Regulation of Synthesis of Alkaline Phosphatase by Deoxyribonucleic Acid Synthesis in a Constitutive Mutant of *Bacillus subtilis*

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ABSTRACT

HIRAGA, SOTA (Osaka University, Osaka, Japan). Regulation of synthesis of alkaline phosphatase by deoxyribonucleic acid synthesis in a constitutive mutant of *Bacillus subtilis*. J. Bacteriol. 91:2192-2199, 1966.—It was found that synthesis of alkaline phosphatase (APase) correlated with deoxyribonucleic acid (DNA) synthesis in a partially constitutive mutant of *Bacillus subtilis*. When cultures of the mutant were made to undergo synchronous growth by germination of spores in an excess-phosphate medium, synthesis of APase was repressed at the beginning of DNA synthesis. If the initiation of DNA synthesis was inhibited by thymine starvation, the repression of APase was not observed. When DNA synthesis, previously initiated, was inhibited by thymine or uracil starvation, or by addition of mitomycin C, the repression was partially released at a later stage. In contrast, this correlation between repression and DNA synthesis was not observed in a repressible strain.

Many investigators have described relationships between the synthesis of specific enzymes and the chromosome in synchronous cultures of various bacteria (9, 10, 19). Synthesis of alkaline phosphatase (APase) in *Escherichia coli* is regulated by concentration of inorganic phosphate present in the medium (13, 27), and the synthesis is genetically controlled (5, 8). It was reported that the synthesis of APase and phosphodiesterase in *Bacillus subtilis* is controlled by common genes (12, 25, 26).

In this paper, the activity of APase in cultures of a partially constitutive mutant of *B. subtilis*, which is able to synthesize APase even in a high concentration of inorganic phosphate, was measured at various intervals during synchronous growth. It was found that the synthesis of this enzyme is not constant but varies with deoxyribonucleic acid (DNA) replication.

MATERIALS AND METHODS

Strain. Three mutant strains, JB76 (*AP^c thy str-r*), JB100 (*AP^c thy ura try*), and JB101 (*AP⁺ thy ura try*), were derived from Marburg's strain 168 of *B. subtilis* by the following procedures. (Abbreviations: *AP^c*, constitutive formation of alkaline phosphatase;

AP⁺, repressed formation of alkaline phosphatase; *thy*, thymine requirement; *ura*, uracil requirement; *try*, tryptophan requirement; *str-r*, resistance to streptomycin.) The mutant which produced APase constitutively was obtained by the methods described by Torriani and Rothman (28); bacteria were first irradiated with ultraviolet light, and then plated on a medium containing a high concentration of inorganic phosphate and β -glycerophosphate as the sole carbon source. Strains JB76 and JB100 carried the same *AP^c* allele, although they differed with respect to the other markers listed. Thymine-requiring mutants were selected in a synthetic minimal glucose medium supplemented with thymine (200 μ g/ml) and aminopterin (1,000 μ g/ml) by the procedures of Okada et al. (20). Then the markers were combined through a technique of transformation, described by Anagnostopoulos and Spizizen (1). JB101 was a spontaneous revertant of JB100.

Media. H medium was a modification of one originally described by Woese and Forro (30). It contained (per liter) tris(hydroxymethyl) aminomethane (Tris), 2.4 g; sodium glutamate, 3.4 g; L-asparagine, 2.6 g; DL- α -alanine, 0.044 g; $\text{MgCl}_2 \cdot \text{H}_2\text{O}$, 1 g; NH_4Cl , 0.5 g; Casamino Acids (Difco), 1 g; orthophosphate as K_2HPO_4 , 10^{-3} M; and sufficient HCl to adjust the pH to 7.2.

P medium contained (per liter) sodium lactate, 10 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g; NaCl, 3 g; Tris, 2.4 g; MnSO_4 , 5 mg; and peptone (Difco), 10 g. X medium was the same as P medium except

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that Casamino Acids (1 g) and L-tryptophan (0.1 g) were substituted for peptone.

Purification of spores. Cultures of bacteria in P medium were shaken at 37 C for 5 to 7 days. Purified spores were prepared by lysozyme treatment and heating (30). They were washed with sterile water and stored in a freezer. These purified spores contained no vegetative cells.

