Effect of Infection with Ribonucleic Acid Bacteriophage R23 on the Inducible Synthesis of β -Galactosidase in *Escherichia coli*

HIROKO WATANABE AND MAMORU WATANABE

Departments of Medicine and Biochemistry, University of Alberta, Edmonton, Alberta, Canada

Received for publication 18 July 1968

Infection by ribonucleic acid (RNA) bacteriophage R23 inhibited the synthesis of β -galactosidase in *Escherichia coli*. The inhibition, although not complete, was apparent shortly after infection and was maximal after the first 20 min of infection. R23 diminished the β -galactosidase-synthesizing capacity when inducer was added after phage infection, but not when infection followed inducer removal. These findings suggested that the primary effect of R23 on enzyme-forming capacity was limitation of synthesis of enzyme-specific messenger RNA. Studies with ultraviolet irradiated phage and amber mutants of R23 indicated that the inhibitory process could be separated into two phases. Early inhibition did not require the expression of the viral genome, whereas late inhibition required the expression of the viral RNA synthetase cistron.

Infection of *Escherichia coli* by different deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) bacteriophages results in an alteration in the metabolism of the host bacterium. However, the degree of inhibition of synthesis of host macromolecules may vary. T-even phage infection produces an immediate and complete inhibition of host protein and nucleic acid synthesis (4, 7, 9, 17, 23, 24, 26, 29, 34-36, 44). Phage λ also inhibits host DNA (50) as well as RNA and protein synthesis (20, 45). The disturbance in the metabolism of the host is more profound after infection by λ than following induction of the prophage (20, 42, 48). After ϕ x174 infection, host DNA synthesis ceases 12 to 14 min after infection, but synthesis of RNA and protein is affected only slightly (27). The inhibitory effect of RNA bacteriophages depends upon the phage strain used to infect the bacterial host (49). Inhibition of synthesis of host DNA (6, 13), RNA (5, 6, 11, 13, 21, 49), and protein (13, 37, 49) has been reported for several RNA phages.

In the case of T-even phage infection, the inhibition of bacterial protein synthesis is due, in part, to an arrest in the initiation of synthesis of messenger RNA (mRNA; 23, 24). Thus, infection with T-even phage at the time of inducer addition completely prevents the synthesis of β -galactosidase (4, 23). In addition, T-even phage infection causes a specific enhancement of mRNA destruction in F⁻ cells (23). Howes (20) reported that

phage λ infection at high multiplicity interrupts protein synthesis prior to any interference with RNA synthesis. On the other hand, studies of Terzi and Levinthal (45) suggest that λ phage interferes with host metabolism at the level of host RNA synthesis.

The mechanism of inhibition of host protein synthesis by RNA phage infection is not yet established. Different observations, however, indicate several mechanisms by which this inhibition might occur. Hotham-Iglewski and Franklin (18) reported an alteration in the distribution of polysomes after R17 infection, with smaller polysome units predominating after phage infection. Codon-specific changes in the ability of transfer RNA (tRNA) to support translation of synthetic polynucleotides are also reported in cells infected with Q β ; these changes apparently result from a decreased ribosomal binding of tRNA (P. P. Hung and L. R. Overby, *unpublished data*). The magnitude of viral RNA synthesis in R23-infected cells (in which practically all of the RNA synthesized after infection is phage-directed RNA) suggests that interference of bacterial protein synthesis is secondary to inhibition of host RNA synthesis (49).

The inhibition of host mRNA synthesis by T-even phage infection does not depend on the expression of viral genes, since the inhibition of β -galactosidase induction occurs promptly upon infection, does not require protein synthesis or

breakdown of host DNA (35, 44), and is unaffected by ultraviolet irradiation of phage (23). This inhibitory effect may be related to the coat protein moiety of the phage particles. Phage ghosts prepared by osmotic shock of intact phage are known to inhibit the synthesis of nucleic acids and protein in host cells (12). Following the proposal of Jacob, Brenner, and Cuzin (22) that bacterial DNA is connected to the cell surface, Kaempfer and Magasanik (23) have hypothesized that changes in surface properties caused by phage may affect the transcription of bacterial DNA into mRNA. However, there is strong evidence to indicate that interference with host RNA synthesis is not due to a protein component of the λ phage and that adsorption itself is not responsible for any membrane alteration causing the reduced cellular synthesis. Host-restricted λ and ghosts of phage λ do not show this inhibitory effect (45). A second factor in T-even phage-infected cells which affects host protein synthesis is the enhancement of mRNA destruction, and this effect appears to require the expression of viral genes (23). Even very small doses of ultraviolet irradiation abolish this effect completely.

In this study we examined the nature of inhibition of bacterial protein synthesis in *E. coli* following infection with the RNA phage R23. R23 has the ability to markedly inhibit host protein and RNA synthesis (49). We attempted to elucidate (i) the mechanism of inhibition of bacterial protein synthesis, (ii) the relationship of inhibition of bacterial protein synthesis to interference with host RNA synthesis, (iii) the requirement of viral genetic information for inhibition of bacterial protein synthesis, and (iv) the specific cistron required for this inhibitory process.

Three cistrons are defined thus far for the RNA phage (14, 19, 46). The proteins specified by these three cistrons are the RNA synthetase (3, 8, 15, 50), the phage coat protein (8, 33, 34, 40, 41) and the "maturation protein." The maturation protein is a histidine-containing polypeptide which has been shown to be a structural component of the viable bacteriophage (33, 43, 47, 49); its absence is associated with noninfective phage particles (2, 16, 28). It was of interest to determine if any of the three known cistrons or an as yet unidentified cistron might be responsible for the ability of R23 to inhibit host macromolecular synthesis.

