RECOVERY OF DEOXYRIBONUCLEIC ACID SYNTHESIS IN BACTERIA AFTER AMINO ACID STARVATION*

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It is well established that, in log phase cultures of bacteria, nucleic acid synthesis will proceed in the presence of chloramphenicol, an antibiotic which blocks protein synthesis (Gale and Folkes, 1953; Wisseman, Smadel, Hahn and Hopps, 1954). However, when a culture of Escherichia coli is starved for a period of time for a required amino acid, a requirement is induced for protein synthesis, so that the addition of chloramphenicol to the culture blocks further deoxyribonucleic acid (DNA) synthesis (Gros and Gros, 1958; Billen, 1960). If the requisite protein synthesis is allowed, however, recovery of the DNA synthetic system occurs and DNA synthesis will again proceed in the presence of chloramphenicol. The present report describes experimental results which suggest that the recovery of cellular capacity for DNA synthesis (involving the synthesis of protein) is not related to the restitution of rate limiting components of the DNA synthetic system but rather reflects the function of an intracellular control mechanism which effectively serves to limit DNA replication.

RESULTS

If a culture of strain WP2 is deprived of tryptophan for one hour, the capacity to make DNA in the presence of chloram-

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phenicol is severly limited (Table 1). The capacity of the culture to form DNA is progressively regained after tryptophan is readded and increasing periods of incubation allowed prior to chloramphenicol addition. If sufficient post-starvation incubation is allowed prior to chloramphenicol addition to allow DNA synthesis to proceed at all, no significant effect on rate of DNA synthesis is observed. The subsequent rate of DNA synthesis appears to be unaffected by prior tryptophan starvation. The limitation of DNA synthesis in chloramphenicol which is imposed by prior amino acid starvation thus appears to be one of amount. Increasing quantities of DNA may be formed with increasing periods of prior incubation with

TABLE 1

The effect of tryptophan starvation on capacity for subsequent deoxyribonucleic synthesis in E. coli strain WP2 (tryptophan-requiring)

Time (min.) of chl. addn.	0	10	20	30	40	50	60	none added
Protein, rel. amt. at chl. addn.	1.0	1.12	1.26	1.38	1.50	1.82	2.16	-
RNA, rel. amt. at chl. addn.	1.0	1.23	1.39	1.52	1.68	2.03	2.43	-
Time (min.) incubation	Relative amt. DNA with time (read down)							
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
20	1.02	1.09	1.11	1.13	1.11	1.10	1.09	1.13
40	1.03	1.24	1.23	1.32	1.29	1.31	1.28	1.29
60	1.05	1.26	1.42	1.47	1.48	1.66	1.60	1.69
80	1.11	1.26	1.44	1.63	1.66	1.84	1.84	1.84
100	1.13	1.27	1.44	1.61	1.74	1.92	1.98	1.98
120	1.11	1.32	1.47	1.58	1.76	1.95	2.29	2.81
140	1.13	1.30	1.47	1.60	1.74	2.02	2.31	3.56
160	1.11	1.29	1.49	1.63	1.79	1.97	2.31	4.44

RNA: 1=92 µg per 5 ml culture based on a purified yeast RNA standard. DNA: 1=25 µg per 5 ml culture based on a purified salmon sperm DNA standard. Protein: 1=528 µg per 5 ml culture based on a bovine albumin standard. The methods used for culture growth and analysis of nucleic acid and protein have been described previously (Doudney, 1961). The culture in log phase was starved for tryptophan for one hour and then tryptophan (5.00 mg per 100 ml) was added at 0 time and incubation begun after dividing into subcultures. Chloramphenicol was added (20 µg per ml) to each subculture at the indicated time and incubation continued.

tryptophan before chloramphenicol limits synthesis. If the culture is allowed to come to log phase after amino acid starvation and prior to chloramphenicol addition, DNA will exactly double and then synthesis will cease.

There appears to be a rough but definite correlation between the relative amount of ribonucleic acid (RNA) formed in the culture after tryptophan starvation and before chloramphenicol addition and relative DNA increase in the culture when protein synthesis is to be limited by chloramphenicol (Table 1). Thus it would appear that prior synthesis of some RNA component or some cellular component formed in correlation with RNA synthesis somehow functions to create conditions in the cell so that an equivalent relative amount of DNA can be formed subsequently, without the necessity of protein synthesis.

DISCUSSION

The data suggest that tryptophan starvation does not affect the DNA synthetic system directly to limit DNA synthesis but rather influences some cellular mechanism of control of DNA synthesis. This is in view of the fact that the rate of DNA synthesis is not modified after tryptophan starvation but that the relative amount of DNA which the culture can form is limited approximately to the relative amount of RNA formed after tryptophan starvation and before chloramphenicol addition. Furthermore, the data suggest that the capacity for DNA synthesis is somehow related quantitatively to prior RNA synthesis in the culture. These conclusions implicating RNA and protein synthesis in subsequent DNA synthesis are in basic agreement with recent publications of Maaloe and Hanawalt (1961), Hanawalt, Maaloe, Cumings, and Schaechter

(1961) and an earlier publication of Doudney (1960). The mechanism would thus effectively function to limit the formation of DNA (i.e., the genetic complement) to prior cytoplasmic increase.

If the cells of the culture are in fair synchrony after tryptophan starvation, so that these gross measurements reflect the synthetic activities of each cell, then the data might imply a relation at the molecular or genetic level between RNA-protein synthesis and subsequent DNA synthesis. If however the data reflect a distribution of cellular activities at different phases of the division cycle, then the limitation on DNA synthesis by chloramphenicol might be due to a requirement for RNA-protein synthesis at the end of each cell growth cycle and prior to cell division, as suggested by Doudney (1960).

Billen (1960a) has shown that there is essentially no difference in activity of the enzymes, DNA polymerase and thymidine kinase, in extracts derived from cells in exponential growth and after amino acid starvation. This would seem to eliminate the possibility that the control mechanism is released through formation of the Kornberg enzyme itself. While the possibility exists that control mechanism could lie in limitation of the synthesis of the nucleotide substrate for the Kornberg system, it seems unlikely that the precision of control indicated by the present data could be achieved through supply of substrate (though certainly, this possibility has not been eliminated).

If we eliminate the Kornberg enzyme and the supply of substrate from consideration, the possibility remains that the control mechanism involves the DNA primer itself, that is to

say, the cellular DNA. The Watson-Crick hypothesis for DNA replication implies that the DNA strands must separate for replication to proceed. Since the end product of a cycle of DNA synthesis by the Kornberg system is the creation of two sets of double stranded DNA molecules from two single DNA strands, it might well be that, in the cell, release of the control mechanism through RNA-protein synthesis is in the generation of DNA capable of functioning in the Kornberg system. Thus, in the cell, limitation of DNA synthesis would consist of the end product of the Kornberg reaction, the formation of double stranded DNA. Presumably then, tryptophan starvation or the limitation of protein synthesis functions to make the DNA unavailable to the Kornberg system, presumably by its coming to the double stranded state.

This need not imply that the DNA strands actually come apart with RNA-protein synthesis in this mechanism. Rather, it may be that formation of the RNA-protein on the DNA template functions somehow to make the DNA available to the Kornberg system subsequently when DNA is actually formed.

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