SYNTHESIS OF TRANSFORMING DNA IN THE PRESENCE OF CHLORAMPHENICOL

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The addition of chloramphenicol to growing bacterial cultures has been shown to inhibit the synthesis of proteins while permitting the synthesis of DNA and RNA to continue for some time thereafter (Wisseman, et al., 1954) (Gale and Folkes, 1953). The question of the function of DNA made in the absence of concurrent protein synthesis has never been rigorously resolved due to restrictions inherent in the test situations. For example, the demonstration that the DNA made in bacteriophage T2 infected cells treated with chloramphenicol subsequently appears in mature phage (Hershey and Melechen, 1957)(Tomizawa, 1958) suffers from the necessity of permitting the resumption of protein synthesis to produce infective phage. The effect of these proteins on previously synthesized DNA is unknown. Similarly, the demonstration that altered chloramphenicol-DNA contributes to genetic material with altered capacity (Tomizawa, 1958)(Glass and Novick, 1959) still leaves unresolved the questions about the genetic capacity of the DNA at the time of its synthesis. The demonstrations that recombination appears to occur in chloramphenicol-DNA (Hershey, et al, 1958) and that it can dilute a gene-pool (Thomas, 1959) remain the most direct pieces of evidence available. A more direct test of the genetic specificity of DNA synthesized in the presence of chloramphenicol under conditions where no detectable protein synthesis occurs, however, is available in bacteria which produce transforming DNA. In this case the genetic potentialities of the chloramphenicol-DNA can be measured directly and without

any interposed protein synthesis. It has been possible to show that DNA synthesized in the absence of protein synthesis (inhibited by chloramphenicol) is functional transforming DNA.

MATERIALS AND METHODS

Hemophilus influenzae type d (Rd) originally obtained from Alexander and Leidy were used as receptor cells for transformations. The cells treated with chloramphenicol and used as donors (after DNA preparation) were Rd resistant to streptomycin (S) and cathomycin (C) (Novobiocin). A final concentration of 250 μg/ml of streptomycin and 2.5 μg/ml of cathomycin were used as selecting agents. The general procedures, medium, etc., have been described (Goodgal and Herriott, 1960).

The experimental procedure consisted of growing a 250 ml culture in broth to a turbidity of 44 by the Klett colorimeter (4×10^8/ml; generation time = 30 minutes) at 37°C. Twenty ml samples were then dispensed into bottles with or without 200 μg chloramphenicol. At 0, 30, 60, and 90 minutes, two 9 ml samples from each bottle were centrifuged and resuspended in 9 ml of citrate-saline (0.05 M citrate pH 7.2 and 0.15 M saline). Three ml of the sample was removed and lysed with 0.2 ml of 0.1 N NaOH and neutralized with 0.2 ml of 0.1 N HCl. This sample was treated at 60° for 10 minutes and used as transforming factor (TF) after diluting 5×10^2 - and 5×10^3 - fold in citrate-saline (0.015 M citrate pH 7.2 and 0.15 M saline). In the transformation procedure, 0.1 ml of competent cells, 2.8 ml of 0.125 M saline and 0.1 ml TF were incubated for 30 minutes at 36°. Platings were made at appropriate dilutions (from 2×10^0 - to 10^2 - fold) incubating for 2 hours at 37°C and adding an additional layer of medium containing the antibiotic S or C or both. The colonies were counted after 18 or 36 hours at 37°C.

The remaining 6 ml of sample was centrifuged again and resuspended in 4.5 ml of H_2O, chilled in ice water and 0.5 ml of 50% TCA added. After 30 minutes the sample was centrifuged and the pellet resuspended in 2 ml of H_2O and used for subsequent DNA and protein analyses. The DNA was determined by the Dische diphenylamine test by the method of Burton (Burton, 1956). Protein

was determined by the method of Lowry (Lowry, et al, 1951).

EXPERIMENTAL RESULTS

In preliminary tests it was found that at 10 μg/ml of chloramphenicol no appreciable protein synthesis occurred for 120 minutes. In the experiments presented here this level was used with the same results. In the absence of chloramphenicol, protein and DNA synthesis proceeded as shown in Table 1. In

TABLE I

The synthesis of DNA and proteins in the presence (+) and absence (-) of chloramphenicol and the measurement of the transforming activity of the DNA.

Time of Sample and Treatment	Protein μg/sample of 6 ml	DNA μg/sample of 6 ml	No. Transformations x 10^{-}	
			S	SC
0	95	4.8	6.5 (8.1*)	3.2 (4.0)
30 (+)	89	7.2	10.2 (8.5)	4.6 (3.8)
30 (-)	184	7.2	12.3 (10.2)	6.1 (5.1)
60 (+)	96	6.9	9.1 (7.9)	4.6 (4.0)
60 (-)	302	14.5	19 (7.9)	8.1 (3.4)
90 (+)	87	8.7	9.6 (6.6)	5.0 (3.5)
90 (-)	372	18.5	22 (7.1)	10.5 (3.4)

the presence of chloramphenicol appreciable DNA was synthesized and for 6 experiments this increase averaged 40% ± 10 in 90 minutes under conditions where no detectable protein synthesis occurred. In the experiment shown in Table 1 the increase in activity was measured for the marker streptomycin resistance and the linked markers streptomycin-cathomycin, i.e., measuring transformation to the double streptomycin-cathomycin. In other experiments the transformations

*Relative transforming efficiency of DNA of sample as number of transformations per μg DNA.

to cathomycin alone were also measured and gave the same results. In Table 1, the transforming activities of a typical experiment are shown to parallel the increase of DNA synthesis. Transforming activity could be determined with a consistency of $\pm$ 15% per determination making the 40% increase due to DNA synthesis highly significant. Most of the DNA increase observed in the chloramphenicol treated cultures occurred during the first 30 minutes, as in the experiment presented. Regardless of the amount of increase in all experiments, the transforming activity remained proportional to the measured total DNA. The relative efficiency of comparable samples (with and without chloramphenicol) was usually very similar. This can be seen from the values in parenthesis in Table 1, indicating that there was no change with incubation in the presence of chloramphenicol.

DISCUSSION

The results presented here are consistent with the findings mentioned previously that DNA synthesized in the presence of chloramphenicol, i.e., in the absence of any measurable proteins synthesis, is functional DNA. In addition, they demonstrate that it is functional at the time of synthesis and, as TF, as efficient as the DNA made in the presence of protein synthesis. The fact that this synthesis reaches a level of only 40% of the uninhibited DNA synthesized (see also Glass and Novick, 1959) suggests that continued protein synthesis is necessary for DNA synthesis (Doudney, 1960). Whether or not it is consistent with the notion that it represents only the completion of synthesis already initiated remains to be seen.

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