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Cell Division and DNA Synthesis in *uvrA recA* Double Mutants of *E. coli* K12

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Summary. Cell division and incorporation of ^{3}H-thymidine into acid-insoluble fraction were investigated for three *uvrA recA* double mutants of *E. coli* K12 irradiated with UV at 1.5 ergs/mm^2, producing about ten pyrimidine dimers per genome (about 0.01% survival). Cell division was measured both in M9 medium and in the same medium which was made very viscous by the addition of Metlose (the same product as Methocel used by Lin *et al.*, 1971). It was found that a major fraction of irradiated bacteria continues to divide once or twice and stops thereafter. Incorporation of ^{3}H-thymidine proceeded at a considerable rate for a short period following irradiation and then stopped. During subsequent incubation, the incorporation gradually decreased and after 4 h incubation most of the early incorporated radioactivity disappeared from the acid-insoluble fraction. These results indicate that cell division occurs after irradiation without parallel DNA synthesis as in a *recA thy* mutant of *E. coli* K12 deprived of thymine (Inouye, 1971). These results suggest that UV irradiation increases lethal sectoring due to the "reckless" cell division without parallel DNA synthesis. Since DNA synthesis took place only for a short period after irradiation, it may be assumed that the *recA* gene normally has at least a dual function; 1. elimination of damage induced by UV to support elongation or initiation of DNA, and 2. maintenance of coordination between DNA synthesis and cell division.

Introduction

Most of the UV-induced lethal damages in *E. coli* DNA can be repaired in the dark by the excision and recombinational repair systems (see reviews: Howard-Flanders, 1968; Witkin, 1969). In fact, a strain of *E. coli uvrA recA* lacking both of these repair systems is killed by less than two pyrimidine dimers per genome (Howard-Flanders *et al.*, 1969). However, the process leading to cell death induced by such a small number of pyrimidine dimers is still unknown, mainly because of the lack of analysis of events induced by a small dose of UV light in the repair-defective cells.

The study presented in this report was performed so as to know the characteristics of cell division and of DNA synthesis of *uvrA recA* double mutants of *E. coli* K12 following irradiation with UV at 1.5 ergs/mm^2, producing approximately ten pyrimidine dimers per genome (Shlaes *et al.*, 1972).

It will be shown here that irradiated mutants undergo cell division once or twice without parallel DNA synthesis, as in a *thyA recA* mutant of *E. coli* K12 deprived of thymine (Inouye, 1971).

Materials and Methods

1. Bacterial Strains

The bacterial strains used in the present study were K284, K106 and URT-4328. K284 is a *uvrA6 recA1* mutant of KMBL 49 (Van de Putte *et al.*, 1965). It was constructed in the follow-

ing way: Bacteriophage P1 grown on AB1886 *uvrA6 malB* (isolated from AB1886 *uvrA6* by treatment with N-methyl-N′-nitro-N-nitrosoguanidine) infected KMBL 49 *metA* (isolated from KMBL 49 by treatment with N-methyl-N′-nitro-N-nitrosoguanidine), and among Met^+ transductants Mal^+UV-sensitive ones were scored. To one of the transductants thus obtained, the *recA1* gene was introduced by mating with N211 HfrC *recA1* (isolated by H. Ogawa from the mating, JC1569 *recA1* × W2252 HfrC). Thy^+ recombinants were isolated first and extremely UV sensitive ones were then scored. K284 requires threonine, leucine and uracil for growth. K106 was a gift from Dr. H. Ogawa, and it carries mutations *rec-42* and *uvrA* (This mutant designated N213 was used also by Horii and Suzuki, 1970). URT-4328 is a *recA* derivative of URT-43 (Shimazu, *et al.*, 1971). The latter strain, exhibiting a temperature-sensitive recovery after UV irradiation, has turned out to carry a mutation in the *uvrA* cistron (Shimazu, manuscript in preparation). URT-4328 requires threonine, leucine and uracil for growth.

