MEMOIRS OF THE FACULTY OF SCIENCE, KYOTO UNIVERSITY, SERIES OF BIOLOGY
Vol. VII, pp. 47–56, March 1976

Early RNA Synthesis of Bacteriophage T4; Relation to DNA Replication and the Gene 55 Product

By

Hirofumi NISHIMOTO*

Department of Botany, Faculty of Science, University of Kyoto, Kyoto, Japan

(Received September 10, 1973)

ABSTRACT RNA synthesis in T4 infected *Escherichia coli* (pm^-) was studied by measuring the rate of ^{3}H-uridine incorporation into acid-insoluble mRNA. When *E. coli* was infected with wild type T4, the rate of ^{3}H-uridine incorporation increased to the maximum at 5 min, then decreased gradually. When an amber mutant of T4 defective in DNA synthesis (DO) or maturation (MD) was used to infect the nonpermissive host, the rate of ^{3}H-uridine incorporation was similar to the rate of incorporation observed after wild type infection only during the first 5 min after infection, but decreased much more drastically thereafter. Chloramphenicol added at 3–8 min after a DO mutant infection inhibited the rapid decrease of the rate of ^{3}H-uridine incorporation. A similar phenomenon was observed when the cells were infected with a DO mutant irradiated with ultraviolet light.

DNA-RNA hybridization-competition test showed that almost all species of early RNA remained to be synthesized at later periods after infection by DO or MD mutants, allthough the amount of phage specific RNA present at that moment was found to be about one fifth of the amounts present in early after infection.

Introduction

Upon infection of *E. coli* by bacteriophage T4, synthesis of host macromolecules stops and T4 specific components such as DNA, RNA and protein start to be synthesized. Replication of phage DNA begins several minutes after infection. Protein which appear before the onset of T4 DNA are called early protein. Most of early protein cease to be synthesized late in infection (11). Protein which apperar after the onset of replication are called late protein. Similarly it was pointed out by Kano-Sueoka and Spiegelman (12) and Minagawa *et al.* (16) that synthesis of some RNA (early RNA) started before the onset of phage DNA reprication and synthsis of some RNA (late RNA) began after the onset of DNA reprication. This was

* Present address: Shionogi Reserch Laboratory, Shionogi & Co., LTD., Osaka, Japan.

clearly proved by Hall *et al.* (9), using their technique of DNA-RNA hybridization-competition. Matsukage and Minagawa (15) and Salser *et al.* (19) indicated that some early RNA ceases to be synshesized late in infection, while other early RNA does not. Those findings lead one to assume that the sequential synthesis of protein is controlled by transcription, allthough the data does not exclude the possibility that the protein synthesis is controled at the transcriptional level as well.

The switch-on mechanism of late synthesis has been shown to be connected with phage DNA replication and the functioning of gene 55 (18). It is not clear whether the shut-off of early protein is dependent on DNA replication or late synthesis (2, 5, 13, 14, 21). Since the temporal sequence of phage protein synthesis seems to be controled with the sequential synthesis of RNA, studies of RNA synthesis may aid in understanding the shut-off mechanisms of early protein synthesis. I have studied early RNA synthesis by measuring the rate of ^{3}H-uridine incorporation into early RNA and the effects of chloramphenicol or ultraviolet light on this process, using amber mutants of phage T4 defective in DNA synthesis or maturation. RNA synthesized after infection of *E. coli* by these amber mutants has been analysed by use of DNA-RNA hybridization-competition experiments.

Materials and Methods

Bacteria and bacteriophage. *Escherichia coli* BB was used as a nonpermissive (pm^{-}) host for amber mutants. *E. coli* BB was also used in studies with wild type T4. Wild type and amber mutants of bacteriophage T4D were kindly given by Dr. R. S. Edgar. An amber mutant designated as amX3 is a triple mutant of amN81 (gene 41), amN122 (gene 42) and amB22 (gene 43). These three mutants are defective in DNA synthesis (DO mutant), respectively. A mutant designated as amBL292 (gene 55) is a maturation defective amber mutant (MD mutant) (7). A quadruple mutant, amX3XBL292 was obtained by crossing amX3 and amBL292.

Ultraviolet light irradiation. Phage was irradiated by ultraviolet light (UV) as follows: Ten ml of phage suspension (10^{12} particles /ml) in a petri dish (diameter 10 cm) was exposed to a 15 watt GE germicidal lamp (Toshiba GL15) for 2 min at a distance of 80 cm. The surviving factor of the phage was 10^{-6}.

