ALTERATION IN THE SEQUENCE OF DEOXYRIBONUCLEIC ACID SYNTHESIS BY EXPOSURE TO ULTRAVIOLET LIGHT*

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Received March 30, 1964

Recent evidence from experiments using a number of techniques (Cairns, 1963; Nagata, 1963; Yoshikawa and Sueoka, 1963; Lark et al., 1963) indicates that the bacterial genome is replicated in a linear and sequential manner. We have undertaken experiments to study the orientation of DNA synthesis in E. coli strain 15T⁻ (555-7) after ultraviolet light irradiation. In this communication data are presented showing that a relatively low dose of UV alters the order of DNA replication.

METHODS

The procedure for analysis of sequential replication was in principle that of Lark et al., 1963. E. coli strain 15T⁻ (555-7) was grown to log phase in minimal medium (Billen, 1959) supplemented with thymidine (4 µg/ml), methionine (30 µg/ml), tryptophane (14 µg/ml) and arginine (38 µg/ml). Cellular DNA was labeled for 5 min (approx. 1/10 generation time) by adding H^3-thymidine (H^3-Tdr, 0.1 µc/ml) to the growing culture. The cells were then chilled, harvested by centrifugation, washed, and resuspended in chilled basal salts medium. Aliquots (20 ml)

*This study was supported in part by grant CA 05047-05 from the U. S. Public Health Service, National Cancer Institute and by contract AT (40-1)-2695 from the U. S. Atomic Energy Commission.

+Postdoctoral Fellow of the Public Health Service supported by grant CA 05047-05 from the National Cancer Institute.

of chilled suspended cells were exposed to ultraviolet light (150 ergs/mm^2) in 150 mm diameter petri dishes. Non-irradiated cells were held for an equal length of time (2.5 hr) at ice bath temperature. After exposure, nutritional supplements were added to the cells, substituting C^{14}-bromouracil (C^{14}-BU, 4.5 µg/ml, 0.04 µc/ml) for thymidine, and incubation at 37°C was resumed. Aliquots of cells were harvested by centrifugation and lysates were prepared for subsequent centrifugation in CsCl. The procedure for buoyant density centrifugation in CsCl and the analysis of DNA density distribution have been described (Billen, 1963).

RESULTS

Replication of the H^3-labeled region of DNA was followed by observing the appearance of H^3-labeled hybrid DNA (1.75 gm/cm^3) in the CsCl gradient (Figure 1). In control cells the labeled portion was replicated between 30 and 60 min. This result agrees qualitatively with that of Lark et al., 1963. The discrepancies observed have been tentatively attributed to the effects of cold shock and the prolonged exposure to low temperature prior to incubation required as a proper control for the irradiated sample. Heavy DNA (1.80 gm/cm^3) was observed (Table 1) only after one generation time (40-45 min) in reasonable agreement with the model for sequential semi-conservative replication (Meselson and Stahl, 1958).

The kinetics observed for irradiated cells indicated a disturbed sequence of replication of the pulsed region. Net DNA synthesis in these cells began after 30 min and immediate replication of the H^3-pulsed region was observed. Heavy DNA first appeared in irradiated cells at 70 min, corresponding to about 40 min of active DNA synthesis. During the observation period it appeared that only 40% of the original DNA participated

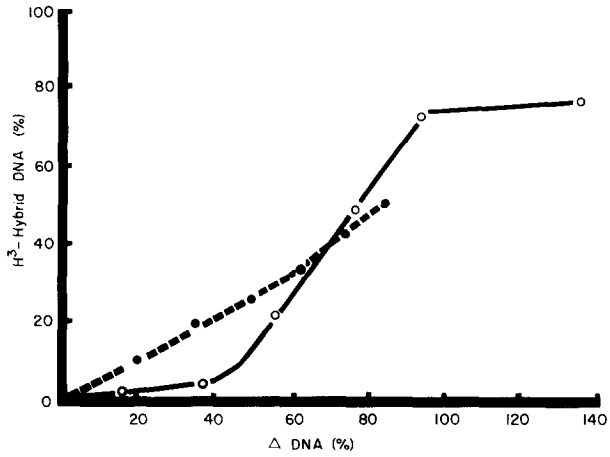

Figure 1. Replication of a previously H^3-labeled portion of cellular DNA during C^{14}-BU incubation. Control cells (o——o). Irradiated cells (● - - ●); exposed to ultraviolet light (150 ergs/mm^2) prior to C^{14}-BU incubation. See text for details.

in DNA synthesis in contrast to control cells in which >90% was replicated.

These findings support a conclusion that the orientation of DNA synthesis is altered by ultraviolet exposure. In such cells the temporal regulation of DNA synthesis does not appear to be grossly altered. A large fraction of the original DNA does not participate in post-irradiation synthesis.

The removal of regions of thymine dimerization has been shown to be related to the resumption of the DNA synthesis in UV-irradiated cells (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). Disorientation of post-irradiation DNA synthesis may be related to thymine dimerization and subsequent repair processes.

It should be pointed out that the UV induced lag in DNA synthesis mimics thymineless incubation (Barner and Cohen, 1956). After the latter treatment an altered pattern of replication was observed (Lark and Pritchard, 1963; Billen, in press). Lark and

TABLE I

ULTRAVIOLET LIGHT EFFECT ON SUBSEQUENT DNA SYNTHESIS IN C^{14}-BU MEDIUM

Time (min)	△DNA[a] (percent)	"Original" DNA replicated[b] (percent)	"Heavy" C^{14}-labeled DNA[c] (percent)	H^3-labeled hybrid DNA[d] (percent)
		CONTROL		
20	16.9	16.9	0.0	2.1
30	37.1	37.1	0.0	5.2
40	56.4	56.4	0.0	22.1
50	76.6	71.9	6.2	49.7
60	95.1	75.5	20.6	72.7
80	134.7	92.7	31.3	76.8
		(UV (150 ergs/mm^2)		
50	20.0	20.0	0.0	10.7
60	35.0	35.0	0.0	20.3
70	50.0	36.2	27.6	26.0
80	62.5	39.4	37.1	32.9
90	73.3	40.8	40.4	42.2
100	84.1	41.6	50.5	50.3

a. △DNA is the increase in DNA. Analysis performed by the method of Burton (1956) suitably reduced in scale. b. "Original" DNA is the content at zero time. The amount replicated was approximated by correcting the newly synthesized DNA (△DNA) for the amount which was present as "heavy" DNA. The difference represents the amount of C^{14}-labeled DNA banded in the "hybrid" region of a CsCl gradient. This is assumed to be equal to the amount of "original" DNA replicated since it represents only half of the double stranded segments banded in this region; the other strand being normal E. coli DNA. c. This entry presents the percentage of C^{14}-labeled DNA appearing in a CsCl gradient which bands in the "heavy" density region. For calculating the amount of DNA it must be kept in mind that:

$$\frac{\text{"heavy" DNA } (\mu g)}{C^{14} \text{ cnt per min}} = \frac{1}{2} \frac{\text{"hybrid" DNA } (\mu g)}{C^{14} \text{ cnt per min}} .$$

d. This entry presents the percentage of H^3-labeled DNA appearing in a CsCl gradient which bands in the "hybrid" density region.

Pritchard (1963) have suggested that in addition to the sites which were active when thymine was withdrawn, replication is apparently initiated at the origins of the incompleted strands of DNA.

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