2. Media

M9 medium (Adams, 1959) supplemented with Casamino acids (0.2%) and necessary requirements (complete M9) was used throughout the study. Bacteria were grown overnight in complete M9 at 37° and the culture was diluted fifty-fold with fresh complete M9 and incubated for 3 to 4 h at 37° to reach about 5×10^8 bacteria per ml. They were washed with M9 buffer before being submitted to experiments.

A very viscous medium used for visualizing chains of bacteria originating from a single ancestor was prepared according to Lin *et al.* (1971). In brief, 1.5 g of Metlose (Shin-etsu Kagaku Kogyo Co. Ltd.; 90 SH, 4000) was dissolved in 50 ml of complete M9 medium and the solution was divided into 2 ml portions. After sterilization by heating for 10 min at 80°, they were stored in a refrigerator before use (Metlose-M9 medium).

3. Cell Counting

The number of cells growing in normal liquid M9 medium was counted with a Petroff-Hauser chamber under a phase-contrast microscope. Counts of the number of cells in chains grown in viscous Metlose-M9 medium was performed according to Ln *et al.* (1971). The washed bacteria were suspended in M9 buffer at a concentration of about 10^9 per ml and a drop of the suspension was mixed with 2 ml of Metlose-M9 medium and a small portion was spread on a slide glass using an aluminium foil-covered slide glass divised by Lin *et al.* (1971). The medium spread on a slide glass was covered with liquid paraffine to avoid evaporation and incubated in the dark. After incubation, liquid paraffine was removed with n-pentane and the slide glass was treated successively with 10% p-toluenesulfonyl chloride in pyridine, 10% sodium carbonate and finally with distilled water. Cell counts were made under a phase-contrast microscope or on photographic prints.

4. Other Methods

Incorporation of ^{3}H-thymidine (methyl-^{3}H, Radiochemical Center, Amersham: 15.3 Ci/m mole) into acid-insoluble fractions was performed by the procedure described previously (Morimyo *et al.*, 1970).

Irradiation with UV light was carried out under a low pressure mercury vapour lamp (5W) emitting mostly the 253.7 nm light. Incident light was filtered through two sheets of wrapping film (Kureha Kagaku Co. Ltd.). Spectrophotometric analysis indicated that a sheet absorbs about 70% of 253.7 nm light. The incident UV energy was measured with a Toshiba UV meter with a full scale of 200 $\mu W/cm^2$. All handlings after irradiation were done in the dark or under a sodium vapour lamp.

Results

A. Cell Division Following UV Irradiation

Increase in the concentration of irradiated (1.5 $ergs/mm^2$) and unirradiated cells of K284 in liquid M9 medium is shown in Fig. 1. The concentration of un-

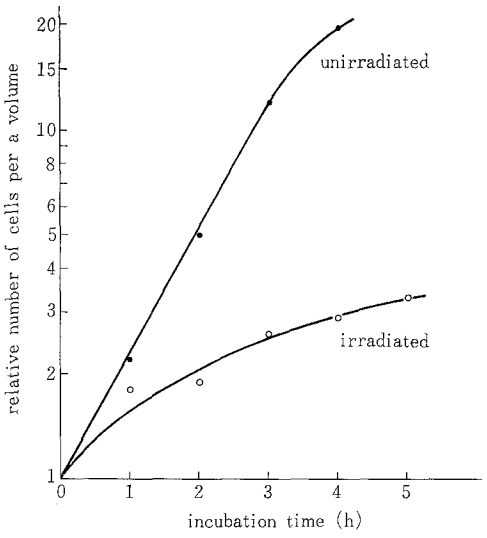

Fig. 1. Effect of UV irradiation on the growth of K284. A portion of the washed cell suspension was irradiated with UV and the rest was used as a control. To both suspensions were added threonine, leucine (30 μg/ml) for each), uracil (20 μg/ml), Casamino acids (0.2%) and glucose (0.2%), and the mixtures were incubated at 37°. At time intervals indicated portions were used for cell counting. The cell concentration at the beginning of incubation was 2.4×10^8/ml by the unirradiated control (closed circles) and 4.4×10^7/ml by the irradiated culture (open circles)

