Theor. Appl. Genet. 55, 119-128 (1979)

THEORETICAL AND APPLIED GENETICS



# Participation of the Intracellular Enzymes in the Control of Mutational Processes*

## Part 4: The Role of UV-Specific Endonuclease and Medium Composition in the Induction of Genic Mutations in *Escherichia coli*

V.N. Soyfer and Y.B. Titov
All-Union Institute of Applied Molecular Biology and Genetics, Moscow (USSR)

**Summary.** The influence of UV-specific endonuclease and medium composition on the frequency and spectrum of genic mutations in *Escherichia coli KI2 uvr*$^+$ (with normal repair enzymes) and *uvr A6* (defective in UV-specific endonuclease) was studied. Mutations at the locus *glu* (gene controlling assimilation of glucose) were induced by ultra-violet irradiation and hydroxylamine treatment. To identify mutant colonies, triphenyl tetrazolium chloride (TTC) was added to the medium since it coloured the mutant colonies bright crimson and readily permitted distinction between pure mutant clones (complete mutations) and mixed clones (mosaic or sector mutations).

A maximum mutation frequency after UV-irradiation was observed in *E. coli uvr*$^+$ cells but not in the *E. coli uvr A6* strain. The curve of mutagenesis with a maximum was found in both studied strains after treatment by hydroxylamine which did not cause DNA damage recognized by UV-specific endonuclease.

The highest frequency of mutations (at the point of maximum) in the series of experiments with enriched growth medium was almost 10 times higher than in the series of the experiments with poor medium.

It was established that in bacteria with normal repair enzymes the frequency of complete mutations was higher than the frequency of mosaic mutations. It was also observed that the rate of UV-mutagenesis was higher in the case of *E. coli uvr*$^+$.

The study of the distribution of mosaic mutant sectors in experiments with bacteria suspended in either a nutrient broth or a buffer during UV-irradiation revealed that the size of mutant sectors was rather variable and that the differences in the number of nucleoids per cell did not always determine the distribution of mutant sector sizes.

**Key words:** UV specific endonuclease – Medium composition – Mutations – *E. coli*

**Abbreviations**

HA Hydroxylamine hydrochloride
TTC Triphenyl tetrazolium chloride
TCA Trichloroacetic acid

## Introduction

The study of the molecular basis of mutations reveals that the stabilisation of premutational lesions into mutations is accomplished in part by intracellular enzymes, especially by repair enzymes. Witkin (1967) divided repair processes into two classes, mutation-prone and mutation-proof, and suggested that mutations are induced only during the former. According to this hypothesis, the product of *uvrA* and *uvrB* genes – UV-specific endonuclease (correndonuclease II) – belongs to mutation-proof enzymes.

Although Nishioka and Doudney (1969, 1970) and Soyfer (1969, 1972a) presented evidence that the product of the *uvrA*-gene may play a role in mutations induced by UV-irradiation, the study of the excision of damaged bases from irradiated DNA suggests that the excision process based on the activity of UV-specific endonuclease is preferentially error-free (Carrier and Setlow 1970). However, these experiments were performed in vitro, with isolated enzymes, and it is obscure how this process is realised in unbroken cells; second, very low doses of UV-light were used; and, third, the authors were unable to check for a small number of enzymatic mistakes during excision. It follows from this that at high doses of irradiation or after prolonged treatment with chemical mutagens the process of excision might be less accurate, as was supposed by Soyfer (1969). At the same time, the analysis of

* Other papers of this series are: Soyfer 1972; Soyfer et al. 1977; Soyfer and Kartel 1978

040-5752/79/0055/0119/$ 2.00

repair replication suggests that this process is error-correcting (Kelly et al. 1969; Kushner et al. 1971) and that during repair the complementary, undamaged strand of DNA serves as a template for error-free replicative synthesis and that exact copying of the template chain in the damaged area results in the restoration of the original nucleotide sequence. Similar data were also obtained in experiments with human and mouse cells (Lieberman and Poirier 1974).

In marked contrast to the above results were data of experiments with normal bacteria, *E. coli* KI2, and with bacteria defective in UV-specific endonuclease. They showed that at elevated doses of UV-radiation the total frequency of mutations and the ratio of complete genic mutations to mosaic mutations increased with doses of UV-irradiation. This was observed only in *E. coli uvr*$^+$, not in *E. coli uvr A6* cells (Soyfer 1972a). Thus, it becomes obvious that in in vivo experiments the total frequency of mutations, the kinetics of mutagenic action and the spectrum of induced mutations could be determined first by the amount of initial damage to DNA molecules and, second, by the general metabolism of the cell (including the activity of repair endonucleases), which would also be changed as a result of irradiation or other treatments.

To estimate the role of UV-specific endonuclease in modifying the mutational process, we performed experiments in which bacterial lines with either normal repair enzymes or with defective UV-specific endonuclease (correndonuclease II) were used. The wide range of doses of UV-irradiation was used to study mutation induction. An attempt was made to answer the following main questions.

1) Are there any differences in mutation rates at low and at high doses of UV-irradiation in both strains of bacteria?

2) What is the frequency of complete and mosaic mutations induced in these bacterial lines in a wide range of doses of UV-irradiation?

3) What is the frequency of mutations in cells irradiated in a rich nutrient broth or in a buffer?

4) Since mosaic mutations may arise not only due to mistakes of repair endonucleases but also due to the presence of several nucleoides in cells, what are the differences in nucleoides in *E. coli uvr*$^+$ and *E. coli uvr A6* cells and in the distribution of mosaic sectors in these experiments?

## Materials and Methods

### *Bacteria*

Bacteria *E. coli KI2 uvr*$^+$ (AB 1157) and a derivate of this line defective in repair excision, *E. coli KI2 uvr A6* (AB 1886), were used in these studies.

