MASTER

A SPECIAL TYPE OF UV-STIMULATED RECOMBINATION IN HAEMOPHILUS INFLUENZAE

Evelyn Waldstein*, Jane K. Setlow and Loretta Santasier

Biology Department, Brookhaven National Laboratory, Upton, NY 11973 (USA)

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

*Present address: Department of Biochemistry, Tel-Aviv University,
Tel-Aviv, Israel

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Running Head: UV-Stimulated Recombination

Send Proofs to:

Jane K. Setlow
Biology Department
Brookhaven National Laboratory
Upton, NY 11973
516-345-3420

Following UV irradiation, the DNA of wild type H. influenzae undergoes extensive exchange between strands synthesized before and after irradiation (Small, 1975). There are apparently many more exchanges than UV-induced pyrimidine dimers (Small, 1975), unlike the situation in Escherichia coli (Rupp et al., 1971). Such UV-induced exchange does not take place in the highly recombination-defective rec1 strain of H. influenzae, but is extensive in the even more recombination-defective rec2 strain. These events are correlated with the filling of the single-strand gaps made in DNA synthesized after UV; in rec2 the gaps are filled normally, but there is little or no gapfilling in rec1 (LeClerc and Setlow, 1972). Normal DNA replication is not required for gapfilling, since it takes place in a temperature-sensitive DNA synthesis mutant at a temperature restrictive for DNA synthesis (Small, 1975). Thus it is probable that in H. influenzae exchange is more important for gapfilling than is de novo synthesis.

In order to find out more about UV-induced exchange, we have examined the question of whether dimers originally in DNA synthesized before irradiation could be shifted into the DNA made after irradiation by way of such a mechanism.

MATERIALS AND METHODS

Wild type H. influenzae (strain Rd) and derivatives of this strain, rec1, uvr1 and rec2 were used. The isolation and properties of these strains have been described (Setlow et al., 1968a, Beattie and Setlow, 1971, Setlow et al., 1972, Notani et al., 1972, Kooistra and Setlow, 1976). Growth medium for cells, conditions for growth and UV irradiation were as before (Setlow et al., 1968a).

In general, the experiments were patterned after those of Ganesan (1974), with some modifications. The general scheme of the experiments is shown in Figure 1. Cells were shifted from radioactively labeled to unlabeled medium by centrifugation. UV doses were approximately 1.5 J/m^2 . Determination of dimers in DNA labeled with $^{14}\text{CdThd}$ ($3 \mu\text{Ci/ml}$, 54 Ci/mole) before UV and labeled with $^3\text{HdThd}$ ($100 \mu\text{Ci/ml}$, 52 Ci/mmole) after UV was done by making the cells permeable to an endonuclease from Micrococcus luteus which makes nicks to UV-induced pyrimidine dimers, then incubating the cells with and without the endonuclease, and finally measuring the single-strand molecular weight of the ^{14}C - and ^3H -labeled DNA by velocity sedimentation in alkali. Permeabilizing the cells was carried out at room temperature in buffer (Setlow et al., 1968a) with 1% Mallinckrodt toluene for 2 min. The M. luteus enzyme, a gift from Dr. Farid Ahmed, was prepared according to Carrier and Setlow (1970) through the ammonium sulfate step, and then thoroughly dialyzed against 0.02 M Tris buffer, pH 8, containing 0.04 M NaCl and 0.002 M EDTA. Treatment of the samples with the enzyme at 0.5 mg/ml was for 30 min at 37°C in the same buffer as used for toluenization. Cells were then layered in a 0.1 ml volume on a 5-20% alkaline sucrose gradient, previously topped by a 0.3 ml layer of 0.5 M NaOH plus 0.01 M EDTA. The sucrose was in a solution of 0.5 M NaCl, 0.2 M NaOH and 0.01 M EDTA. Centrifugation was for 90 min at 30,000 rpm in a SW 50.1 rotor of a Beckman ultracentrifuge. Fractions were dripped onto paper, processed as previously described (Carrier and Setlow, 1971) and counted in a toluene-based scintillator in a Searle Analytic 92 counter. Weight average molecular weights (M_w) were obtained from the distribution of radioactivities, and single-strand

breaks per unit molecular weight were calculated as $1/(M_w/2)$. The number of endonuclease-sensitive sites were computed from the difference in the number of single-strand breaks in samples treated and untreated with the enzyme. In unirradiated cells the enzyme preparation caused around 0.3 breaks/ 10^8 daltons in the DNA. Corresponding values for irradiated cells in these experiments were at least an order of magnitude higher.