Germination of spores. The purified spores in distilled water (turbidity, 0.4 to 0.6 units in an ERMA photoelectric colorimeter) were heated at 65 C for 2.5 hr, transferred to 0.175% Penassay Broth (Difco), and aerated for 2 hr. More than 95% of the spores germinated during the incubation, and the turbidity decreased by 60%. They were then centrifuged, suspended in warm H medium supplemented with L-tryptophan, uracil, and thymine (each at 50 μ g/ml), and shaken at 37 C in a flask.

Enzyme assay. Activity of APase was measured by the rate of hydrolysis of *p*-nitrophenyl phosphate (Daiichi Pure Chemical Co., Ltd., Tokyo, Japan), by a modification of the method of Schlesinger and Levinthal (23). The reaction mixture contained 2 ml of 0.02% *p*-nitrophenyl phosphate in 1 M Tris-HCl buffer (pH 8.0) containing 0.63 mM magnesium acetate and 0.5 ml of diluted toluene-treated culture. It was incubated at 37 C, and the reaction was terminated by the addition of 0.5 ml of K_2HPO_4 (13%). The absorbance at 410 $m\mu$ was then measured in a Beckman spectrophotometer; 1 unit of enzyme activity was that amount of enzyme which led to a change of 1.0 unit of absorbancy per min.

Determination of rate of DNA synthesis. To determine the rate of DNA synthesis, 1 μ Ci/ml of thymidine-2- C^{14} (26 mc/mmole; obtained from the Radiochemical Center, Buckinghamshire, England) was added to the medium. Samples were removed at various intervals, and ice-cold 5% trichloroacetic acid was immediately added. After standing in the cold for at least 30 min, the precipitate was collected on a membrane filter (Millipore, 0.45- μ pore diameter) and washed with cold 5% trichloroacetic acid. Radioactivity was determined with an end-window gas-flow counter.

Pulse-labeling of ribonucleic acid (RNA) with C^{14} -uracil. To pulse-label RNA in cells, C^{14} -uracil (45 mc/ml; The Radiochemical Center) was added to 0.2 ml of culture (1 μ Ci/ml) and incubated for 1 min. Then the reaction was terminated with 3 ml of cold 5% trichloroacetic acid containing unlabeled uracil (100 μ g/ml). The acid-insoluble material was collected, washed, and measured as described above.

RESULTS

Synthesis of APase in germinated cells. When cells of JB100 were transferred (zero-time) to H medium containing uracil, thymine, and tryptophan after germination in Penassay Broth, the turbidity of the culture increased almost logarithmically for approximately 9 hr. The APase activity, however, increased rapidly during the first 2 hr of incubation, and reached a distinct plateau thereafter (Fig. 1). These observations are also summarized in terms of the specific

FIG. 1. Synthesis of APase in germinated cells of a constitutive mutant, JB100. The inset shows the decrease in turbidity during germination of spores in Penassay Broth. After 2 hr, the cells were washed and transferred to H medium supplemented with tryptophan, uracil, and thymine (zero-time). The specific activity was obtained by dividing the APase activity by the turbidity of the cultures.

activity of APase in such a culture. There was a marked increase in specific activity, which then decreased after 2 hr of growth.

When actinomycin D, which has been shown to inhibit synthesis of messenger RNA (mRNA) (17), was added to the culture at various times, synthesis of APase ceased and the enzyme activity decreased (Fig. 2). Thus, it was possible to measure the stability and decay of enzyme activity in cells at any point by the use of actinomycin D. The decay of APase was not constant during the growth cycle of the organism. It was noticed that enzyme activity was markedly stable late in the growth cycle in comparison with the enzyme activity found in early culture. These results are not in agreement with the idea that the cessation of synthesis of APase was caused by an increase of some proteinase activity which inactivated APase. Further evidence against such a possibility was obtained as follows: toluene-treated samples of cultures from the early and late periods of growth were mixed and assayed

FIG. 2. (a) Decay of APase activity after addition of actinomycin D in germinated cells of JB100. At the various times indicated by arrows, samples were removed from the culture and were incubated with actinomycin D (10 μ g/ml). (●) Control culture; (○) cultures incubated with actinomycin. (b) At intervals, samples were removed from the culture, and were incubated with actinomycin D (10 μ g/ml) at 37 C for 2 hr. (●) Enzyme activity before the addition; (○) activity after 2 hr of incubation with actinomycin D.

for enzyme activity. The observed APase activity was the sum of the activity found in each fraction.