### *Media*

The minimal medium M9 ($NH_4Cl$ – 1 g, $Na_2HPO_4 \cdot 7H_2O$ – 11 g, $KH_2PO_4$ – 3 g, NaCl – 5 g, $MgSO_4$ – 120 mg, $CaCl_2$ – 10 mg, glucose – 8 g) supplemented (per liter) with 10 g bacto peptone (Difco), 1 g yeast extract (Merck), 0.5 g caseine hydrolysate (Difco), 8 g NaCl, 2 g sodium citrate and 0.01 g DL-tryptophan, was used for growing the bacteria at 37°C before UV-irradiation in the first series of experiments (irradiation in an enriched medium). In the second series (irradiation in a buffer), bacteria were grown in another medium (per liter): 0.4 g $Na_2HPO_4 \cdot 12\ H_2O$, 0.9 g $KH_2PO_4$, 4 g NaCl, 7.5 g bactopeptone, 1.5 g beef extract and 10 g glucose. For the determination of the number of mutant colonies in both experiments bacteria were plated onto solid agar medium containing (per liter of distilled water) 6 g peptone, 3 g yeast extract, 1.5 g beef extract (Difco), 8 g NaCl, 10 g glucose, 15 g bacto agar (Difco) and 50 mg TTC (Difco). All media and solutions in which bacteria were grown, suspended, irradiated and plated were of pH 6.6.

### *HA Treatment*

Twice recrystallised preparations of $NH_2OH \cdot HCl$ (Koch-Light, England) were used. Bacteria synchronised by the modified method of Helmstetter and Cummings (1963) were treated by 1M HA + 1M NaCl (pH 6.6) at 25°C. Mutagenic treatment was interrupted by the addition of 4.5 ml of stopping mixture (acetone – 10 ml, 4M NaCl – 25 ml, peptone – 2 g, water – up to 100 ml) to 0.5 ml bacterial suspension.

### *UV-Irradiation*

Ultraviolet irradiation was performed with three I5 W germicidal lamps in the first experiment (enriched medium) and with one lamp in the second experiment (buffer). Irradiation was made at distance of 26 cm with a dose rate 6.5 erg·mm$^{-2}$·sec$^{-1}$. In the first series of experiments about $5 \times 10^7$ cells/ml (at log phase) were irradiated in growth media in 9 cm Petri dishes innoculated with 3 ml of bacterial suspension. In the second series $2 \times 10^7$ cells/ml of bacteria were twice centrifuged and then resuspended in minimal medium M9 without glucose before irradiation. During irradiation the suspension was agitated with a magnetic stirrer. Samples were taken at various times during irradiation, immediately titrated in M9 medium and plated for mutant assay. Irradiation and succeeding treatments were performed under conditions preventing photoreactivation.

### *Determination of Mutations*

Both lines of bacteria used glucose as a carbohydrate source (*glu*$^+$). UV-irradiation induces mutant cells which are unable to assimilate glucose. The presence of TTC in the solid medium makes it possible, therefore, to visualise colonies forming from these mutant bacteria. Colonies formed from nonmutant cells have a grayish colour in the presence of TTC, while bacteria not assimilating glucose are stained bright crimson due to the precipitation of insoluble formasan (Ogur et al. 1957). Such a method provides the opportunity for determining complete (if the whole colony is stained crimson) and mosaic mutant colonies (crimson sectors of colonies of different sizes). Any methods of pre-selection or enrichment of mutants are unacceptable for these purposes. Therefore all bacterial colonies formed after mutagenic treatment had to be scored at the

plates. The number of colonies on individual plates did not exceed 300. To avoid accidental mistakes in such experiments it was determined whether the distribution of mutants on separate plates agreed with the Poisson distribution.

*The Determination of the Number of Nucleoids in Cells*

For the staining of nucleoids, the bacteria were first washed from the growth media with a physiological solution and then concentrated by centrifugation. A drop of the concentrated bacterial suspension was layered on the surface of a microscope slide and fixed for 20 minutes with a solution of 3 parts ethanol and 1 part glacial acetic acid. In order to remove the RNA, the preparations were incubated with 1M HCl at 60°C for 7 min or were treated with 1 mg/ml of pancreatic RNAse (Wortington, USA). After washing with distilled water the preparations were stained by azur-eosine and covered with a cover glass and Canada Balsam. The number of nucleoids in the cells were measured microscopically.

*The Study of DNA Synthesis*

Bacteria were transferred into the growth medium containing 2 μCi/ml $^3$H-thymidine and incubated at 37°C for 5 hours. Samples were treated with 10% TCA and washed, with 5% TCA and 70% ethanol. The radioactivity was measured in toluene scintillator in a Mark II Nuclear Chicago radiospectrometer. At the same time aliquots of bacterial suspension were withdrawn for a calculation of exact data on colony-forming ability.

## Results

### *The Influence of UV-Specific Endonuclease on Mutation Kinetics after UV-Irradiation and Hydroxylamine Treatment*

It is generally known that linear kinetics of mutation induction takes place only under relatively low doses. When doses of UV-irradiation are further increased, the mutation frequency reaches the maximal level, and then begins to decrease. This phenomenon is termed 'the maximum of mutating'.

Earlier it was shown that under UV-irradiation of bacteria suspended in a rich medium the maximum of mutating was observed only in *E. coli uvr*$^+$, but not in *E. coli uvr A6* cells.

In the present work the maximum of mutating was studied in experiments with UV-irradiation and HA treatment of *E. coli uvr*$^+$ and *uvr A6* bacteria suspended in a buffer.

The substitution of enriched growth medium by the buffer provided the possibility, first, to perform the dosimetry more precisely, and, second, to modify the activity of *uvr*-dependent repair enzymes, to decrease the action of the *exr, rec*-dependent systems of repair needed for their activity in a rich growth medium (Sedgwick and Bridges 1972; Smith 1975; Youngs and Smith 1973).