RESULTS

Redistribution of dimers. Figure 2 shows a typical set of sedimentation profiles for DNA of wild type cells irradiated with a dose of about 1.5 J/m^2 at 254 nm. The labeling scheme is that of Fig. 1, except that there was no incubation time between UV and the ^3H pulse. The left-hand panels are profiles for cells labeled with $^{14}\text{CdThd}$ before UV, and then the DNA was sedimented after UV but before the 10 min incubation with $^3\text{HdThd}$. The endonuclease-treated sample sediments much more slowly than the untreated sample, showing that the enzyme has made nicks in the DNA. The breaks made by the enzyme are about 3/4 of the number of dimers (the latter calculated as 3.5 per 10^8 daltons from previous measurements). The other panels show profiles of DNA prelabeled with ^{14}C and pulse labeled with ^3H after UV, from cells immediately after the pulse and also 30 min later.

In the case of both time points the enzyme makes approximately as many breaks in the ^3H DNA as in the ^{14}C DNA, indicating that the dimers become rapidly redistributed between old and new strands. The size of the ^3H DNA at the early time is rather heterogeneous, without enzyme treatment. The enzyme eliminates the faster sedimenting ^3H

DNA. This faster sedimenting material presumably has had gaps filled with dimer-containing parental DNA. At the later time there are apparently no more gaps in the ^3H DNA, since without enzyme treatment it sediments like the parental DNA.

Excision of dimers from parental and new DNA. Since the wild type cells are capable of excision of pyrimidine dimers (Setlow et al., 1968a,b), the number of dimers in the DNA decreases with time of incubation after UV irradiation. Figure 3 shows the kinetics of this process in the DNA labeled before and after UV. Although there is considerable uncertainty in the number average molecular weight of the ^3H -labeled DNA at the earliest time because of the biphasic type of curve, nevertheless it appears that the rate of loss of dimers is similar from the daughter as from the parental DNA, suggesting that excision rate is independent of the distance of dimers from the replication fork.

Earliest time by redistribution of dimers. We have interpreted Figure 2 to mean that even by 1 min following UV irradiation there has been extensive exchange between parental DNA and DNA synthesized after UV, resulting in very early redistribution of dimers. An alternative explanation is that the ^3H DNA made during the pulse is added on to unlabeled parental DNA, and that the enzyme-induced breaks observed in the ^3H DNA are caused by breaks made in the adjoining unlabeled DNA. If this interpretation is correct, then one might expect to find breaks in the ^3H strand even in the absence of recombination between new and old strands.

Table 1 shows results of an experiment in which the time between UV and the ^3H pulse was varied, and the samples were taken immediately after the pulse. The experiment was done with two strains, the wild type and the rec1 mutant. Since dimer redistribution would not be expected to occur in the latter strain because of its inability to fill single-strand gaps in DNA synthesized after irradiation (LeClerc and Setlow, 1972), and because of the lack of UV-induced exchange between new and old strands observed in density shift experiments (Small, 1975), it is of interest that breaks in the ^3H strand by the endonuclease are observed in this strain only when the pulse is given immediately after the irradiation. In the case of the wild type strain, however, there is evidence of such breakage at all the times shown. Since the synthesis of DNA after UV is inhibited almost a factor of ten more strongly in rec1 than in the wild type (Modak and Setlow, 1969), it is probable that the dimers are redistributed among the old and new DNA strands in the wild type at times earlier than 15 min. It is clear, however, that when the interval is 15 min or more between UV irradiation and pulse, the redistribution of dimers in the wild type strain is finished by the completion of the pulse. Therefore in most experiments 20 min was allowed to elapse between irradiation and pulse (Fig. 1). Note that the data of Table 1 show evidence of excision in rec1, in accord with previous work (Setlow et al., 1968b).