Effects of thymine deprivation, uracil deprivation, and mitomycin C on APase synthesis. If thymine was removed from the medium at a time when the culture showed no further increase in APase activity, a marked increase of the enzyme activity was observed (arrow B in Fig. 3). However, deprivation of thymine at 30 min after transfer to H medium (arrow A in Fig. 3), when the rate of increase in APase activity was high, produced no change in the rate of increase of the enzyme activity; however, the increase continued after the control reached a plateau, and synthesis did not end until lysis of the cells occurred. Effects of thymine deprivation at various times on the synthesis of APase are shown in Fig. 4b.

Incubation of the bacteria with mitomycin C

FIG. 3. Effect of thymine deprivation on synthesis of APase in germinated cells of a constitutive mutant, JB100. At various times (indicated by arrows in the inset), samples of the culture were removed and centrifuged. The harvested cells were washed three times with three volumes of warm H medium. The cell suspension was divided into two parts and shaken at 37 C with thymine, L-tryptophan, and uracil (●), and with L-tryptophan and uracil but without thymine (○).

(3 μ g/ml) at the late period caused an increase in APase activity. However, there was a lag period of about 100 min before the increase in enzyme activity (Fig. 5). Ultraviolet irradiation was found to have no effect upon the enzyme synthesis (Fig. 5). Uracil deprivation in the late period gave rise to an induction of the enzyme synthesis (Fig. 6). Deprivation of both uracil and thymine caused induction of the enzyme, as did thymine deprivation alone. Addition of actinomycin D (10 μ g/ml) inhibited the increased enzyme activity caused by removal of these growth factors. In germinated cells of a repressible strain, JB101, synthesis of APase was repressed even before the initiation of DNA synthesis in the medium containing a high concentration of inorganic phosphate, and neither uracil deprivation nor thymine deprivation had any effect on synthesis of the enzyme.

Correlation of DNA synthesis and the synthesis of APase. Synthesis of DNA in germinated

FIG. 4. Effect of thymine deprivation upon synthesis of APase in germinated cells of JB100. At various times, indicated by arrows, thymine was removed from the medium as described in the legend of Fig. 3. (a) Control culture. (b) Thymine-deprived cultures ($\circ$); cultures with thymine ($\bullet$).

FIG. 5. Effect of mitomycin C and ultraviolet irradiation on synthesis of APase in a germinated culture of JB100. After 105 min of growth in H medium, the culture was divided into three parts. ($\bullet$) Without treatment; ($\blacksquare$) with mitomycin C ($3 \mu\text{g/ml}$); ($\triangle$) with irradiation (germicidal lamp, 15 w, 50 cm, 30 sec).

FIG. 6. Effect of uracil and thymine deprivation on synthesis of APase in germinated cells of JB100. Germinated 3-hr cells were centrifuged and washed as described in the legend to Fig. 3. Resuspended cells were incubated with the following supplements: ($\bullet$) control culture with thymine, uracil, and L-tryptophan (each $50 \mu\text{g/ml}$); (∇) without uracil; ($\blacktriangle$) without thymine; ($\blacksquare$) without uracil and thymine. At times indicated by arrows, portions of culture were removed and actinomycin D ($10 \mu\text{g/ml}$) was added (broken lines).

cells began 2 to 3 hr after transfer to H medium (Fig. 7), whereas synthesis of APase declined at about the same time. A portion removed from the culture at the 3rd hour, in which synthesis of APase was declining, was centrifuged, washed, and suspended in the following three media: (i) the complete medium supplemented with thymine, uracil, and L-tryptophan; (ii) medium i with cytidine instead of uracil; and (iii) medium i without uracil. The kinetics of synthesis of APase and DNA in these three cultures is shown in Fig. 8. DNA synthesis occurred after a short lag in the first two media, and the synthesis of APase was limited. On the other hand, in a medium deprived of pyrimidines, DNA synthesis declined after 3 hr, and the enzyme synthesis continued at a high rate.