Culture and radioisotope labeling. *E.* coli BB was grown in TG medium (10) to a concentration of 5×10^{8} cells per ml at 37 C infected with phage at a multiplicity of about 8 in most experiments, but 4 in the UV-irradiated phage experiments. Uninfected cells at 1 min after infection with UV-irradiated phage were less than 2.0 % of cells before infection. The rate of incorporation of 5-^{3}H-uridine into RNA was determined by measuring the radioactivity in the 5 % cold trichloroacetic acid (TCA) insoluble material. Samples (0.2 ml) of the culture were re-

moved at appropriate intervals and put into test tubes containing 0.01 ml of 5-^{3}H-uridine (200 nCi/0.8 nmoles) and incubated at 37 C with shaking. One min after the addition of the isotope 0.2 ml of 2 % sodium-dodecylsulfate (SDS) containing 0.05 M NaCN was added to stop the incorporation. 0.1 ml of the lysate was pipetted on to a filter paper disk of 2.4 cm diameter. The disks were dried and washed with ice-chilled 0.3 M TCA three times, and acetone once. After dried again with hot air, the disks were counted radioactivity in toluene scintillation fluid with a Packard liquid scintillation spectrophotometer. Nonspecific adsorption of 5-^{3}H-uridine on the filter disk was determined as follows and subtracted from the counts on each disk: Infected cells were lysed in 0.2 % SDS containing 0.05 M NaCN and then 5-^{3}H-uridine was added. measurment of the radioactivity was done as described above.

DNA-RNA hybridization. ^{3}H-labeled and unlabeled RNA were extracted from infected cells by the SDS-phenol method (17) and pruified by the Dowex-Sephadex column method (1). Hybridization was carried out by the method of Gillespie and Spiegelman (8) with a slight modification: 0.1 % SDS was added to the hybridizing medium for the purpose of decreasing nonspecific adsorption of ^{3}H-RNA on the filter.

Chemicals. 5-^{3}H-uridine (13.6 Ci/mM) and chloramphenicol were purchased from Daiichi Pure Chemicals Co., LTD. and from Sankyo Co., LTD., respectively.

Results

The rate of ^{3}H-uridine incorporation into RNA after phage infection.

As shown in Fig. 1, the rate of ^{3}H-uridine incorporation into RNA increased during the first 5 min. Thereafter it decreased. Differences in this decrease were remarkable between infection with wild type and am mutants. In celles infected with wild type phage rapid decrease occured from 5 min to 8 min, then get a shoulder of ^{3}H-uridine incorporation to 15 min. After 15 min the rate of ^{3}H-uridine incorporation decreased again graduately (Fig. 1 a). When am mutants were used to infect *E. coli* (pm^{-}) rapid decrease continued from 5 min to 20 min, thereafter small amount of ^{3}H-uridine incorporation remained. Since no late RNA is synthesized during the infection of *E. coli* (pm^{-}) with amber mutants of phage T4 defective in DNA synthesis (DO) or in gene 55 (MD) (2), the shoulder of ^{3}H-uridine incorporation observed in cells infected with wild type phage may be attributed to ^{3}H-uridine incorporation into late RNA. Rapid decrease in the rate of ^{3}H-uridine incorporation observed in *E. coli* (pm^{-}) infected with amX3 mutant (DO) or BL292 mutant (MD) means that ^{3}H-uridine incorporation into early RNA stops in the absence of DNA replication or the function of gene 55.