Exchange of dimers in mutants. Table 2 shows that in excision-defective uvr1 and in rec2 there is also exchange leading to appearance of endonuclease-sensitive sites in DNA synthesized after irradiation. The redistribution of dimers is apparently similar to that in the wild type, although no excision takes place in uvr1.

DISCUSSION

We have seen that dimers in wild type, uvr1 and rec2 rapidly become equally distributed between daughter and parental strands. This phenomenon is in some respects similar to that found in E. coli (Ganesan, 1974), except that there appears to be considerably more exchange of material between new and old strands in H. influenza and E. coli (Rupp et al., 1971, Small, 1975). It is not clear whether the exchanges that seem to take place at sites other than those of dimers contribute to the dimer exchange.

The dimer exchange in bacteria is in marked contrast to the situation in human cells, in which endonuclease-sensitive sites in daughter strands probably don't occur, since breaks are seen in daughter strands only when there is a relatively short interval between irradiation and pulse, indicating that under these conditions the pulse label is attached to unlabeled parental strand during centrifugation (Lehmann and Kirk-Bell, 1978).

The recombination that leads to dimer exchange, which we consider to be the same process that closes single-strand gaps in DNA caused by synthesis from a dimer-containing template, is apparently a different type from the recombination of transforming DNA or phage DNA, since the UV-induced type of recombination is normal in the highly recombination-defective rec2. Table 3 summarizes some of the properties of two different Rec⁻ strains. The UV sensitivity of rec1 clearly results from its defect in postreplication repair. This strain is considered to be recombination-defective because of local breakdown at the site of recombination (LeClerc and Setlow, 1974). The rec2 mutant has been found to be unable to form the special type of DNA

containing single-strand regions, mostly as tailed structures, characteristic of newly-synthesized DNA in highly competent cells, and considered to be essential for recombination between transforming DNA and cell DNA (LeClerc and Setlow, 1974, 1975). It is possible that similar conversions of the form of intracellular phage DNA occur in wild type cells (but not in rec2) as a prelude to recombination. This may take place more readily in competent cells, which indeed show considerably higher phage recombination than do exponentially-growing cells (Boling, Setlow and Allison, 1972).

If the UV-induced type of recombination had certain steps in common with recombination of transforming DNA or phage DNA, it might be expected that the latter two types could be stimulated by UV. However, they are not (Boling and Setlow, 1969). Furthermore, since rec2 is normal in UV-induced recombination, it also might be expected that transformation in this strain could be enhanced by UV irradiation of the cells. However, transformation in this strain is even further depressed by such treatment of competent cells (J. Setlow, unpublished experiments). Thus although UV induces recombination between parental and daughter strands, it does not stimulate other types of recombination in H. influenzae.

ACKNOWLEDGMENTS

This research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Department of Energy. E.W. was partially supported by grant #636 awarded by the Israel-U.S.A. Binational Grant Foundation.

REFERENCES

- BEATTIE, K. L. and J. K. SETLOW. 1971. Transformation-defective strains of Haemophilus influenzae. Nature New Biol. 231: 177.
- BOLING, M.E. and J. K. SETLOW. 1969. Dependence of vegetative recombination among Haemophilus influenzae bacteriophage on the host cell. J. Bacteriol. 4: 240.
- BOLING, M. E., J. K. SETLOW and D. P. ALLISON. 1972. Bacteriophage of Haemophilus influenzae I. J. Mol. Biol. 63: 335.
- CARRIER, W. L. and R. B. SETLOW. 1970. Endonuclease from Micrococcus luteus which has activity toward ultraviolet-irradiated deoxyribonucleic acid: purification and properties. J. Bacteriol. 102: 178.
- CARRIER, W. L. and R. B. SETLOW. 1971. Paper strip method for assaying gradient fractions containing radioactive macromolecules. Anal. Biochem. 43: 427.
- GANESAN, A. 1974. Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated Escherichia coli K12. J. Mol. Biol. 87: 103.
- KOOISTRA, J. and J. K. SETLOW. 1976. Similarity in properties and mapping of three Rec mutants of Haemophilus influenzae. J. Bacteriol. 127: 327.
- LECLERC, J. E. and J. K. SETLOW. 1972. Postreplication repair of ultraviolet damage in Haemophilus influenzae. J. Bacteriol. 110: 930.
- LECLERC, J. E. and J. K. SETLOW. 1974. Transformation in Haemophilus influenzae. In: Mechanisms in Recombination (ed. R. F. Grell), p. 187. Plenum Press, New York.