irradiated cells increased almost linearly on a semilogarithmic scale, hence the mean division time was easily determined to be 54 min. The mean division time of cells growing in Metlose-M9 medium could be estimated to be 56 min from the number of total cell in chains and the number of cell chains scored. Therefore, cell division proceeded at a similar rate in both media. Nearly the same results were obtained for URT-4328; the mean division time in liquid M9 and in Metlose-M9 medium were 55 min and 52 min, respectively.

When K284 was irradiated (0.01% survival) and incubated in liquid M9 medium, the concentration increased two-fold in about 2 h and the rate of increase slowed down thereafter. At 5 h or later, the cell concentration was from 3 to 4 times the initial concentration. This means that, as an average, a fraction of the cell population had divided twice and the rest once. Similar results were obtained for URT-4328 and K106 (data not shown). Therefore, it may be concluded that mutants defective in both excision and recombinational repair are capable of continuing cell division once or twice following UV irradiation.

However, the above estimations may only be true on average, and there is a possibility that only a small fraction of the cell population continued to divide very rapidly, while the rest did not. In order to check this possibility, experiments were carried out in which successively dividing cells were fixed to form a cell chain of descendants (Lin *et al.*, 1971). At definite times after the beginning of incubation, the culture in Metlose-M9 medium on slide glasses was treated as described briefly in Materials and Methods. Microscopic photographs were taken and the number of