Pulse-labeling of RNA with C^{14} -uracil. Portions of germinated culture were removed at intervals,

FIG. 7. Synthesis of DNA and of APase in germinated cells of JB100. At zero-time, germinated spores in Penassay Broth were transferred to H medium containing L-tryptophan, uracil, thymine (each 50 μ g/ml), and C^{14} -thymidine (1 μ Ci/ml). These cultures were incubated at 37 C with constant shaking.

and were incubated with C^{14} -uracil for 1 min. The incorporation of C^{14} -uracil into acid-insoluble material was not constant over the growth period. Moreover, no correlation was observed between the synthesis of APase and the degree of RNA pulse-labeling (Fig. 9).

Effect of thymine deprivation on synthesis of APase in an unsynchronized culture. Synthesis of APase was stimulated about twofold by thymine deprivation in a nonsynchronous-growth culture of a constitutive mutant, JB76 (Fig. 10). When thymine was added to a culture at 90 min after thymine deprivation, no significant effect was observed. Although similar results would be expected with strain JB100, this experiment has not been performed to date.

DISCUSSION

Strain JB100 of *B. subtilis* is a constitutive mutant which is able to synthesize APase in a medium containing an excess of orthophosphate. In synchronous culture started by germination of spores, synthesis of APase ceased with the initiation of DNA replication. Levinthal et al. (17) reported that addition of actinomycin D inhibits synthesis of mRNA and, accordingly, synthesis of protein. The results summarized in Fig. 2 indicate that the apparent decrease in

FIG. 8. Effect of uracil deprivation on synthesis of DNA and of APase. A portion from the unlabeled culture was removed at the time indicated by the arrow in Fig. 7, and was washed three times with H medium. The washed cells were resuspended in the medium, and were divided into three parts: (●) supplied with thymine, thymidine, L-tryptophan, and uracil; (Δ) with thymine, thymidine, and tryptophan; (○) thymine, thymidine, and cytidine (each 50 μ g/ml). Each cell suspension was then divided into two parts. One was used for assay of APase; to the other was added C^{14} -thymidine (1 μ Ci/ml). These cultures were incubated at 37 C with constant shaking.

enzyme synthesis is not due to the action of proteinases. In fact, when the synthesis of APase was most active, the decay of the enzyme activity after addition of actinomycin D was most pronounced. The decrease in enzyme activity after addition of actinomycin D is not due to a direct action of the antibiotics upon the enzyme (Hiraga, *in preparation*). It has been shown that the decay rate of APase, after repression by addition of inorganic phosphate in a wild-type strain, was equal to that observed after inhibition of synthesis by actinomycin D or by chloramphenicol.

The cessation of the increase in enzyme activity was not due to the presence of an inhibitor or an activator of the enzyme. Further, the results in Fig. 6 suggest that induction of APase by thymine or uracil deprivation is due to the de novo synthesis of the enzyme via synthesis of mRNA.

Yoshikawa et al. (31) have shown, with syn-

FIG. 9. Synthesis of DNA, and pulse-labeling of RNA, in germinated cells of JB100. Germinated spores were transferred to H medium supplemented with thymine, uracil, and L-tryptophan, and the suspension was divided into two parts. To one part was added C^{14} -thymidine ($10 \mu\text{C}/\text{ml}$). At intervals, 0.1-ml samples of the culture were removed, and cold 5% trichloroacetic acid was added. The other part was incubated at 37°C with shaking. At intervals, 0.2-ml samples of the culture were removed, incubated with C^{14} -uracil ($0.2 \mu\text{C}/\text{ml}$) for 1 min at 37°C , and cold 5% trichloroacetic acid containing unlabeled uracil ($100 \mu\text{g}/\text{ml}$) was added.

chronous culture of *B. subtilis* W168 started by germination of spores, that replication of the chromosome begins from a constant point and has polarity. If the initial synthesis of APase observed in the present work is due to replication of the structural gene as described by Gorman et al. (9), no increase in APase should be observed when thymine is removed from the medium. However, the synthesis of APase was actually induced by thymine starvation.

