OBSERVATIONS ON THE REGULATION OF DNA BIOSYNTHESIS

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It has been established that bacteria in stationary phase do not incorporate radioactive thymidine into DNA (see Lark, 1963). Three of the possible explanations for the failure to incorporate thymidine in stationary phase have been considered: (1) inactive DNA polymerase, (2) depletion of one or more of the deoxyribonucleoside triphosphates, and (3) non-functionality of DNA as a primer.

Experiments with E. coli starved of an essential amino acid (Billen, 1962) and the slime mold P. polycephalium (Guttes and Guttes, 1961) were interpreted to indicate that factors other than the enzyme and substrate were responsible for the regulation of DNA synthesis. This communication reports on the relative inability of DNA in sonicates of stationary phase Myxococcus xanthus to function as an active primer for DNA synthesis.

MATERIALS AND METHODS. M. xanthus** were grown at 30°C in aerated 2% casitone (generation time, 5 hr.), harvested by centrifugation, washed in 0.01 M phosphate buffer, pH 7.0, and resuspended in buffer to give a final $O.D._{560\ m\mu}$ of 20 (20×10^9 cells/ml). Sonicates from log phase were obtained from cultures which had reached an $O.D._{560\ m\mu}$

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**A culture of strain FB of M. xanthus was kindly supplied by M. Dworkin.

of 0.9. Sonicates from stationary phase were obtained from cultures whose O.D. had remained constant for at least two generation times (final O.D. = 2.5).

Ten ml suspensions of the cells were broken in a Branson Model S-75 Sonifier with a one-half inch probe for 30 seconds (final temperature 5°C). Microscopic examinations demonstrated complete breakage in both log and stationary cells.

DNA polymerase was assayed using a modification of the Lehman et al. (1958) procedure. DNAase activity was determined by the conversion of acid insoluble DNA to acid soluble oligonucleotides. Analyses were carried out by micromodifications (one-tenth scale) of existing methods for the determination of DNA and RNA (Dische, 1955) and protein (Lowry, et al., 1951). M. xanthus DNA* was isolated from log phase cells according to the method of Marmur (1961). RNA, extracted from Bacillus subtilis by the method of Kirby (1956), was a gift of Dr. J. Péne.

RESULTS AND DISCUSSION. The synthesis of DNA by sonicates of stationary phase M. xanthus was stimulated by added Mg^{++}, dATP, dGTP, TTP, and homologous DNA (Table 1). In these respects, the crude DNA polymerase of M. xanthus behaved like the well studied DNA polymerase of E. coli (Lehman, 1958). Ninety-five percent of the radioactivity of the acid insoluble product was solubilized by DNAase.

Fig. 1 demonstrates that added homologous DNA was more effective than sonicate DNA in stimulating DNA synthesis. From the slope of the curve, it may be seen that DNA synthesis was stimulated to the extent of approximately 8 cpm/µg added DNA. Since there were 50-60 cpm incorporated with no added DNA, one

*The M. xanthus DNA contained 69% CG and had a M.W. of approximately 2×10^6 (unpublished results).

Table 1

Requirements for DNA Synthesis

Components	Incorporation of dATP-8-C^{14} (cpm)
Complete system*	155**
minus TTP	71
minus dGTP	28
minus dCTP	28
minus TTP, dGTP, dCTP	10
minus Mg^{++}	5
minus added DNA	87
minus added DNA + 9 μg RNA	89

*Complete system contains glycine buffer, pH 9.0, 20 μm; $MgCl_2$, 2 μm; 2-mercaptoethanol, 0.3 μm; TTP, dATP, dGTP, and dCTP each 5 μμm; DNA, 9 μg; dATP-8-C^{14}, 5.65 μc/μm, 16,000 cpm; and stationary sonicate, 0.15 ml (53 mg protein/ml), all in 0.3 ml; incubation time 15 minutes at 37°C.