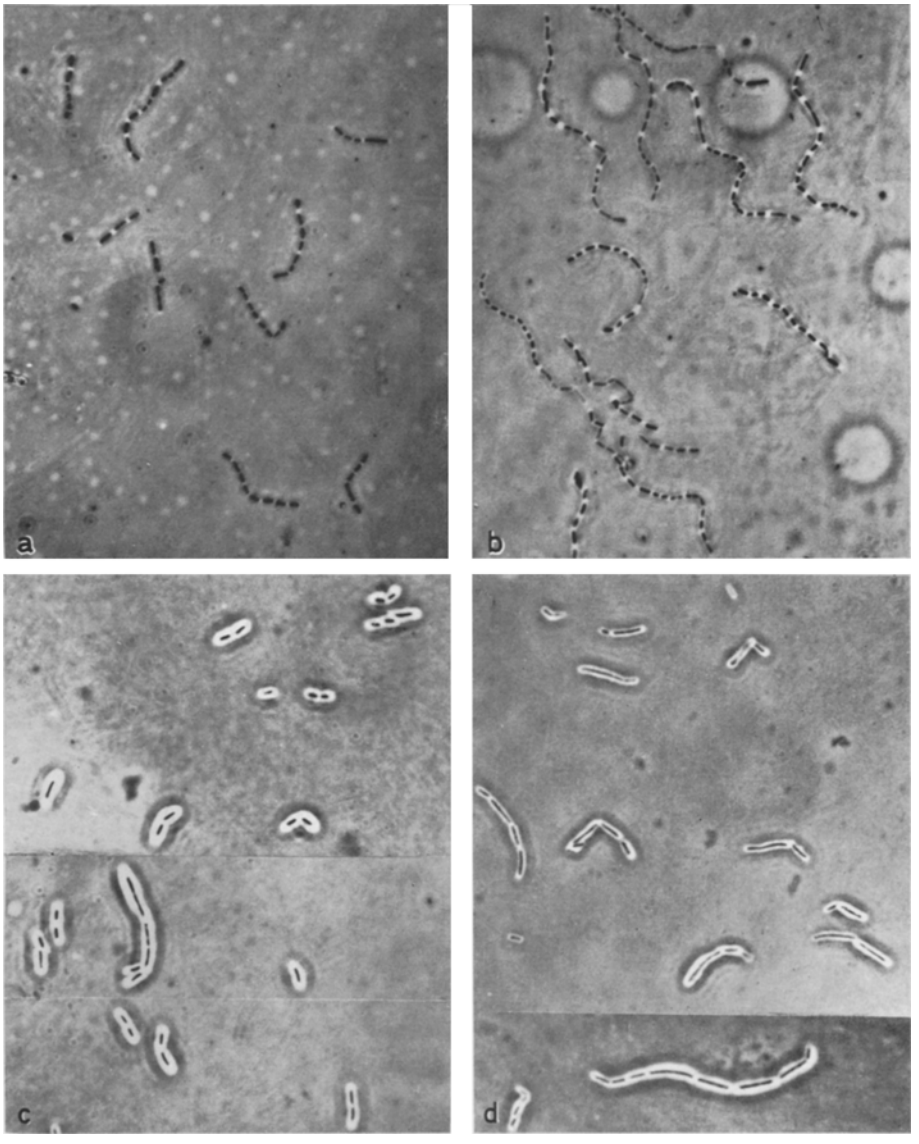

Fig. 2a—d. Effect of UV irradiation on the growth of cell chains in Metlose-M9 medium (K284). The supplements to the medium have been described under Fig. 1 and the method is described in Materials and Methods. Photographs were taken with a magnifying factor of 600 and enlarged to photoprints by about five times in length. (a) unirradiated, 2 h, (b) unirradiated, 3 h, (c) irradiated, 2 h, (d) irradiated, 4 h

cell in chains was counted. Typical examples of chain growth from unirradiated and irradiated K284 cells are shown in Fig. 2. In Fig. 3 are shown histograms indicating on the abscissa the number of cells in a chain and on the ordinate the fraction of the chains carrying the indicated number of cells. It can be seen in Fig. 2a