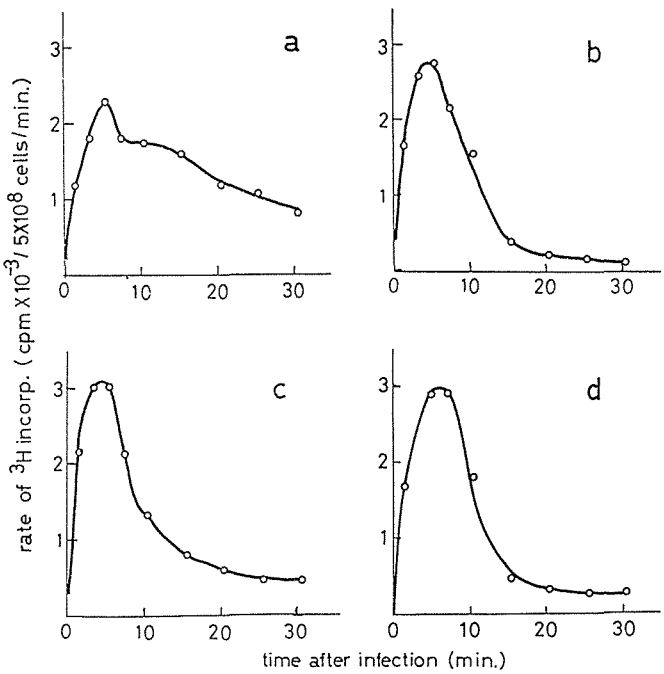

Fig. 1. The rate of ^{3}H-uridine incorporation into acid-insoluble RNA in phage-infected cells.

E. coli BB (pm^-) were grown in TG medium at 37C and infected with phage (moi 8), and then sampled at the times indicated and pulse labelled with 5-^{3}H-uridine (1μCi/4 mμmoles/ml) for one min. The radioactivity of cold trichloroacetic acid inscoluble material was measured as described in Materials and Methods. Nonspecific adsorption of ^{3}H-compounds to the filter was 80 cpm and was subtracted from each experimental value. (a) *E. coli* BB infected with wild type. (b) *E. coli* BB infected with amX3. (c) *E. coli* BB infected with amBL292. (d) *E. coli* BB infected with am×3×BL292.

Effect of chloramphenicol on the rate of ^{3}H-uridine incorporation into RNA.

To determine whether protein synthesis is required for the rapid decrease in the rate of ^{3}H-uridine incorporation observed in *E. coli* (pm^-) infected with amX3, chloramphenicol (CM) was added (100μg/ml) to cultures of cells infected with phage, and the rate of ^{3}H-uridine incorporation at different times was measured. As shown in Fig. 2, the rapid decrease in the rate of ^{3}H-uridine incorporation was inhibited dependently on the time of addition of CM. When CM was added at 2.5 or 4.5 min after infection the decrease was blocked almost completely (Fig. 2 a and b). However, the addition of CM at 6.5 or 8.5 min after infection, when the rate of ^{3}H-uridine incoporation started to decrease, prevent a further decrease after a short lag (Fig. 2 c and d). These results indicate that protein plays an important role in the rapid decrease of the rate of ^{3}H-uridine into early RNA. And the main-

tenance of ^{3}H-uridine incorporation after the short lag shown in Fig. 2 c and d may indicate that the protein works stoichiometrically or is unstable.

Effect of UV on the rate of ^{3}H-uridine incorporation into RNA.

It is well known that the arrest of the synthesis of early proteins which normally observed in T4 infected cells does not occur in cells infected with UV irradiated T even phage (6). Therefore I studied the effect of UV on the rapid decrease in the rate of ^{3}H-uridine incorporation. Cells were infected with UV irradiated T4 (surviving factor 10^{-6}). Allthough the rate of ^{3}H-uridine incorporation during the first 5 min was smaller than the rate observed in cells infected with unirradiated phage, the decrease in the rate of ^{3}H-uridine incorporation after 5 min was gradual both in cells infected with UV irradiated wild type T4 (Fig. 3 a) and with UV irradiated amX3 (Fig. 3 b). At the period later than 10 min after infection the rate of ^{3}H-uridine incorporation in cells infected with UV irradiated amX3 was higher

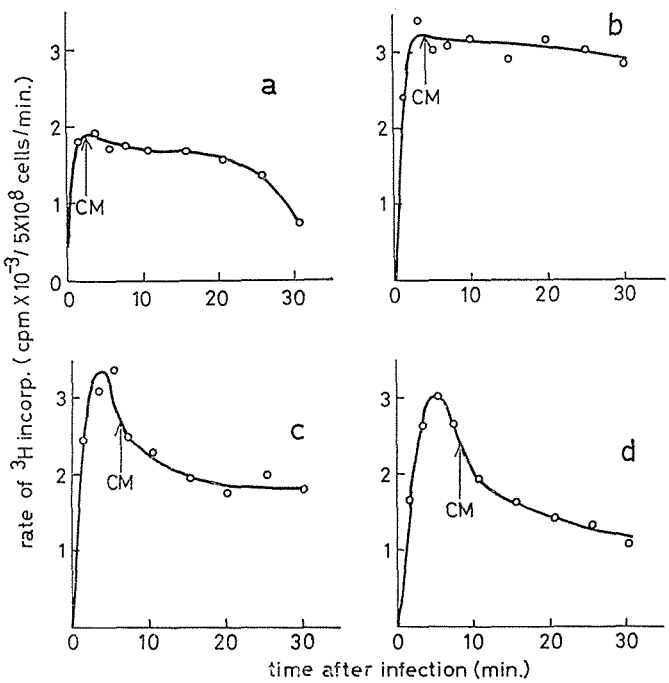

Fig. 2. Effect of chloramphenicol on the rate of 3H-uridine incorporation into RNA.