**Incorporation at zero time has been subtracted.

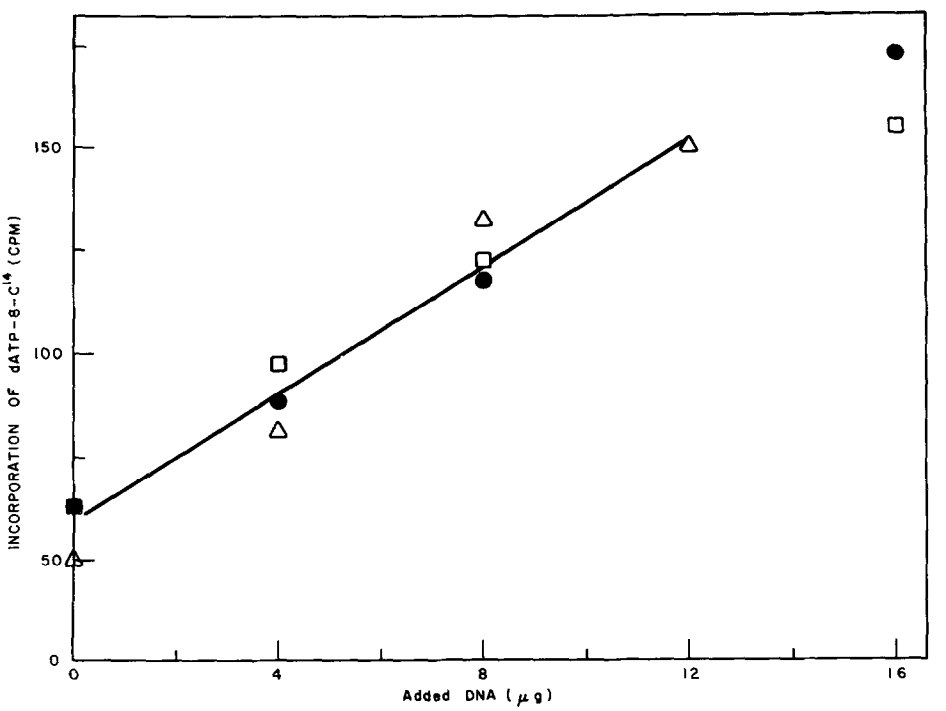

Fig. 1. Stimulation of DNA synthesis by added DNA. The assay system is identical to that described in Table 1 except that the quantities of added DNA were varied. The curve represents experiments done on stationary sonicates prepared at 3 different times, all adjusted to contain 70 μg sonicate DNA.

would predict that there were approximately 7 μg DNA in the stationary sonicates. However, chemical analysis demonstrated that the sonicates contained 70 ± 3 μg DNA. This suggests that the stationary sonicate DNA is approximately 90% nonfunctional with regard to priming activity. Preliminary experiments have revealed that log phase sonicate DNA is a more active primer than the stationary phase sonicate DNA.

Fig. 2 demonstrates that at a concentration of 3 mg protein/ml (1:4 dilution), the specific activities of DNA polymerase of the

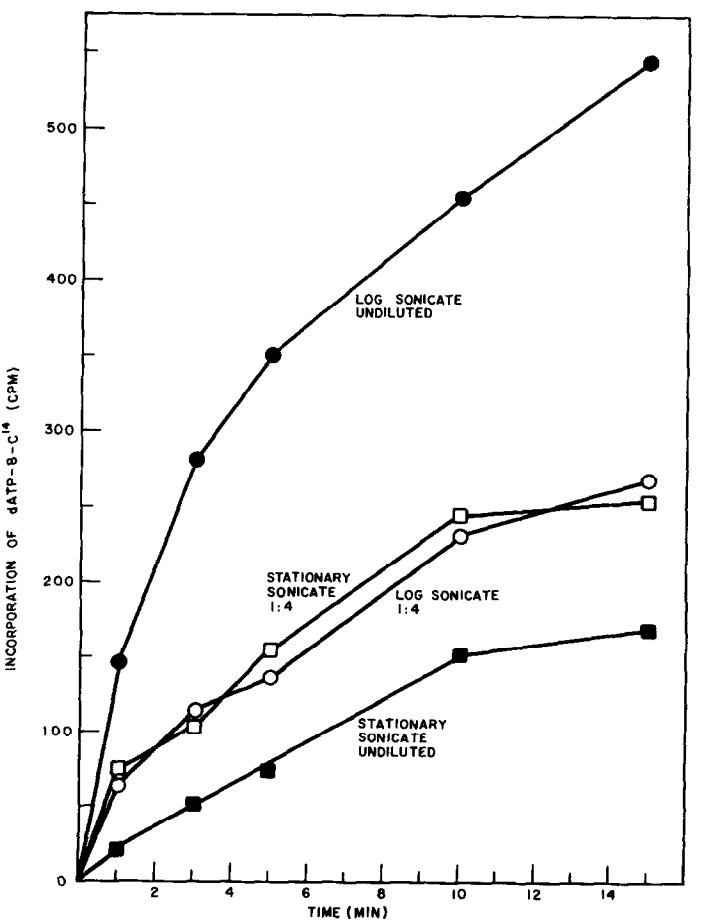

Fig. 2. The kinetics of DNA synthesis by sonicates of stationary and log phase M. xanthus. The assay system was as described in Table 1 except that excess salmon sperm DNA (32 μg) and ATP (0.5 μm) were added. Multiple amounts were used and 0.5 ml aliquots were withdrawn at various times. Dilutions of the sonicates were made with .01 M phosphate buffer.

stationary and log phase sonicates are identical. Therefore, the failure of the resting cells to synthesize DNA can not be attributed simply to the absence of DNA polymerase.

Increasing the concentration of log phase sonicates in the incubation mixtures from 3 to 12 mg protein/ml result in approximately a threefold increase in the rate of DNA synthesis (Fig. 2). However, identical increases in the concentration of stationary phase sonicates result in approximately a twofold decrease in rate. In similar experiments it was found that the rate of dATP-8-C^{14} incorporation increased with increasing concentrations of log phase sonicates from 0-24 mg protein/ml, while in stationary phase sonicates, the activity increased from 0-3 mg/ml and then decreased at higher protein concentrations.

The inhibition of DNA polymerase at the higher protein concentrations could not be due to DNAase activity since the specific activity of DNAase in log phase sonicates is slightly higher than stationary phase sonicates under the conditions used to assay for the DNA polymerase.

In conclusion it has been observed that stationary sonicates of M. xanthus contain:

(1) As active a DNA polymerase as log phase sonicates if measured at low protein concentrations,

(2) an inhibitor for the DNA polymerase system (not DNAase) which is not expressed in log phase sonicates, and

(3) DNA which is 90% nonfunctional with respect to priming activity for DNA polymerase.

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