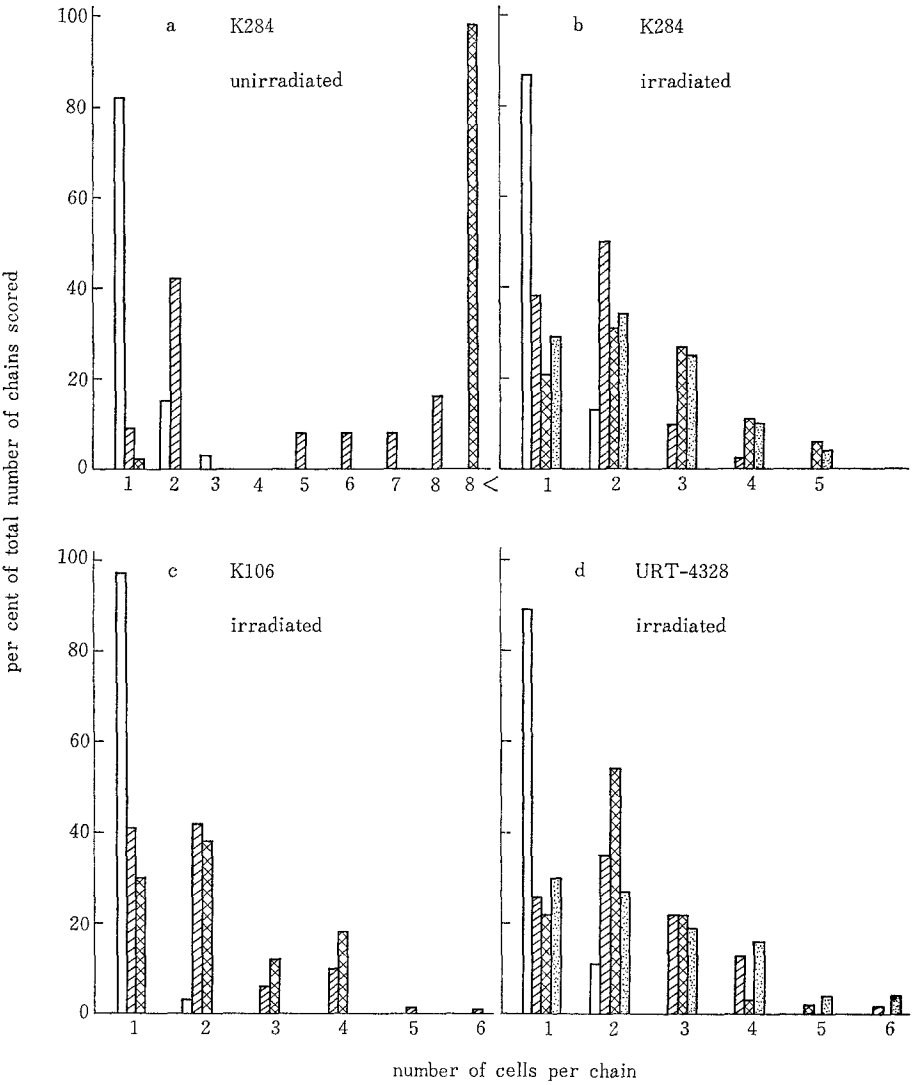

Fig. 3a—d. Effect of UV irradiation on the growth of cell chains in Metlose-M9 medium (K284, K106 and URT-4328). The method has been described in Materials and Methods and under Fig. 2. The number of cell was counted with photoprints. Actual number of cell chains scored was more than 100 for zero-time control and more than 200 for other cases. 0 h, open bar; 2 h, singly shadowed bar; 4 h, doubly shadowed bar; 6 h, bar shadowed with dotts

and b that unirradiated bacteria form long chains containing cells of nearly uniform size, and Fig. 3a shows that at 2 h most of the unirradiated cells have completed the first division (more than two cells in a chain) and a considerable fraction (about fifty per cent) has finished the second or third division. At 3 h, most of the chains contain more than eight cells, indicating that the third division has been

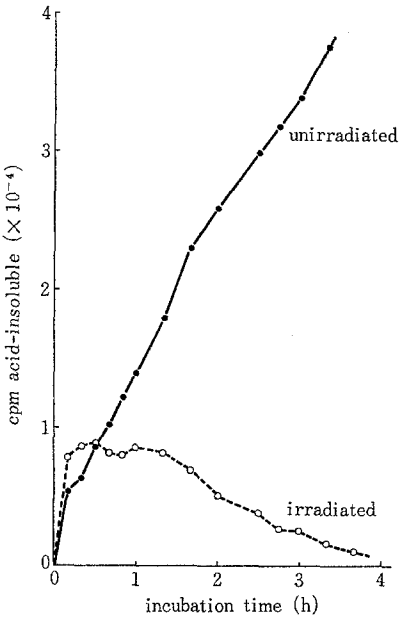

Fig. 4. Effect of UV irradiation on the incorporation of ^{3}H-thymidine into K284 cells. A portion of washed cell suspension was UV irradiated and the rest was used as a control. After addition of requirements described under Fig. 1, ^{3}H-thymidine was added (17 μCi/ml) and the mixtures were incubated at 37°. At time intervals, 0.1 ml portions were used for assay of the radioactivity in acid-insoluble fraction. Unirradiated, closed circles with a solid line; irradiated, open circles with a broken line

completed. These results are in good agreement with those obtained in experiments using liquid M9 medium instead of Metlose-M9 medium (see Fig. 1). Similar results were aslo obtained for unirradiated cells of K106 and URT-4328 (data not shown).