The results of these experiments with UV-irradiation are presented in Table 1. As was observed in the experiments with enriched media, the maximum of mutating in experiments with a buffer was found only in bacteria with normal repair enzymes (Fig. 1A), but this maximum was absent in the strain defective in the *uvr A*-gene (Fig. 1B). As is known, the system of *uvr*-dependent repair does not take part in the repair of damage induced by 1M HA (Soyfer 1969). Using HA, the level of mutation in the *uvr*

**Table 1.** The number of induced mutants and the frequency of mutations in bacteria irradiated with UV-rays

| Dose of irradiation sec. | Survival % | No. of scored colonies | No. of observed mutations | Frequency of mutations ($\times 10^{-4}$) |
|---|---|---|---|---|
| *E. coli uvr*$^+$ (AB 1157) | | | | |
| 0 | 100 | 13609 | 1 | 0.73 ± 0.7[a] |
| 30 | 100 | 76642 | 8 | 1.0 ± 0.4 |
| 60 | 79 | 20908 | 9 | 4.3 ± 1.4 |
| 90 | 55 | 18760 | 10 | 5.3 ± 1.7 |
| 120 | 2.2 | 52492 | 55 | 10.0 ± 1.4 |
| 150 | 0.7 | 50083 | 67 | 13.0 ± 1.6 |
| 180 | 0.12 | 22787 | 27 | 12.0 ± 2.3 |
| 240 | 0.02 | 35529 | 15 | 4.6 ± 1.2 |
| 300 | 0.013 | 8580 | 2 | 2.3 ± 1.6 |
| 360 | 0.01 | 8950 | 2 | 2.2 ± 1.6 |
| *E. coli uvr A6* (AB 1886) | | | | |
| 0 | 100 | 37152 | 9 | 2.4 ± 0.8 |
| 3.3 | 38.6 | 63649 | 27 | 4.2 ± 0.8 |
| 6.6 | 1.4 | 17665 | 12 | 6.8 ± 2.0 |
| 10 | 0.9 | 39863 | 67 | 17.0 ± 2.0 |
| 17 | 0.052 | 51982 | 70 | 14.0 ± 1.8 |
| 23 | 0.0076 | 33960 | 72 | 21.0 ± 2.5 |

[a] Frequency of mutations with standard errors

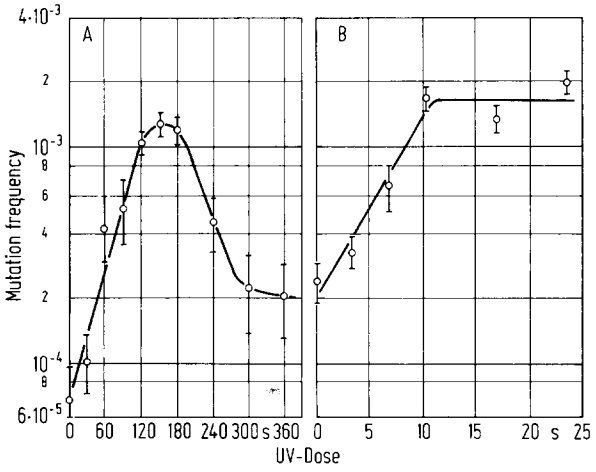


**Fig. 1A and B.** Induction of *glu*-mutations by UV-irradiation in *E. coli uvr*$^+$ (A) and *uvr A6* (B) cells. Standard deviations according to Poisson are indicated

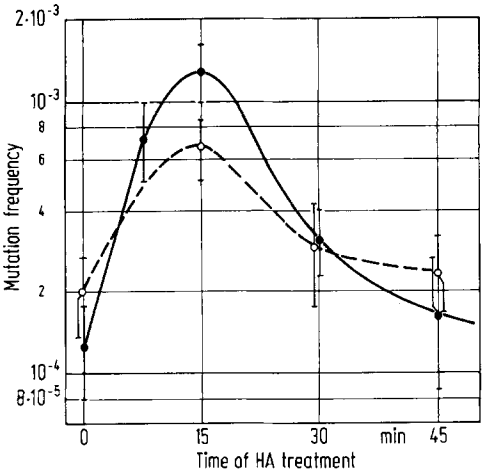


**Fig. 2.** Induction of mutations by 1M hydroxylamine treatment in *E. coli uvr*$^+$ (•) and *uvr A6* (○) cells

*A6*-strain was nearly two times lower than in the *uvr*$^+$ strain (Table 2), and the maximum for this mutagen was observed in both strains of bacteria (Fig. 2).

We suggest that these data indicate both the possibility of the participation of repair endonucleases in the appearance of the maximum phenomenon in the case of UV-irradiation of *E. coli* K12 cells and the increase in the level of mutation frequency in the case of HA-treatment of these bacteria.

### *The Influence of Repair Endonucleases on the Spectrum of Genic Mutations Induced by UV-Irradiation*

The analysis of complete and mosaic mutations revealed essential differences in mutagenesis between the lines with normal and defective UV-specific endonucleases. Inasmuch as bacteria of the *uvr A6* strain are highly sensitive to UV-irradiation, we first studied the kinetics of inactivation of both bacterial strains (Soyfer 1972a) and on this basis dose ranges giving a similar degree of inactivation were chosen. Only under this condition could the mutation frequencies of the two lines be compared quantitatively.

The results of the experiments on the induction of pure and mosaic mutations in bacteria with normal and defective repair enzymes are given by Table 3. Because repair of pre-mutational lesions is determined by systems of enzymes which are dependent on the presence of nutrient components in media (Sedgwick and Bridges 1972), all experiments were divided into two series.

In the first series bacteria were irradiated in enriched growth media. Under these conditions repair apparently proceeds successfully in the first 4-6 minutes after UV-irradiation (Paul et al. 1976). In this series of experiments when up to 8 min doses of UV-irradiation were given, the ratio of complete and mosaic mutation frequencies remained rather constant in *E. coli K12 uvr*$^+$ (see the last column of the Table 3). At higher doses, when the number of induced thymine dimers increases abruptly, the ratio of complete to mosaic mutation frequencies increased sharply in *uvr*$^+$ cells. In the *E. coli K12 uvr A6* cells which lacked UV-dependent endonuclease and which were unable to excise from DNA the pyrimidine dimers induced by UV-irradiation, the induction of complete and mosaic mutations proceeded differently than in *E. coli uvr*$^+$ cells. There was no increase of complete mutations compared to mosaics: their ratio remained rather constant within the whole dose range used.

