RECOVERY OF DEOXYRIBONUCLEIC ACID SYNTHESIS IN ULTRAVIOLET-LIGHT-EXPOSED BACTERIA*

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Received July 17, 1961

It has been demonstrated that the ultraviolet light (UV) induced lag in deoxyribonucleic acid (DNA) synthesis in bacteria (Kelner, 1953) is overcome through the synthesis of ribonucleic acid (RNA) and protein (Harold and Ziporin, 1958; Doudney, 1959; Drakulic and Errera, 1959). In studies of the effects of chloramphenicol on induced mutation and on nucleic acid synthesis in "synchronized" cultures of Escherichia coli exposed to UV, Doudney and Haas (1960a; 1960b) demonstrated that a relation exists between the amount of postirradiation RNA which has been synthesized at the time of chloramphenicol addition and the relative rate of DNA synthesis in the presence of chloramphenicol. The present report extends these studies to log phase cultures of E. coli strain B/r and describes experimental results which suggest that the recovery of cellular capacity for DNA synthesis (involving the synthesis of RNA and protein) is related to the institution of a rate limiting component of the postirradiation DNA synthetic system. The results further show that the presence of chloramphenicol after UV exposure does not limit the amount of

*This work was supported in part by U. S. Atomic Energy Commission contract no. AT-(40-1)-2139.

DNA formed as is the case with unirradiated cells (Doudney, 1961a).

RESULTS

If a log-phase culture of strain B/r is exposed to UV, it is incapable of DNA synthesis in the presence of chloramphenicol when added during the first 15 minutes after UV exposure (Table 1). With increasing periods of postirradiation incubation (15-60 minutes) prior to chloramphenicol addition, increasing rates of subsequent DNA synthesis in chloramphenicol are observed. Furthermore, a relation exists between the amount of RNA formed with incubation after radiation exposure and before chloramphenicol addition and the subsequent rate of DNA synthesis in chloramphenicol. After doubling of RNA in the culture (60 minutes), further increase in RNA does not lead to any further increase in rate of DNA synthesis in chloramphenicol. The RNA-protein system involved in recovery of DNA synthesis thus is formed in correlation with the initial doubling of RNA in the culture.

No effect of chloramphenicol in limiting the amount of DNA formed after UV exposure is observed, if chloramphenicol is added during the period that RNA doubles after exposure (Table 1). In log phase cultures, the increase in DNA in chloramphenicol is never more than double (Doudney, 1961a). In UV-exposed cultures, where RNA has been allowed to just double prior to chloramphenicol addition, DNA has been observed to increase over four times in chloramphenicol in some experiments.

DISCUSSION

The data presented in this report suggest that ultraviolet radiation inactivates some component of the DNA synthetic

TABLE 1

The effect of UV exposure on subsequent deoxyribonucleic acid synthesis in chloramphenicol in E. coli strain B/r

Time (min.) of chl. addn.	15	30	45	60	75	none added
Protein, rel. amt. at chl. addn.	1.08	1.16	1.32	1.53	1.84	-
RNA, rel. amt. at chl. addn.	1.12	1.39	1.68	1.98	2.27	-
Time (min.) incubation	Relative amt. DNA with time (read down)					
0	1.00	1.00	1.00	1.00	1.00	1.00
45	1.02	1.01	1.02	1.02	1.01	1.01
60	1.03	1.18	1.24	1.30	1.27	1.32
75	1.01	1.32	1.53	1.65	1.63	1.71
90	1.02	1.45	1.80	2.01	2.04	2.09
105	1.03	1.66	2.01	2.39	2.42	2.48
120	1.02	1.80	2.39	2.75	2.79	2.88
135	1.02	1.93	2.66	3.02	3.06	3.35
150	1.02	2.10	2.92	3.29	3.33	3.61

RNA: 1=76 μg per 5 ml culture based on a purified yeast RNA standard. DNA: 1=28 μg per 5 ml culture based on a purified salmon sperm DNA standard. Protein: 1=420 μg per 5 ml culture based on a bovine albumin standard. The basic techniques for culture growth, UV-exposure and analysis of nucleic acid and protein have been described previously (Doudney, 1961c). The culture in log phase was exposed to UV and divided into subcultures. Incubation was begun at 0 time. Chloramphenicol (20 μg per ml) was added to each subculture at the indicated time and the incubation continued.

system. Restitution of DNA synthesis would appear to be dependent on formation of some RNA-protein moiety which is rate limiting. It is evident that the first cycle of DNA replication after UV exposure is unique in that during this period continued DNA synthesis does not require RNA-protein synthesis with each replication cycle as appears to be the case with non-UV-exposed cells (Doudney, 1961a).

Recent work by Doudney and Billen (1961) seems to eliminate the possibility of any effect of UV on the enzymes, DNA polymerase or thymidine kinase, being involved in the UV induced block to DNA synthesis. If there is no effect of doses of UV which block DNA synthesis on DNA polymerase, then possibly the *in vivo* UV induced block to DNA formation is caused by some

UV effect directly on the cellular DNA. Recently, Marmur and Grossman (1961) demonstrated that the complementary strands of DNA are bound together in vitro by some photochemical effect of UV absorption by DNA. It has been suggested that, if this is true in the cell at the proper doses of UV, then the UV-induced block to DNA synthesis might be based on the fact that the strands of DNA can no longer be separated by cellular mechanisms so as to make them available as primer for action of the Kornberg system (Doudney, 1961b). Thus, the recovery of DNA synthesis after UV exposure (through RNA and protein synthesis) would have to be due to one of two mechanisms (Doudney, 1961b). The indicated RNA-protein could be involved either in repair of the UV-induced lesions which hold the complementary strands of DNA together or in the adoption of an alternate DNA replication mechanism not involving separation of the DNA strands.

The repair hypothesis seems unlikely in consideration of the fact that after UV exposure DNA synthesis can proceed in the presence of chloramphenicol for more than one replication cycle. The effect of such repair would be to allow the DNA strands to separate normally in replication. Double stranded DNA daughter molecules would then be formed. Thus, the increase in DNA in chloramphenicol should be limited to a single replication cycle even after UV exposure, if we assume that RNA-protein formation is a requisite component of each normal DNA replication cycle (Doudney, 1961a).

In view of the correlation of rate of DNA synthesis in chloramphenicol with amount of RNA formed prior to chloramphenicol addition, the hypothetical alternate DNA replication mechanism might involve transfer of information from double stranded DNA to some intermediate RNA-protein structure and

back to daughter DNA. The proposed function of the RNA-protein structure as a template in formation of DNA would fit the data presented in this report. Thus, the amount of formation of this intermediate RNA-protein structure prior to chloramphenicol addition would limit the rate of subsequent DNA synthesis in chloramphenicol. Presumably, the RNA-protein template would continue to form DNA in the presence of chloramphenicol, without effective limitation.

While present evidence is not sufficient to support the hypothesis for an alternate DNA replication mechanism following UV exposure, the utility of such a hypothesis in explaining certain findings with UV-induced mutation in bacteria has been pointed out (Doudney and Haas, 1960a; 1960b; Doudney, 1961b). The hypothesis at least has the advantage of being subject to test, in that it makes a definite prediction as to the distribution of DNA subunits with replication following UV exposure. Thus the UV-exposed DNA strands should remain together and the daughter DNA be formed de novo, if the hypothesis in its simplest form is valid.

ACKNOWLEDGEMENT

The author wishes to thank Miss Janet Allison for capable technical assistance.

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