E. coli BB was grown, infected with am×3 (moi 8) and then RNA was pulse pabeled with 5-^{3}H-uridine and measured as described in the legend to Fig. 1. Chloramphenical (CM)was added at a concentration of 100 μg/ml. (a) CM was added at 2.5 min after infection. (b) CM was added at 4.5 min after infection. (c) CM was added at 6.5 min after infection. (d) CM was added at 8.5 min after infection.

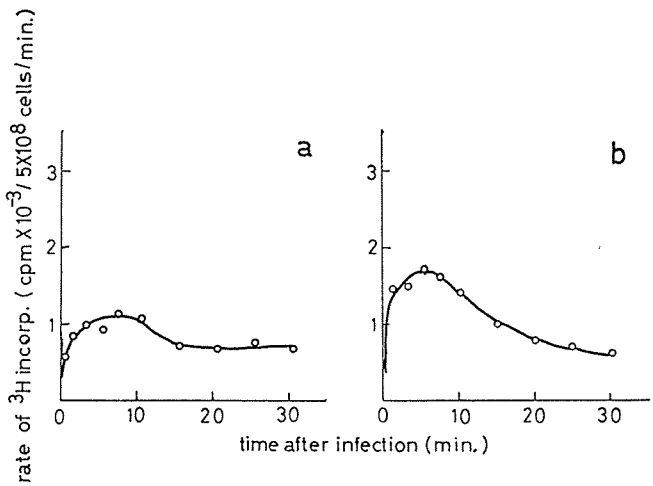

Fig. 3. Effec of ultraviolet light on the rate of ^{3}H-uridine incorporation into RNA.

E. coli BB was grown, infected with ultraviolet light (UV)-irradiated phage (the surviving factor 10^{-6}, moi 4), pulse labeled with 5-^{3}H-uridine for one min and radioactivity was measured as described in the legend to Fig. 1. (a) *E. coli* BB infected with UV-irradiated wild type. (b) *E. coli* BB infected with UV-irradiated am×3.

than that observed in cells infected with unirradiated amX3 (Fig. 1 b). This result indicates that UV irradiation of the phage interferes with the rapid decrease of the rate of ^{3}H-uridine incorporation into early RNA.

DNA-RNA hybridization-competition.

To compare the amount of amX3 RNA at early and later periods (4 min and 35 min) of infection, pulse labeled RNA was put in competition with unlabeled RNA of the same species (self-competition) in DNA-RNA hybridization. As shown in Fig. 4, RNA extracted at later periods of infection competed less well against itself (curve 2) than did early period RNA itself (curve 3). To obtain 50 % competition only 10 μg of unlabeled early period RNA was required, under the same condition 5 times more later period RNA was required to obtain the same level of self-competition. This indicates that the amount of phage RNA late in infection is decreased, probably to about one fifth of the amount present in early after infection. This observation suggests that the decreased rate of ^{3}H-uridine incorporation into RNA reflects the decrease in the rate of RNA synthesis.

To compare the RNA species of amX3 at early and later periods of infection, ^{3}H-labeled RNA extracted at an early period of amX3 infection was put in competition with unlabeled RNA extracted at a later period of amX3 infection. As shown in Fig. 4 curve 1, the degree of competion is almost the same as that of curve

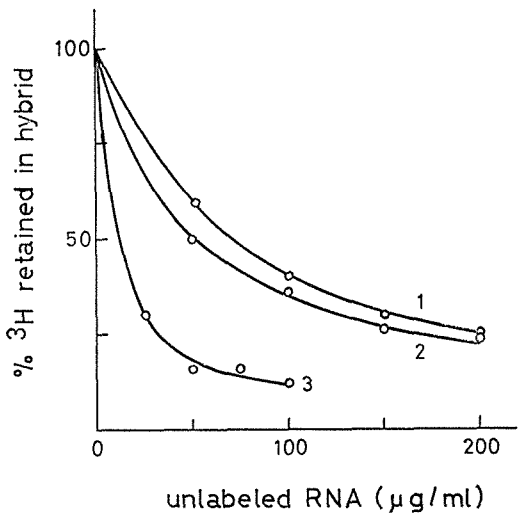

Fig. 4. DNA-RNA hybridization-competition tests of am×3 RNA.