In the second series of experiments bacteria were centrifuged, resuspended in minimal medium without a carbohydrate source, and then, after irradiation, were transferred to a nutrient broth. In these conditions it was also observed that in cells with normal repair enzymes frequency ratio of complete mutations to mosaic mutations was higher as compared with bacteria *E. coli uvr A6*. Another interesting result of this series of experiments was the decreasing level of maximal mutability for *uvr*$^+$

**Table 2.** Frequency of *glu*$^-$ mutations induced with HA

| Bacteria | Dose of HA treatment, min | Number of experiments | Number of scored colonies | Number of mutant colonies | Frequency of mutations *glu*$^-$ (× $10^{-4}$) |
|---|---|---|---|---|---|
| *E. coli uvr*$^+$ | 0 | 8 | 59063 | 8 | 1.3 ± 0.5 |
| | 7.5 | 4 | 12384 | 9 | 7.3 ± 2.4 |
| | 15 | 5 | 20524 | 25 | 13.0 ± 2.4 |
| | 30 | 8 | 34744 | 11 | 3.2 ± 0.9 |
| | 45 | 4 | 17454 | 3 | 1.7 ± 1.0 |
| *E. coli uvr*$^-$ | 0 | 7 | 34277 | 7 | 2.0 ± 0.8 |
| | 15 | 5 | 34969 | 22 | 6.7 ± 1.3 |
| | 30 | 4 | 16319 | 5 | 3.0 ± 1.4 |
| | 45 | 4 | 21428 | 7 | 2.4 ± 1.2 |

**Table 3.** Influence of medium composition on the frequency of induced mutations

| Medium composition in the time of irradiation | Strain of bacteria | Dose of UV-irradiation sec | Survival % | Number of scored colonies | Number of mutant colonies | | Frequency of mutations ($\times 10^{-4}$) | | Ratio of frequencies of complete to mosaic mutation mutations |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | complete | mosaic | complete | mosaic | |
| Enriched growth medium | *uvr*$^+$ | 0 | 100 | 8523 | 2 | no | 2.2 ± 1.6 | < 0.1 | > 23 |
| | | 60 | 68 | 113810 | 133 | 6 | 12.0 ± 1.0 | 0.53 ± 0.21 | 24 |
| | | 480 | 15 | 19952 | 211 | 23 | 110.0 ± 7.3 | 12.0 ± 2.4 | 9.2 |
| | | 600 | 8.7 | 62936 | 33 | no | 5.3 ± 0.9 | < 0.1 | > 53 |
| | | 900 | 3.0 | 31692 | 39 | no | 12.0 ± 2.0 | < 0.1 | > 120 |
| | *uvr*$^-$ | 0 | 100 | 67662 | 4 | no | 0.6 ± 0.3 | < 0.1 | > 6 |
| | | 30 | 15 | 27210 | 19 | 3 | 7.0 ± 1.6 | 1.1 ± 0.6 | 6.4 |
| | | 75 | 2.2 | 58360 | 50 | 5 | 8.6 ± 1.2 | 0.9 ± 0.4 | 10 |
| | | 135 | 0.6 | 26043 | 11 | 5 | 4.2 ± 1.3 | 1.9 ± 0.8 | 3.2 |
| Minimal medium without glucose (a buffer) | *uvr*$^+$ | 0 | 100 | 10745 | 2 | 1 | 1.9 ± 1.3 | 0.9 ± 0.9 | 2 |
| | | 60 | 79 | 15154 | 10 | 2 | 6.6 ± 2.1 | 1.3 ± 1.0 | 5 |
| | | 120 | 2.2 | 5694 | 15 | 3 | 26.0 ± 6.5 | 5.3 ± 3.0 | 5 |
| | | 150 | 0.7 | 30568 | 45 | 10 | 14.7 ± 2.0 | 3.3 ± 1.0 | 4.5 |
| | | 210 | 0.04 | 29448 | 34 | 9 | 11.5 ± 2.0 | 3.0 ± 1.0 | 3.8 |
| | | 270 | 0.025 | 28194 | 29 | 8 | 10.0 ± 2.0 | 2.8 ± 1.0 | 3.6 |
| | *uvr*$^-$ | 0 | 100 | 37152 | 7 | 2 | 1.9 ± 0.7 | 0.5 ± 0.4 | 3.5 |
| | | 3.3 | 38 | 41599 | 14 | 4 | 3.4 ± 0.9 | 1.0 ± 0.5 | 3.5 |
| | | 10 | 0.9 | 12315 | 36 | 18 | 29.2 ± 5.0 | 14.6 ± 3.4 | 2 |
| | | 17 | 0.05 | 13586 | 42 | 15 | 31.0 ± 4.8 | 11.0 ± 2.9 | 2.8 |
| | | 23 | 0.008 | 8875 | 45 | 18 | 50.0 ± 7.5 | 20.3 ± 4.8 | 2.5 |

cells as compared with their irradiation in enriched growth medium. In addition, it must be emphasized that in both experiments the maximum of mutability was observed only in *uvr*$^+$, but not in *uvr A6* cells (Fig. 3).

However, bearing in mind that such differences in the frequency of mosaic and complete mutations might be a consequence of a different content of DNA per cell and different numbers of nucleoids in cells, we decided to find out if there were differences in both patterns between the studied strains of bacteria.

### *Content of DNA per Cell of Two Studied Strains of Bacteria*

We observed earlier (Titov and Soyfer 1977) that before UV-irradiation *uvr*$^+$ and *uvr A6* cells had an equal content of DNA per cell in both strains of bacteria. For present experiments it is very important to know if this ratio will be preserved under further cultivation of both bacterial strains. It was found that during 5 hours of cultivation of unirradiated bacteria in nutrient broth the mean content of DNA per cell was constant (Fig. 4).

Therefore, it seems that the differences in complete and mosaic mutations are not connected with different content of DNA per cell of both strains.

