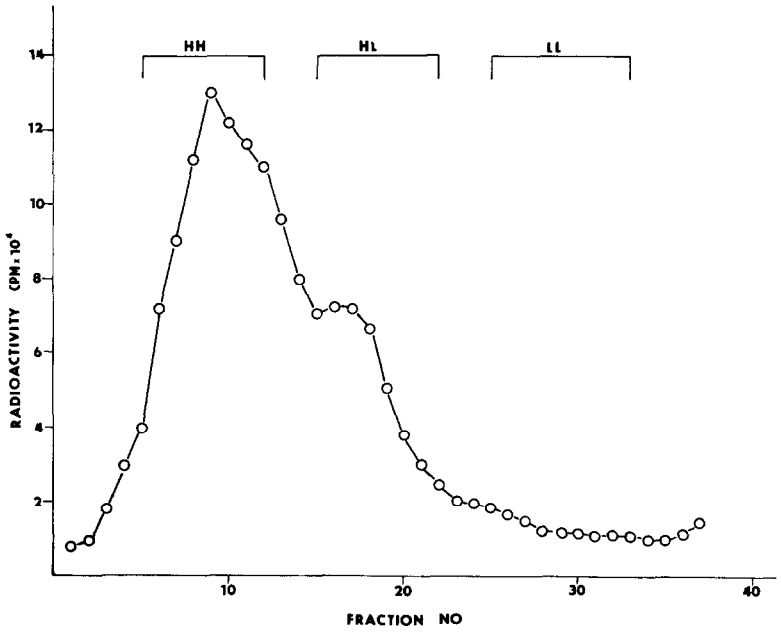

Fig.1. CsCl gradient profiles of native DNA from thymineless aminoacidless pretreated and UV irradiated cells of *E. coli* B/r Hcr$^+$ thy$^-$ trp$^-$. 3 h after irradiation samples were withdrawn, the DNA was isolated and separated by centrifugation in CsCl gradient. After fractionation, the indicated fractions of HH and HL double stranded DNAs were pooled, dialysed and submitted to denaturation.

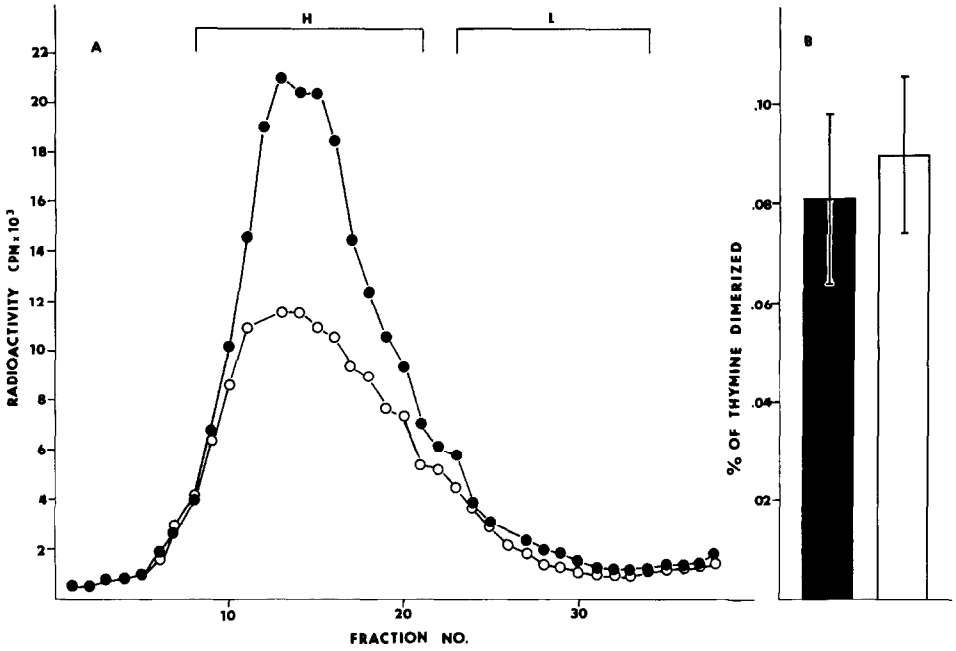

Fig.2. CsCl gradient profiles (A) and thymine dimer content (B) of denatured DNAs isolated from unreplicated HH double stranded (●———●, ■) and replicated HL double stranded forms (○———○, □). Native HH and HL DNAs were denatured and centrifuged in CsCl gradients. Denatured DNAs were centrifuged separately and the results are drawn together in the figure. Fractions from the indicated H regions were pooled for the determination of thymine dimers.

unreplicated (HH) and replicated (HL) DNAs (fig.2B).

The finding that the $\hat{T}\hat{T}$:T ratio was similar in both types of parental chains (with deviations of about ± 15% in both cases; for the details see fig.2b) indicates that dimers were not transferred into DNA daughter chains selectively. This, by itself, does not exclude the possibility that dimers were transferred unselectively, i.e. in the original $\hat{T}\hat{T}$:T ratio. However, in the latter case a substantial increase of ^{3}H-cpm should have been detected in the L region when the replicated HL-DNA was denatured and rebanded, which was not observed. On the contrary, it appears from fig.2A that in the L-region the percentage of the total amounts of cpm is similar in both single stranded DNA derived from unreplicated (HH) and replicated (HL) DNA double-strands.

Since no decrease in the $\hat{T}\hat{T}$:T ratio was found in the replicated parental strands and no increase of ^{3}H-cpm could be detected in the L-region of the replicated denatured DNA, we conclude that detectable amounts of dimers were transferred into DNA daughter chains neither selectively nor in the original dimer-to-monomer ratio. Thus, it appears that under the given conditions *E. coli* B/r Hcr^{+} cells tolerate unexcised dimers, or at least the bulk of them, without transferring the dimers into DNA daughter chains.

Recently, it has been shown that, similarly to thymine starvation, dimer excision may be repressed by a smaller dose of UV-radiation applied prior to a lethal UV dose. Simultaneously, it has been shown that the resumption of DNA replication in UV-irradiated cells requires (an) inducible protein(s). When this protein is available (i.e. when its formation is induced by previous fluence) DNA replication is resumed after the second fluence, even in the presence of chloramphenicol [10], templates containing dimers are replicated, and dimers remain 'in situ'; they are neither transferred nor excised [11].

Since, contrary to excision-proficient cells, excision-deficient cells cannot tolerate so many unexcised dimers (this follows from the finding that the latter

cannot replicate templates containing about 0.05% of thymine dimerized, while the former can do so), it has been concluded that in UV irradiated cells the UV endonuclease fulfills another function beside dimer excision [12]. Data are available indicating that dimers remaining in excision-proficient cells are less mutagenic [13] and postreplication repair taking place in these cells is more accurate than that occurring in excision-deficient cells [14]. This may indicate that the mechanisms of 'postreplication repair' are different in both types of cells. If so, results obtained in excision-proficient cells need not be valid for excision-deficient cells; thus, our conclusions need not necessarily be in contradiction with those of Ganesan [4,5].

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