^{3}H-labeled and unlabeled RNA from *E. coli* BB infected with am×3 was prepared as described in Materials and Methods. Hybridization was carried out as described in Materials and Methods. Two ml of hybridizing medium contained 2.0 μg ^{3}H-labeled RNA, 0.5 μg denatured T4 DNA immobilized on a Millipore filter membrane and unlabeled RNA as shown on abscissa. In this experiment 100% hydbridization denotes the radioactivity hybridized to T4 DNA on the membrane in the absence of unlabeled RNA: hybridization efficiency was 28% (curve 1 and 3) or 24% (curve 2) of input ^{3}H-labeled RNA, respectively (Each efficiency was constant even if the amount of ^{3}H-labeled RNA was changed from 0.1 to 3.0 μg. So, although 0.5 μg of denatured DNA was less amount in weight than 2.0 μg of ^{3}H-labeled RNA, the hybridizing medium without unlabeled RNA was a DNA-excess condition.). The nonspecific adsorption of ^{3}H-labeled RNA to a membrane on which 0.5 μg denatured T3 DNA was immobilized was negligible (less than 0.08% of input radioactivity). Crurve 1, competition of ^{3}H-RNA labeled between 2 and 4 min of am×3 infection (4230 cpm/μg) by unlabeled am×3 35 min RNA. Curve 2, competition of ^{3}H-RNA labeled between 28 and 32 min of am×3 infection (1000 cpm/μg) by unlabeled am×3 35 min RNA. Curve 3, competition of ^{3}H-RNA labeled between 2 and 4 min of am×3 infection by unlabeled am×3 4 min RNA.

2 which is self-competition of amX3 RNA extracted at a later period. This result indicates that unlabeled RNA extracted at a later period of amX3 infection included the ^{3}H-RNA extracted early in amX3 infection, in other words almost all RNA species present at an early period of amX3 infection remained at a later period. Similer results were obtained during infection of *E. coli* (pm^{-}) with amBL292 (data not shown).

Discussion

The DNA-RNA hybridization-competition experiments suggest that the decrease in ^{3}H-uridine incorporation during the course of infection reflects a decrease

in RNA synthesis. So the experiments on the rate of ^{3}H-uridine incorporation lead to the following conclusion concerning the report that no late RNA was observed during the infection of *E. coli* (pm^-) by am mutant of phage T4 defective in DNA synthesis (DO) or in gene 55 (MD) (2): In pm^- cells infected with an am mutant of T4 defective in genes 41, 42 and 43, synthesis of early RNA reaches a maximum at 5 min and then decreases rapidly to 20 min, after which only minor synthesis remains. The situation is the same in the case of infection of pm^- cells with an am mutant defective in gene 55. Since gene 41, 42 and 43 are essential for phage DNA replication and gene 55 controls late RNA synthesis, it is evident that neither replication of phage DNA, nor the product of gene 55, nor late protein is responsible for the decrease in the synthesis of early RNA. The finding that phage DNA replication is not essential for the decrease of early RNA synthesis is agrees with the recent results of Sköld (20) and Bolund and Sköld (3). However, I found no requirement for the gene 55 product, apparently contradicting Sköld (20), who showed from the experiments of DNA synthesis with fluorouracil that the gene 55 product is needed for the shut-off of the mRNA for early enzymes. This discrepancy may arise from the fact that different proteins are shut-off at different times. For example the patterns of gel-electrophoresis of early proteins by Hosoda and Levinthal (11) revealed that early protein synthesis ceased at different periods.

Addition of chloramphenicol before the decrease of RNA synthesis blocked this decrease almost completely, while addition during the decrease period blocked further decrease after a lag. This finding implies that some protein synthesized at around 5 min is responsible for the decrease in early RNA synthesis, and that the protein may be unstable or function stoichiometrically. The action of CM together with the UV-phage experiments where the decrease was found to be sensitive to UV suggests that the protein is controled by phage gene.

The RNA extracted late in infection of pm^- cells by DO or MD mutant reported to belong to early RNA (2). I found that small amounts of RNA which synthesized in late infection of DO or MD mutant does not consist of a portion of early RNA but consists of almost all species of early RNA.