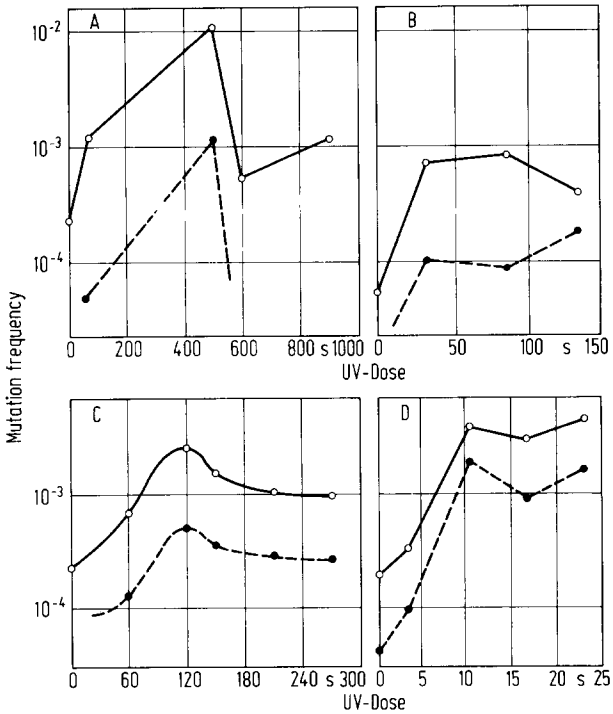


**Fig. 3A-D.** Induction of complete (○) and mosaic (●) mutations by UV-irradiation of *E. coli uvr*$^+$ in an enriched nutrient broth (A) and in a buffer (C), and *E. coli uvr A6* in an enriched nutrient broth (B) and in a buffer (D)

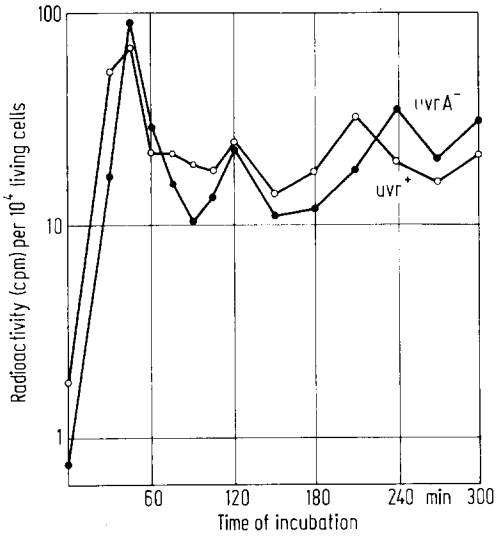


**Fig. 4.** Incorporation of $^3$H-thymidine into DNA of *E. coli uvr*$^+$ (○) and *uvr A6* (●) cells during incubation of un-irradiated cells

### *Number of Nucleoids in the Cells of the Two Strains*

As is well known, bacteria may have not one but several nucleoids per cell. Determination of the number of nucleoids in the populations of *E. coli uvr*$^+$ and *uvr A6* cells demonstrated that the overwhelming majority (93.9 per cent) of *uvr*$^+$ cells have one or two nucleoids, about 5 per cent of the cells have 3 and only 1 per cent have 4 or more nucleoids (Table 4). In *E. coli uvr A6* the number of cells with one nucleoid was approximately half as many as that in the *uvr*$^+$ strain; the number of cells with two nucleoids was higher than in the latter (50.3 and 66.5 per cent respectively), and the total number of bacterial cells with 1-2 nucleoids was approximately the same in both strains (93.9 and 87.1 per cent respectively). The number of cells with three nucleoids in the two strains was also rather similar (5.1 and 6.8 per cent, respectively).

Thus, the quantitative study of nucleoid number revealed some differences between the investigated strains. The decrease in the number of cells with one nucleoid in the population of bacteria *E. coli uvr A6* owing to the increase of the proportion of cells with two and more nucleoids per cell may have led to a change in the ratio of complete to mosaic mutations in *uvr A6* cells irradiated by UV-light – in particular an increase of half-sectored mosaic mutants in *uvr A6* cells. To check whether the differences in the frequencies of complete and mosaic mutations might be connected with nucleoid patterns in both strains we have analyzed the distribution of sector sizes of mosaic mutations in both bacterial strains.

**Table 4.** Nucleoid number in *E. coli uvr*$^+$ and *uvr A6* cells

| No. of nucleoids | No. of cells | | | | | | | | | | Total of cells with number of nucleoids | % of the number of cells |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | expt 1 | expt 2 | expt 3 | expt 4 | expt 5 | expt 6 | expt 7 | expt 8 | expt 9 | expt 10 | | |
| 1 | 38 | 38 | 52 | 54 | 34 | 44 | 43 | 50 | 41 | 42 | 436 | 43.6 |
| 2 | 54 | 54 | 37 | 41 | 60 | 53 | 53 | 45 | 52 | 54 | 503 | 50.3 |
| 3 | 7 | 8 | 10 | 4 | 5 | 2 | 2 | 4 | 6 | 3 | 51 | 5.1 |
| 4 | 1 | – | 1 | – | 1 | – | – | 1 | 1 | 1 | 6 | 0.6 |
| 5 | – | – | – | 1 | – | 1 | – | – | – | – | 2 | 0.2 |
| 6 | – | – | – | – | – | – | 1 | – | – | – | 1 | 0.1 |
| 7 | – | – | – | – | – | – | 1 | – | – | – | 1 | 0.1 |
| 1 | 21 | 22 | 17 | 19 | 27 | 22 | 25 | 22 | 11 | 17 | 206 | 20.6 |
| 2 | 68 | 45 | 69 | 69 | 59 | 69 | 65 | 68 | 78 | 75 | 665 | 66.5 |
| 3 | 8 | 19 | 9 | 6 | 5 | 7 | 6 | 4 | 6 | 5 | 68 | 6.8 |
| 4 | 1 | 17 | 5 | 4 | 4 | 1 | 1 | 1 | 3 | 2 | 39 | 3.9 |
| 5 | 2 | 3 | – | 1 | 1 | – | 1 | – | 2 | – | 10 | 1.0 |
| 6 | – | – | – | – | – | – | 1 | – | – | – | 1 | 0.1 |
| 7 | – | – | – | – | 2 | – | – | – | – | – | 2 | 0.2 |
| 8 | – | – | – | – | – | – | – | 1 | – | – | 1 | 0.1 |
| 9 | – | 1 | – | – | – | – | – | – | – | – | 1 | 0.1 |
| 10 | – | – | – | – | – | – | 1 | – | – | – | 1 | 0.1 |
| 11 | – | – | – | 1 | 2 | – | – | – | – | 1 | 4 | 0.4 |
| 12 | – | – | – | – | – | – | – | 1 | – | – | 1 | 0.1 |
| 13 | – | – | – | – | – | 1 | – | – | – | – | 1 | 0.1 |