- LECLERC, J. E. and J. K. SETLOW. 1975. Single-strand regions in the deoxyribonucleic acid of competent Haemophilus influenzae. J. Bacteriol. 122: 1091.
- LEHMANN, A. R. and S. KIRK-BELL. 1978. Pyrimidine dimer sites associated with the daughter DNA strands in UV-irradiated human fibroblasts. Photochem. Photobiol. 27: 297.
- MODAK, S. P. and J. K. SETLOW. 1969. Synthesis of deoxyribonucleic acid after ultraviolet irradiation of sensitive and resistant Haemophilus influenzae. J. Bacteriol. 98: 1195.
- NOTANI, N. K., J. K. SETLOW, V. R. JOSHI and D. P. ALLISON. 1972. Molecular basis for the transformation defects in mutants of Haemophilus influenzae. J. Bacteriol. 110: 1171.
- RUPP, W. D., C. E. WILDE III, D. L. RENO and P. HOWARD-FLANDERS. 1971. Exchanges between DNA strands in ultraviolet-irradiated Escherichia coli. J. Mol. Biol. 61: 25.
- SETLOW, J. K., M. E. BOLING, K. L. BEATTIE and R. F. KIMBALL. 1972. A complex of recombination and repair genes in Haemophilus influenzae. J. Mol. Biol. 68: 361.
- SETLOW, J. K., D. C. BROWN, M. E. BOLING, A. MATTINGLY and M. P. GORDON 1968a. Repair of deoxyribonucleic acid in Haemophilus influenzae I. J. Bacteriol. 95: 546.
- SETLOW, J. K., M. L. RANDOLPH, M. E. BOLING, A. MATTINGLY, G. PRICE and M. P. GORDON. 1968b. Cold Spring Harbor Symp. Quant. Biol. 33: 209.
- SMALL, G. D. 1975. Mechanism of gap-filling during postreplication repair of ultraviolet damage in Haemophilus influenzae. J. Bacteriol 124: 176.

Table 1. Endonuclease sites per 10^8 daltons

Time between UV and ^3H pulse (min)	Wild type		<u>rec1</u>	
	^{14}C	^3H	^{14}C	^3H
0	1.3	1.2	3.6	3.5
15	0.8	0.8	2.0	0
30	0.6	0.5		

All samples were taken immediately following the pulse given at various times after UV. Otherwise the labeling scheme was as in Figure 1.

Table 2. Endonuclease sites per 10^8 daltons in the excision-defective uvr1 and recombination-defective rec2 strains

Time after pulse (min)	<u>uvr1</u>		<u>rec2</u>	
	^{14}C	^3H	^{14}C	^3H
0	1.9	2.0	1.8	1.8
30	1.6	2.1	0.8	1.0

Labeling scheme as in Figure 1. Dose to uvr1 was 0.7 J/m^2 , to rec2 was 1.4 J/m^2 .

Table 3. Properties of Rec mutants of Haemophilus influenzae

	<u>UV sensitivity</u>	Postreplication repair <u>(gap-filling)</u>	Dimers appearing in DNA made <u>after UV</u>	Transformation relative to <u>wild type</u>	Phage <u>Recombination</u>
<u>rec1</u>	+	-	-	10^{-6}	0
<u>rec2</u>	-	+	+	10^{-7}	0

FIGURE LEGENDS

Figure 1. Scheme of experiments.

Figure 2. Sedimentation profiles of DNA from wild type cells treated according to the scheme of Figure 1 except that there was no incubation between irradiation and pulse. The horizontal scale represents fraction numbers.

Figure 3. Endonuclease (UVendo) sites in wild type DNA labeled with ^{14}C before irradiation ($\blacktriangle$) or ^3H after irradiation ($\bullet$) as a function of time after irradiation. Data from Figure 2.

FIGURE 1

PERCENT TOTAL COUNTS ($\bullet$ — ^{14}C , $\triangle$ -- ^{3}H)

FIGURE 2

FIGURE 3