Strain 168, from which strain JB100 was derived, is lysogenic for phage PBSX, and could be induced to produce phage particles by brief exposure to mitomycin C (24).

In the present experiments, it was found that addition of thymine to a thymine-starved culture of JB100 results in lysis of these cells, presumably owing to induction of PBSX; it has been shown

FIG. 10. Effect of thymine deprivation on synthesis of APase in a nonsynchronous culture of a constitutive mutant, JB76. An overnight culture in P medium supplemented with thymine ($50 \mu\text{g}/\text{ml}$) was diluted 1:20 with fresh medium, and was incubated at 37°C . After 140 min, a sample of the culture was harvested, and was washed with X medium three times. The cells were resuspended in the medium, which was then divided into three parts. (●) Supplemented with thymine ($50 \mu\text{g}/\text{ml}$); (△) without thymine; (■) without thymine for 100 min, after which thymine was added.

with other temperate phages that induction is caused by mitomycin C, ultraviolet irradiation, or thymine starvation (2, 15, 16). After a brief treatment (10 min) with mitomycin C, the antibiotic was removed by centrifugation, and washed cells were incubated in a medium without mitomycin C. After 1 hr, cells were lysed by induction with PBSX. However, synthesis of APase was not induced (*unpublished data*). Furthermore, ultraviolet irradiation had no effect on the induction of synthesis of APase (Fig. 5). It has been reported that, in ultraviolet-irradiated cells, DNA synthesis resumes after a time lag (14). These results suggest that the induction of APase requires an extended period of inhibition of DNA synthesis.

Uracil deprivation caused a decline in DNA synthesis and an induction of APase (Fig. 8). If uracil was removed from the medium, DNA synthesis was repressed after a lag period, probably owing to exhaustion of the precursor pool.

The strain used requires thymine, uracil, and tryptophan for growth. One of the enzymes which converts carbamyl phosphate to uridine monophosphate, and thymidylate synthetase, which converts deoxyuridine monophosphate to deoxythymidine monophosphate (7), are presumably blocked because of mutations in JB100. The synthesis of deoxycytidine nucleotides depends directly upon synthesis of cytidine nucleotides (3, 22). Bulk RNA synthesis was blocked by uracil deprivation in JB100. Turbidity increased for 2 hr in the absence of uracil. Synthesis of bulk RNA was limited by uracil deprivation, so that DNA synthesis was limited. Cytidine or cytosine can be converted into uridine or uracil by deamination. Therefore, the supplementation of the medium with cytidine, instead of uracil, allows the synthesis of DNA as well as of RNA (Fig. 8).

The synthesis of APase was induced by thymine deprivation, uracil deprivation, or addition of mitomycin C, all of which are known to inhibit DNA synthesis. These results suggest that synthesis of APase in the constitutive mutant is related to DNA synthesis. The induction of APase by thymine deprivation is not a special case for a synchronous culture started by germination; induction was also observed in a nonsynchronous culture (Fig. 10). However, the phenomenon was not as pronounced as in synchronous cultures.

Cohen et al. (4) showed that incorporation of C^{14} -uracil, measured in the absence of an amino acid, was more rapid in the absence of thymine than in its presence. They interpreted this as being due to a competition between synthesizing DNA and RNA for DNA strands as templates. However, the same mechanism does not seem to apply to the present data. The decline in synthesis of mRNA did not correspond to the time of DNA synthesis (Fig. 9).

Wiberg et al. (29) observed that synthesis of early phage enzymes in *E. coli* ceases 10 min after infection with wild-type phage T4. On the other hand, they showed, in some amber mutants in which DNA synthesis was restrained, that the synthesis of early enzymes continued even after 10 min. Therefore, in these mutants, no synthesis of late enzymes occurred (6). The same observation was reported with λ phage (21) and with f2 phage (18). The results obtained in the present experiment are consistent with these observations.