In view of the histograms shown in Fig. 3 b, c and d, irradiated K284, K106 and URT-4328 cells give a qualitatively similar pattern of distribution. Namely, during incubation for 2 h the fraction of undividing single cells is markedly decreased, while the fraction of cell chains containing two cells is increased. Prolonged incubation increases somewhat the fraction of chains carrying more than two cells, but the fraction of longer cells chains remains very small. These results may indicate that a large portion of irradiated cells of double mutants *uvrA recA* continue to divide once or twice within 2 to 6 h and these data are in agreement with those shown in Fig. 1. Therefore, it may be concluded that the increase in the concentration of irradiated bacteria shown in Fig. 1 is not due to the presence of a small fraction of very rapidly dividing cells, but due to a rather large fraction (about 70%, see Fig. 3 b, c and d) which undergoes division.

It must be noted in this connection that the uniformity of cell size was lost in irradiated bacteria as compared to unirradiated bacteria. As can be seen in Fig. 2 c and d, there are cells with a length several times the normal size.

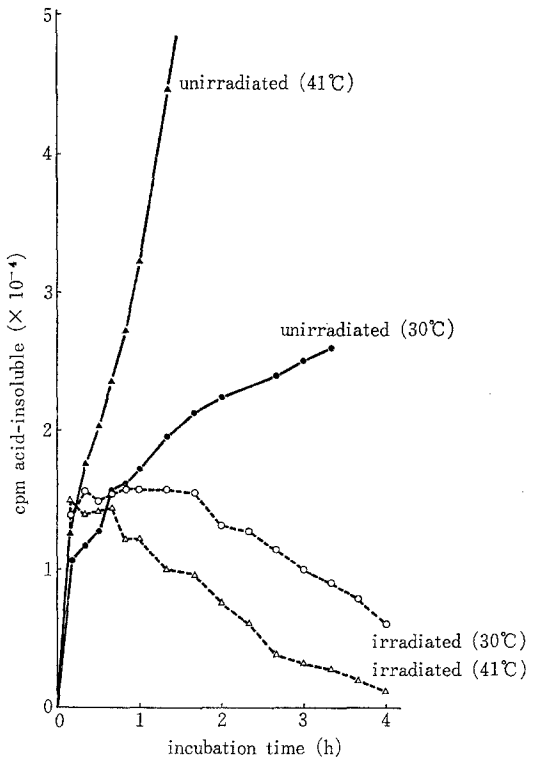

Fig. 5. Effect of UV irradiation of the incorporation of ^{3}H-thymidine into URT-4328. The method was the same as has been described under Fig. 4, except for the temperature of incubation (30° or 41°). Unirradiated, closed circles (30°) or closed triangles (41°) with solid lines; irradiated, open circles (30°) or open triangles (41°) with broken lines

B. DNA Synthesis in UV Irradiated uvrA recA Mutants

According to Howard-Flanders, *et al.* (1969), DNA synthesis in a *uvrA recA* mutant of *E. coli* K12 is blocked almost completely after irradiation with UV at 20 ergs/mm^2. Since a much smaller dose of UV was applied in the present study, there was a possibility that the residual cell division described in the above section would be dependent on a residual DNA synthesis. In order to check this possibility, incorporation of ^{3}H-thymidine into acid-insoluble fraction was investigated with K284 cells irradiated with UV. The results are shown in Fig. 4.

It can be seen that, while the incorporation into unirradiated bacteria increases for more than 3 h, the incorporation into irradiated bacteria proceeds at a rate almost similar to the rate of unirradiated bacteria for 20 min, and stops thereafter. After about 90 min, the incorporated radioactivity begins to decrease gradually, and at 4 h the majority of the radioactivity is released from the acid-insoluble fraction.

Similar results were obtained with URT-4328. As can be seen in Fig. 5, unirradiated cells incorporated ^{3}H-thymidine more rapidly at 41° than at 30°, while the incorporation into irradiated cells took place only for the first 10 min

and the radioactivity gradually decreased thereafter irrespective of the temperature of incubation. Therefore, despite the potential ability for recovery of this double mutant at a low temperature (Shimazu *et al.*, 1971), post-irradiation DNA synthesis was blocked in a short time and the newly incorporated radioactivity was eventually released from the acid-insoluble fraction even at 30°.