### *The Distribution of Sizes of Sectors in Mosaic Colonies of uvr⁺ and uvr A⁻ Strains of Bacteria*

To reveal whether the arisal of mosaic mutations depends on the activity of intracellular enzymes of repair in bacteria, or on only the presence of one, two or several nucleoids, we studied the distribution of mosaic mutations in relation to types of sectors. Such analysis of sector sizes was made for data summarized in Table 3. The distribution of mosaic colonies' sector sizes was studied in the case of mutation induction in bacterial populations suspended in an enriched nutrient broth (Table 5), and in the case of a poor mineral medium (Table 6).

It appeared that the size of mutant sectors was rather variable. Colonies were found in the experiments with enriched medium having 1/16, 1/8, 1/4 or 1/2 non-mutant parts, as well as colonies with 1/16, 1/8, 1/4 or 1/2 mutant parts of whole space of the colony (Table 5). In *uvr⁺* cells the number of colonies with mutant sectors occupying half of the colony's space was one-half as large as other types of sectors after 60 sec of irradiation and was roughly similar to the number of mutant colonies with other sizes of sectors (after 480 sec of irradiation). In the *uvr A6* strain the number of half-mutant colonies was less than that for other types of sectors at 30 sec doses of irradiation, and this number was increased with the enhancing of the irradiation dose.

At the same time the study of the number of nucleoids per cell showed that in both bacterial strains the overwhelming majority of multi-nucleoid cells were represented by cells with two nucleoids. Therefore, if a direct correlation exists between the number of nucleoids and the type of arising mutations then the overwhelming part of the mosaic colonies will belong to half-sectored colonies. However, data presented in Table 5 do not agree with this suggestion.

The same result was obtained in the series of experiments in which bacteria were suspended in a buffer without glucose during UV-irradiation. A wide distribution of types of sectors was also revealed in this case (Table 6) and it was found that the wide spectrum of different sizes of mosaic colonies remained relatively constant in *uvr⁺* and *uvr A⁻* cells irradiated by different doses of UV-rays. Thus, these experiments demonstrated that no direct rela-

**Table 5.** Distributions of sizes of *glu⁻* sectors in colonies of *uvr⁺* and *uvr⁻* strains suspended in enriched nutrient broth during UV-irradiation

| Strain | UV dose sec | Total *glu⁻* colonies | Number of colonies with mutant sector size | | | | | | | | The ratio of half-sectors to other types of sectors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 15/16 | 7/8 | 3/4 | 1/2 | 1/4 | 1/8 | 1/16 | |
| *uvr⁺* | 60 | 139 | 133 | | | 2 | 2 | 2 | | | 2 : 4 |
| | 480 | 234 | 211 | 4 | 2 | 2 | 10 | 3 | 1 | 1 | 10 : 13 |
| *uvr⁻* | 30 | 22 | 19 | | | | 1 | | 2 | | 1 : 2 |
| | 75 | 55 | 50 | | 2 | | 3 | | | | 3 : 2 |
| | 135 | 16 | 11 | | | | 5 | | | | 5 : 0 |

**Table 6.** Distribution of sizes of *glu⁻* sectors in colonies *uvr⁺* and *uvr⁻* cells suspended in a buffer during UV-irradiation

| Strain | UV dose sec | Number of mosaic mutant colonies | Number of colonies with a certain mutant sector | | | | | | The ratio of half-sectors to other types of sectors |
|---|---|---|---|---|---|---|---|---|---|
| | | | 3/4 | 1/2 | 1/3 | 1/4 | 1/6 | 1/8 | |
| *uvr⁺* | 0 | 1 | | | 1 | | | | 1 : 0 |
| | 60 | 2 | 1 | 1 | | | | | 1 : 1 |
| | 120 | 3 | | 1 | 1 | 1 | | | 1 : 2 |
| | 150 | 10 | 1 | 4 | 1 | 3 | | 1 | 4 : 6 |
| | 210 | 9 | 1 | 5 | | 2 | 1 | | 5 : 4 |
| | 270 | 8 | | 1 | | 3 | 2 | 2 | 1 : 7 |
| *uvr⁻* | 0 | 2 | | 1 | | 1 | | | 1 : 1 |
| | 3.3 | 4 | | 2 | | 1 | 1 | | 2 : 2 |
| | 10 | 15 | | 6 | 3 | 1 | 3 | 2 | 6 : 9 |
| | 17 | 15 | 1 | 8 | | 4 | 1 | 1 | 8 : 7 |
| | 23 | 18 | 2 | 12 | | 3 | 1 | | 12 : 6 |

tionship existed between the number of nucleoids in appropriate strains and the types of induced mutations. The more preferential explanation is that intracellular enzymes of repair are involved in the process of changing of the types of induced mutations.

## Discussion

In this paper we presented data on the role of UV-specific endonuclease (correndonuclease II) in affecting mutation induction by UV-irradiation and hydroxylamine treatment. Probably the presence of normal *uvr*-genes plays an important role both in the kinetics of mutagenesis and in the change of the spectrum of the induced mutations.

As indicated by the findings presented here, a maximum mutation frequency after UV-irradiation was observed only in *E. coli uvr*$^+$ cells but not in the *E. coli uvr A6* strain. Of course, while the doses of UV-irradiation used in experiments with *uvr*$^+$ and *uvr A*$^-$ strains are markedly different, it may be assumed that the maximum occurs only at sufficiently high UV-doses (i.e. beyond those received by the *uvr A*$^-$ cells). However, this suggestion is hardly correct because in the experiments with 1 M hydroxylamine (the damage caused to the DNA was not repaired by UV-specific endonuclease (Soyfer 1972a)) the maximum of mutation was demonstrated in both strains of bacteria at the same doses of mutagenic treatment and at the same levels of survival. Therefore, the data from experiments with mutagenesis under UV-irradiation and HA-treatment confirmed the hypothesis of the role of repair enzymes (Soyfer 1969) and, mainly, repair endonucleases (Soyfer 1972a) in the formation of the maximum.