The constitutive mutant was able to synthesize APase at a more rapid rate in a limited-phosphate medium than in an excess-phosphate medium (Hiraga, *in preparation*). These results show that the mutant was partially constitutive. In purified

spores of the mutant, the repressor of APase, which may be synthesized at the start of DNA replication and may repress the synthesis of APase, might have been absent. The inhibition of DNA replication by thymine starvation, accompanying APase synthesis, may then be interpreted as a result of inhibition of synthesis of this repressor. The necessity of DNA synthesis for the basal-level synthesis of β -galactosidase and APase in *E. coli* (11) is in agreement with such an interpretation. The lag period and the continued rise in APase activity that followed thymine starvation applied at the late period of APase synthesis further suggest either the dilution of the existing repressor owing to cell multiplication or its gradual decay.

In germinated cells of a repressible strain, JB101, there was no synthesis of the enzyme, not even before the beginning of chromosome replication. Furthermore, when synthesis of DNA was inhibited by thymine or uracil starvation, no induction of APase was observed. These results with the repressed strain suggest the following possibilities: the repressor in this strain may be synthesized independently of DNA synthesis; or, synthesis of repressor depends upon DNA synthesis, but the repressor may be more stable than that of the constitutive mutant so that no derepression occurs as a result of thymine deprivation. In this event, the repressor must be assumed to exist even in spores treated at high temperature.

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LITERATURE CITED

1. ANAGNOSTOPOULOS, C., AND J. SPIZIZEN. 1961. Requirements for transformation in *Bacillus subtilis*. *J. Bacteriol.* **81**:741-746.
2. BERTANI, G. 1958. Lysogeny. *Advan. Virus Res.* **5**:151-193.
3. BERTANI, G., L. E. HÄGGMARK, AND P. REICHERD. 1961. Synthesis of pyrimidine deoxyribonucleoside diphosphates with enzymes from *Escherichia coli*. *J. Biol. Chem.* **236**:PC67-68.
4. COHEN, S. S., M. SEKIGUCHI, J. L. STERN, AND H. D. BARNER. 1963. The synthesis of messenger RNA without protein synthesis in normal and phage-infected thymineless strains of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **49**:699-707.