It may be concluded, therefore, that UV irradiation does not block DNA synthesis completely, but that the synthesis continues for some time at a considerable rate and is followed by a breakdown of the newly synthesized DNA. The instability of the newly synthesized DNA in stationary phase cells of K106 has already been demonstrated by means of pulse-labeling-chase experiments (Horii and Suzuki, 1970). The present results confirm their results using other *uvrA recA* mutants and at a much lower UV dose.

Discussion

Mutants of *E. coli* defective in both excision and recombinational repairs are extremely sensitive to UV irradiation. Like the mutant used by Howard-Flanders *et al.* (1969), the mutants used in the present study lost their viability with less than two pyrimidine dimers per genome. Then, why one or two dimers can kill a cell?

Of some possibilities, degradation of DNA is not a likely candidate, because the characteristic "reckless" DNA breakdown in *recA* cells (Clark *et al.*, 1966; Horii and Suzuki, 1968) is partially diminished in the *uvr recA* double mutants (Horii and Suzuki, 1970; Yonei and Nozu, 1972), although the latter mutants are much more sensitive to UV irradiation than the former. A possible cause for cell death would be the block in DNA synthesis. The present data show that a very small UV dose seriously affects the incorporation of ^{3}H-thymidine in a characteristic fashion, suggesting that there is a residual DNA synthesis for a short period after irradiation which is followed by a breakdown of newly synthesized DNA.

As for the early termination of DNA synthesis, it could be assumed that the replication is blocked at a pyrimidine dimer (Horii and Suzuki, 1968, 1970) or at the initiation point. Although we have no evidence to support either one of these two opinions, it would be possible to imagine that an unexcised pyrimidine dimer can not allow replication to get over the dimer in *uvrA recA* cells, while the *uvrA* mutant can continue replication producing strands with gaps (Rupp and Howard-Flanders, 1968; Smith and Meun, 1970). If this view is correct, the *recA* gene operates to allow the replication enzyme to jump over a dimer, presumably by a mechanism related to the gap filling (Smith and Meun, 1970). Replication of the healthy strand opposite the dimer-carrying strand appears to be blocked also. This would be due to a symmetry required for the replication machine. There is also a possibility that DNA replication stops at the terminal end or at the initiating point. If this were the case, we must consider some disturbance in DNA initiation induced by the presence of only ten pyrimidine dimers. On the basis of the idea that DNA initiation or replication requires certain structural assembly involving RNA and membrane fraction in addition to DNA template (Lark, 1972), it would be possible to think that a small number of unrepaired pyrimidine dimers alters the tertiary structure of the DNA initiation apparatus.

The present results give an additional information on the cell division of irradiated *uvrA recA* cells. Despite a very small amount of DNA synthesized, these cells were capable of undergoing cell division once or twice during post-irradiation incubation. These results are consistent with those of Inouye (1971) on a *recA thyA* mutant of *E. coli* K12 deprived of thymine. It could be assumed, then, that daughter cells originated from an irradiated parent must be different from normal daughter cells at least in the contents of DNA. According to Haefner (1968) and Capaldo-Kimball and Barbour (1971), the viability of unirradiated *recA* cells is low as compared to *recA*$^+$ cells. Then it may be possible to think that UV irradiation increases lethal sectoring due to the "reckless" cell division without parallel DNA synthesis. If this view is correct, the *recA* gene would normally have at least a dual function in UV irradiated cells; 1) elimination of damage induced by pyrimidine dimers in initiation or elongation of DNA, and 2) maintenance of coordination between DNA synthesis and cell division. Further investigations will be necessary to confirm the validity of these views.

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