Another result of our experiments is that the prevalence of the frequency of complete mutations over mosaics was observed on the model of *E. coli uvr*$^+$. This is in total agreement with earlier observations of Soyfer (1972a). Such a prevalence was not observed in bacteria defective in UV-specific endonuclease.

It is rather difficult to explain more efficient recombinational repair in *uvr A*$^-$ cells compared to *uvr*$^+$ cells by the differences in the number of nucleoids per cell or by the quantity of DNA per cell. Three groups of facts speak for the conclusion on the role of UV-endonuclease in the mutagenesis: (i) the absence of maximum of mutation in *uvr A6* cells irradiated by UV-rays; (ii) the absence of correlation between the distribution of cells with different number of nucleoids per cell and the distribution of mosaic sectors in *uvr*$^+$ and *uvr A*$^-$ cells irradiated both in enriched and poor media; and (iii) the presence of a wide distribution of types of mosaic mutations (from 15/16 to 1/16).

The point of view that the *uvr*-dependent branch of repair does not take part in mutation production because the product of *uvr A* and *uvr B* genes, i.e., correndonuclease II, works without mistakes (Carrier and Setlow 1970) was widely held among geneticists (Witkin 1976). This view-point can be substantiated by the experimental data. Experiments with crude extracts revealed that this enzyme has barely 10% activity when located on the DNA strand opposite to that containing the pyrimidine dimers (Carrier and Setlow 1970). Another experiment showed that a maximum of one break in the unirradiated, as opposed to 70 breaks in the UV-irradiated, strands occurred when heavily UV-irradiated bacterial DNA was analysed in vitro (Paribok and Tomilin 1971). More precise studies with T4 phage UV-endonuclease has revealed that less than 2% of the breaks occur in the strand complementary to the strand containing the dimers (Simon et al. 1975). Thus, these data clearly demonstrate that mistakes of UV-specific endonuclease may take place and, therefore, may result in mutation production, especially at high UV-doses, as was assumed by Soyfer (Dubinin and Soyfer 1969; Soyfer 1969) and as was experimentally confirmed by Soyfer (1972a), by the works of other authors (Makino and Okada 1974; Roberts et al. 1974; Verly 1974) and by the present work.

It is now proven that different groups of repair enzymes of *E. coli* require various components of growth medium for their work. Apparently, with the transfer of the bacteria into a buffer repair proceeds mainly due to the activity of enzymes which are synthesized under the control of *uvr* genes and the *pol A* gene (Town et al. 1973). It was supposed that these enzymes did not insert many mutational mistakes into the repaired chains of DNA and, in addition, they finished their work within a very few minutes after irradiation (Paul et al. 1976).

Another type of repair depends on the activity of products of *exr*, *rec* and *pol C* genes. The enzymes involved in this type of repair require growth medium (Sedgwick and Bridges 1972; Smith 1975; Youngs and Smith 1973). Hence, it follows that under irradiation of bacteria in a rich nutrient broth the conditions will be favourable to the second group of enzymes and the frequency of mutation will be higher in comparison with the experiment in which bacteria were resuspended in a buffer without carbohydrates. This supposition was confirmed experimentally in the present work. First, the highest frequency of mutations (at the point of maximum) in the first series of experiments (an enriched growth medium) was almost 10 times higher than in the second series (with a buffer); second, this higher frequency was registered against the background of higher survival. The last effect may be seen, apparently, as a result not only of more active work by a system of *exr*, *rec* and *pol C* genes but also of more active work of *uvr* and *pol A* genes.

Yet, as was shown theoretically (Soyfer 1971), induc-

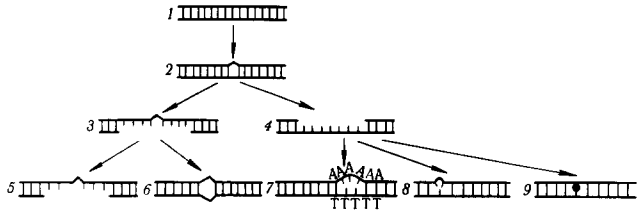

**Fig. 5.** Formation of mutations due to the errors of repair enzymes. *1* original DNA, *2* DNA with lesions recognized by repair endonucleases, *3* error of excision resulting in the removal of the region opposite the damaged one, *4* normal repair event at the point of a gap formation at the site of the exased damaged region, *5* a double-strand break according to the mechanism of a long-lived gap, *6* formation of a complete mutation, *7* insertion of an extra base under repair replication, *8* insertion of an extra base at the end of the repaired fragment during repair replication, *9* insertion of a wrong base during repair replication

tion of mutations dependent on the activity of repair enzymes may presumably result not only from the errors of endonucleases cutting the DNA but also from other repair reactions (Fig. 5). Many of these events come from the erroneous recognition of certain regions in DNA by repair enzymes or from 'conformational errors' as Soyfer called them earlier (1972b).

On the whole the results presented in this communication indicate an important role of UV-specific endonuclease in the mutagenesis of prokaryotes. Of course, we cannot say which concrete biochemical mechanism involved in the mutagenesis is mediated by the *uvr A* gene although many speculative hypothesis are presented recently (Cole et al. 1976; Sedgwick 1976; Witkin 1976) may be considered in this relation. At the present time data have been accumulated which indicate the influence of repair enzymes in mutagenesis not only in bacterial and animal cells, but in higher plants as well (Soyfer and Kartel 1978).

## Acknowledgement

We are indebted to Professor P. Howard-Flanders for providing us with the bacteria used in these studies.