5. ECHOLS, H., A. GAREN, S. GAREN, AND A. TORRIANI. 1961. Genetic control of repression of alkaline phosphatase in *E. coli*. *J. Mol. Biol.* **3**:425-438.
6. EPSTEIN, R. H., A. BOLLE, C. M. STEINBERG, E. KELLENBERGER, E. BOY DE LA TOUR, R. CHEVALLEY, R. S. EDGAR, M. SUSMAN, G. H. DEHARDT, AND A. LIELAUSIS. 1963. Physiological studies of conditional lethal mutants of bacteriophage T₄D. Cold Spring Harbor Symp. Quant. Biol. **28**:375-392.
7. FRIEDKINS, M., AND A. KORNBERG. 1957. The enzymatic conversion of deoxyuridylic acid to thymidylic acid and the participation of tetrahydrofolic acid, p. 609-613. In W. McElroy and B. Glass [ed.], *The chemical basis of heredity*. Johns Hopkins Press, Baltimore.
8. GAREN, A., AND H. ECHOLS. 1962. Genetic control of induction of alkaline phosphatase synthesis in *E. coli*. *Proc. Natl. Acad. Sci. U.S.* **48**:1398-1402.
9. GORMAN, J., P. TARUO, M. LABERGE, AND H. HALVORSON. 1964. Timing of enzyme synthesis during synchronous division in yeast. *Biochem. Biophys. Res. Commun.* **15**:43-49.
10. HALVORSON, H., J. GORMAN, P. TARUO, R. EPSTEIN, AND M. LABERGE. 1964. Control of enzyme synthesis in synchronous cultures of yeast. *Federation Proc.* **23**:1002-1008.
11. HANAWALT, P., AND R. WAX. 1964. Transcription of a repressed gene: evidence that it requires DNA replication. *Science* **145**:1061-1063.
12. HIRAGA, S. 1964. Synthesis of alkaline phosphatase and phosphodiesterase of mutants in *Bacillus subtilis*. *Japan. J. Genet.* **39**:342-343.
13. HORIUCHI, T., S. HORIUCHI, AND D. MIZUNO. 1959. A possible negative feedback phenomenon controlling formation of alkaline phosphomonoesterase in *Escherichia coli*. *Nature* **183**:1529-1530.
14. HOROWITZ, N. H., AND R. L. METZENBERG. 1965. Biochemical aspects of genetics. *Ann. Rev. Biochem.* **34**:527-564.
15. JACOB, F., AND E. L. WOLLMAN. 1959. Lysogeny, p. 319-351. In F. M. Burnet and W. M. Stanley [ed.], *The viruses*, vol. 2. Academic Press, Inc., New York.
16. KORN, D., AND A. WEISSBACH. 1962. Thymineless induction in *Escherichia coli* K12 (λ). *Biochim. Biophys. Acta* **61**:775-790.
17. LEVINTHAL, C., A. KEYNAN, AND A. HIGA. 1962. Messenger RNA turnover and protein synthesis in *B. subtilis* inhibited by actinomycin D. *Proc. Natl. Acad. Sci. U.S.* **48**:1631-1638.
18. LODISH, H. F., S. COOPER, AND N. D. ZINDER. 1964. Host-dependent mutants of the bacteriophage f2. IV. On the biosynthesis of a viral RNA polymerase. *Virology* **24**:60-70.
19. MASTERS, M., P. L. KUEMPEL, AND A. B. PARDEE. 1964. Enzyme synthesis in synchronous cultures of bacteria. *Biochem. Biophys. Res. Commun.* **15**:38-42.
20. OKADA, T., J. HOMMA, AND H. SONOHARA. 1962. Improved method for obtaining thymineless mutants of *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* **84**:602-603.
21. RADDING, G. M. 1964. Nuclease activity in defective lysogens of phage λ . II. A hyperactive mutant. *Proc. Natl. Acad. Sci. U.S.* **52**:965-973.
22. REICHARD, P., A. BALDESTEN, AND L. RUTBERG. 1961. Formation of deoxycytidine phosphates from cytidine phosphates in extracts from *Escherichia coli*. *J. Biol. Chem.* **236**:1150-1157.
23. SCHLESINGER, M. J., AND C. LEVINTHAL. 1963. Hybrid protein formation of *E. coli* alkaline phosphatase leading *in vitro* complementation. *J. Mol. Biol.* **7**:1-12.
24. SEAMAN, E., E. TARMY, AND J. MARMUR. 1964. Inducible phage of *Bacillus subtilis*. *Biochemistry* **3**:607-613.
25. TAKADA, R., AND Y. HIROTA. 1964. Genetic studies on synthesis of phosphatases in *Bacillus subtilis*. *Japan. J. Genet.* **39**:370-371.
26. TAKADA, R., M. TANIGUCHI, Y. HIROTA, AND A. TSUGITA. 1963. Genetic studies on synthesis of phosphatases in *Bacillus subtilis*. *Japan. J. Genet.* **38**:208.
27. TORRIANI, A. 1960. Influence of inorganic phosphate in the formation of phosphates by *Escherichia coli*. *Biochim. Biophys. Acta* **38**:460-479.
28. TORRIANI, A., AND F. ROTHMAN. 1961. Mutants of *Escherichia coli* constitutive for alkaline phosphatase. *J. Bacteriol.* **81**:835-836.
29. WIBERG, J. S., M. L. DIRKSEN, R. H. EPSTEIN, S. E. LURIA, AND J. M. BUCHANAN. 1962. Early enzyme synthesis and its control in *E. coli* infected with some amber mutants of bacteriophage T₄. *Proc. Natl. Acad. Sci. U.S.* **48**:293-302.
30. WOESE, F. R., AND J. R. FORRO. 1960. Correlations between ribonucleic acid and deoxyribonucleic acid metabolism during spore germination. *J. Bacteriol.* **80**:811-817.
31. YOSHIKAWA, H. A. O'SULLIVAN, AND N. SUEOKA. 1964. Sequential replication of the *Bacillus subtilis* chromosome. III. Regulation of initiation. *Proc. Natl. Acad. Sci. U.S.* **52**:973-980.