Acknowledgments

The author expresses his sincere thanks to Prof. T. Minagawa for his valuable advice during the course of this study.

References

1) Bautz, E.K.F. and Hall, B.D. 1962. The isolation of T4-specific RNA on a DNA-cellulose column. Proc. Nat. Acad. Sci. **48**: 400–408.

2) Bolle, A., Epstein, R.H., Salser, W. and Geiduschek, E.P. 1968. Transcription during bacteriophage T4 development: Requirements for late messenger synthesis. J. Mol. Biol. **33**: 339–362.
3) Bolund, C. and Sköld, O. 1971. Formation of RNA in T4 phage-infected bacteria: Transcriptional regulation of early enzyme synthesis. Virology **43**: 390–402.
4) Bose, S.K. and Warren, R.J. 1967. *In vitro* stability of phage messenger ribonucleic acid. Proc. Nat. Acad. Sci. **58**: 2408–2414.
5) Cohen, P.S. 1968. Reversible repression of early enzyme synthesis in bacteriophage T4 infected *Escherichia coli*. J. Virol. **2**: 192–197.
6) Dirksen, M.L., Wiberg, J.S., Koerner, J.F. and Buchanan, J.M. 1960. Effect of ultraviolet irradiation of bacteriophage T2 on enzyme synthesis in host cells. Proc. Nat. Acad. Sci. **46**: 1425–1430.
7) Edgar, R.S. and Wood, W.B. 1966. Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. Proc. Nat. Acad. Sci. **55**: 498–505.
8) Gillespie, D. and Spiegelman, S. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. **12**: 829–842.
9) Hall, B.D., Nygaard, A.P. and Green, M.D. 1964. Controle of T2-specific RNA synthesis. J. Mol. Biol. **9**: 143–153.
10) Hershey, A.D. and Melechen, N.E. 1957. Synthesis of phage-precursor nucleic acid in the presence of chloramphenicol. Virology. **3**: 207–236.
11) Hosoda, J. and Levinthal, C. 1968. Protein synthesis by *Escherichia coli* infected with bacteriophage T4D. Virology. **34**: 709–727.
12) Kano-Sueoka, T. and Spiegelman, S. 1962. Evidence for a nonrandom reading of the gene. Proc. Nat. Acad. Sci. **48**: 1942–1949.
13) Lembach, K.J., Kuninaka, A. and Buchanan, J.M. 1969. The relationship of DNA replication to the control of protein synthesis in protoplasts of T4-infected *Escherichia coli* B. Proc. Nat. Acad. Sci. **62**: 446–453.
14) Mathews, C.K. and Kessin, R.H. 1967. Control of bacteriophage-induced enzyme synthesis in cells infected with a temperature-sensitive mutant. J. Virol. **1**: 92–96.
15) Matsukage, A. and Minagawa, T. 1967. Shut-off of early messenger RNA synthesis in *E. coli* infected with phage T2. Biochem. Biophys. Res. Comm. **29**: 39–44.
16) Minagawa, T., Okamoto, T., Aono, H., Uchida, A. and Mizuno, N. 1964. Messenger RNA of ultraviolet light irradiated phage T2. Biochim. Biophys. Acta **91**: 158–160.
17) Okamoto, K., Sugino, Y. and Nomura, M. 1962. Synthesis and turnover of phage messenger RNA in *E. coli* infected with bacteriophage T4 in the presence of Chloromycetin. J. Mol. Biol. **5**: 527–534.
18) Puritzer, J.F. and Geiduschek, E.P. 1970. Function of T4 gene 55 II. RNA synthesis by temperature-sensitive gene 55 mutants. J. Mol. Biol. **49**: 489–507.
19) Salser, W., Bolle, A. and Epstein, R. 1970. Transcription during bacteriophage T4 development: A demonstration that distinct subclasses of the "early" RNA appear at different times and that some are "turned-off" at late times. J. Mol. Biol. **49**: 271–295.
20) Sköld, O. 1970. Regulation of early-RNA synthesis in bacteriophage T4-infected *Escherichia coli*. J. Mol. Biol. **53**: 339–356.
21) Wiberg, J.S., Dirksen, M-L., Epstein, R.H., Luria, S.E. and Buchanan, J.M. 1962. Early enzyme synthesis and its controle in *E. coli* infected with some amber mutants of bacteriophage T4. Proc. Nat. Acad. Sci. **48**: 293–302.