## Literature

Carrier, W.L.; Setlow, R.B.: Endonucleases from *Micrococcus luteus* which has activity toward ultraviolet-irradiated deoxyribonucleic acid: purification and properties. J. Bacteriol. **102**, 178-186 (1970)

Cole, R.S.; Levitan, D.; Sinden, R.R.: Removal of psoralen interstrand crosslinks from DNA of *Escherichia coli*: mechanism and genetic control. J. Mol. Biol. **103**, 39-59 (1976)

Dubinin, N.P.; Soyfer, V.N.: Chromosome breakage and complete genic mutation production in molecular terms. Mutation Res. 8, 353-365 (1969)

Helmstetter, C.E.; Cummings, D.J.: Bacterial synchronization by selection of cells at division. Proc. Natl. Acad. Sci. (USA) **50**, 767-773 (1963)

Kelly, R.B.; Atkinson, M.R.; Huberman, J.A.; Kornberg, A.: Excision of thymine dimers and other mismatched sequences by DNA polymerase of *Escherichia coli*. Nature **244**, 495-501 (1969)

Kushner, S.R.; Kaplan, I.C.; Ono, H.; Grossman, L.: Enzymic repair of deoxyribonucleic acid. IV. Mechanism of photoproduct excision. Biochemistry **10**, 3325-3334 (1971)

Lieberman, M.W.; Poirier, M.C.: Base pairing and template specificity during deoxyribonucleic acid repair synthesis in human and mouse cells. Biochemistry **13**, 5384-5388 (1974)

Makino, R.; Okada, S.: Comparative studies of the effects of carcino-genic and antitumor agents on the DNA replication of cultured mammalian cells. Mutation Res. **23**, 387-394 (1974)

Nischioka, H.; Doudney, C.O.: Different modes of loss of photoreversibility of mutation and lethal damage in ultraviolet-light resistant and sensitive bacteria. Mutation Res. 8, 215-228 (1969)

Nischioka, H.; Doudney, C.O.: Different modes of loss of photoreversibility of ultraviolet-light induced true and supressor mutations to tryptophan independence in an auxotrophic strain of *E. coli*. Mutation Res. 9, 349-358 (1970)

Ogur, M.; John, R.; Nagai, S.: Tetrazolium overlay technique for population studies of respiration deficiency in yeast. Science **125**, 928-929 (1957)

Paribok, V.P.; Tomilin, N.V.: Recognition of pyrimidine dimers in DNA by the incision enzyme from *Micrococcus lysodeikticus*. Nature **230**, 210-211 (1971)

Paul, C.; Taylor, R.; Friedberg, E.C.: Kinetics of thymine dimer excision from phage T4 DNA in vivo. Biophys. J. **16** (2) part 2, pp. 58 (1976)

Roberts, J.J.; Sturrock, J.F.; Ward, K.N.: Enchancement by caffeine of alkylation induced cell death, mutations and chromosome aberrations in Chinese hamster cells as result of inhibition of postreplicative repair. Mutation Res. **26**, 129-143 (1974)

Sedgwick, S.G.: Misrepair of overlapping daughter strand gaps as a possible mechanism for UV-induced mutagenesis in *uvr* strains of *Escherichia coli*: a general model for induced mutagenesis by misrepair (SOS repair) of closely spaced DNA lesions. Mutation Res. **41**, 185-200 (1976)

Sedgwick, S.G.; Bridges, B.A.: Survival, mutation and capacity to repair single-strand DNA breaks after gamma irradiation in different *exr*$^-$ strains of *E. coli*. Molec. Gen. Genet. **119**, 93-102 (1972)

Simon, T.J.; Smith, C.A.; Friedberg, F.C.: Action of bacteriophage T4 ultraviolet endonuclease on duplex DNA containing one ultra-violet-irradiated strand. J. Biol. Chem. **250**, 8748-8752 (1975)

Smith, K.C.: The inhibition of DNA repair processes. Proc. XI. Intern. Cancer Congr. 5, 105-110 (1975)

Soyfer, V.N.: The Molecular Mechanisms of Mutagenesis. (in Russian) Moscow: Publ. House 'Nauka' 1969

Soyfer, V.N.: Neueste Forschungsergebnisse und Perspektiven der Mutationstheorie. Moderne Medizin **1**, 134-141 (1971)

Soyfer, V.N.: Participation of the intracellular enzymes in the control of mutation process. In: Molecular mechanisms of genetic processes. (in Russian) pp 364-377. Moscow: 1972a

Soyfer, V.N.: The role of the DNA molecules conformation in the initiation of dark repair and possible consequences of 'conformational mistakes' of the repair systems. In: Abstr. IV. intern. biophys. congr., Sections V-VII. pp. 320-321 (1972b)

Soyfer, V.N.; Kartel, N.A.: Participation of the intracellular enzymes in the control of mutation process. Part 2: Influence of

inhibition of repair and replication on X-ray induced chromosomal aberrations in barley. Theor. Appl. Genet. **53**, 9-15 (1978)

Soyfer, V.N.; Titov, Yu.B.; Kartel, N.A.; Sen', L.A.: The role of the mistakes of repair enzymes in the induction of genic and chromosome mutations. Bull. Acad. Sci. USSR Biol. **3**, 363-373 (1977)

Titov, Yu.B.; Soyfer, V.N.: Differences in the inhibition of cell division and DNA synthesis by ultraviolet irradiation of *E. coli* *uvr*$^+$ and *uvr A6*. Effects of hydroxyurea on DNA synthesis in irradiated cells. Mol. Biol. **11**, 84-91 (1977) (transl. by Plenum Publ. Co.)

Town, C.D.; Smith, K.C.; Kaplan, H.S.: Repair of X-ray damage to bacterial DNA. Current topics Radiat. Res. **8**, 351-399 (1973)

Verly, W.G.: Monofunctional alkylating agents and apurinic sites in DNA. Biochem. Pharm. **23**, 3-8 (1974)

Witkin, E.M.: Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli* B differing in sensitivity to UV-light. Brookhaven Symp. Biol. **20**, 17-55 (1967)

Witkin, E.M.: Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli* Bacter. Rev. **40**, 869-907 (1976)

Youngs, D.A.; Smith, K.C.: X-ray sensitivity and repair capacity of a *pol AI exr A* strain of *E. coli* KI2. J. Bacter. **114**, 121-127 (1973)

Received February 15, 1978
Accepted February 28, 1979
Communicated by D.K. Belyaev

Dr. V.N. Soyfer
Chertanovskayastr. 39
Building 2, apt. 131
Moscow 113519 (USSR)