MATERIALS AND METHODS

Bacteria and bacteriophages. *E. coli* K38 (51) was used as bacterial host for these studies. Bacteriophage R23, an RNA bacteriophage of *E. coli*, has been previously described (49). Amber mutants of R23 were isolated after nitrous acid mutagenesis. Their characterization will be reported in a subsequent publication.

For ultraviolet irradiation of phage, suspensions diluted in tris(hydroxymethyl)aminomethane (Tris) buffer to a titer of 10^{12} phage/ml were exposed for 4 min to a GE germicidal lamp at a distance of 13.5 cm. Under these conditions, the titer of survivors was 5×10^6 phage/ml.

Media. Medium A (10) was used for most of the studies; this medium contained 7 g of Na_2HPO_4 , 3 g of KH_2PO_4 , 1 g of NH_4Cl , 0.5 g of NaCl , 0.6 g of MgSO_4 , 0.11 g of CaCl_2 , 6 g of Casamino Acids (Difco), 10 ml of 30% glycerol, and 1,000 ml of demineralized water. Tryptone medium, the medium used for plating RNA phage, and the solutions used for diluting phage suspensions have been previously described (49).

Materials. Isopropyl- β -D-thiogalactopyranoside (IPTG) and *o*-nitrophenyl- β -D-galactopyranoside (ONPG) were purchased from Mann Research Laboratories, Inc., New York, N.Y.; ^{14}C -IPTG (25 $\mu\text{C}/\mu\text{mole}$) from Calbiochem, Los Angeles, Calif.; and ^{14}C -methionine (12.5 $\mu\text{C}/\mu\text{mole}$) from New England Nuclear Corporation, Boston, Mass. Membrane filters (type DA) were obtained from Millipore Corp., Bedford, Mass.

Bacterial growth and phage assay. Cultures of *E. coli* K38 grown overnight at 37 C in medium A were diluted 300-fold with the same fresh medium, and incubated in a gyrotory shaker at 37 C until the exponentially growing culture reached a density of 2×10^8 cells/ml. RNA phage was then added at a multiplicity of 10 to 20 phage/cell.

Phage was assayed as plaque-forming units by the agar overlay technique as described by Adams (1).

Induction of β -galactosidase. The enzyme was induced by the addition of IPTG to a final concentration of 5×10^{-4} M to cultures incubated in a gyrotory shaker at 37 C.

Inducer was removed from the medium by filtration on a membrane filter of pore size 0.65 μm (Millipore Corp., grade DA). The cells were washed with an equal volume of phosphate buffer at room temperature, and resuspended in fresh medium A by blowing liquid on the submerged membrane. The entire procedure took 2 to 3 min.

β -Galactosidase was assayed as described by Pardee, Jacob, and Monod (40). One unit of enzyme activity has been defined as that amount of enzyme which catalyzed the hydrolysis of 1 nmole of ONPG per min at 37 C (pH 6.8).

Synthesis of protein. Synthesis of protein was determined by the incorporation of radioactive amino acids into acid-insoluble material as previously described (49).

RESULTS

Effect of phage R23 on protein and β -galactosidase synthesis. A study of the effect of R23 phage infection on bacterial protein synthesis was complicated by the fact that rapid infection of an entire bacterial population could not be obtained. Maximal phage adsorption required 4 min, and 10% of the bacterial population survived the initial infection (Watanabe and Watanabe, *un-*

published data). Despite this limitation, it was possible to examine the effect of R23 on the synthesis of β -galactosidase in *E. coli*. The synthesis of β -galactosidase after R23 infection, although diminished in amount, was not completely inhibited (Fig. 1). The enzyme activity observed in an R23-infected culture was greater than that which could be accounted for by the population of uninfected bacteria although the latter, which varied from one culture to the other affected the final enzyme activity observed. The inhibitory effect was evident shortly after infection, increased gradually for the first 20 min, and then became more pronounced (Fig. 2). This period of maximal inhibition corresponds to the time of maximal phage coat protein synthesis (49).

Infection with T-even phages completely and instantly halts host mRNA synthesis (24). In cells carrying the F episome, the decay of enzyme-forming capacity proceeds at the same rate whether induction is arrested by phage infection or by inducer removal (23). The synthesis of β -galactosidase continued for a longer period after R23 infection than after inducer removal (Fig. 3), suggesting that R23 differed from T2 in its ability to inhibit mRNA synthesis.

Pardee and Prestidge (41) have observed that about 3 min elapse between addition of inducer and appearance of active enzyme. This lag period is related to the synthesis of β -galactosidase-specific mRNA. Removal of the inducer at the end of the lag period by filtration of the culture and subsequent resuspension in inducer-free medium

FIG. 1. Kinetics of β -galactosidase induction and the effect of R23 infection. To one-half of a culture of *E. coli* K38 growing in medium A, R23 was added at a multiplicity of 10 phage/cell at 0 min. IPTG was added 3 min later. Control (●); R23-infected culture (○).

FIG. 2. Rate of β -galactosidase synthesis in bacteria infected with R23. A culture of *E. coli* K38 growing in medium A was infected with R23 at a multiplicity of 20 phage/cell. At various periods after infection, IPTG was added and the culture was shaken at 37°C in a gyrotory shaker. The cultures were analyzed for β -galactosidase activity 15 min after the addition of inducer.

allows the cells to express their enzyme-forming capacity. In uninfected and in R23-infected cultures, enzyme synthesis ceased 7 to 10 min after inducer removal (Fig. 4). In R23-infected cells, however, the final level of enzyme attained after inducer removal was lower than that in uninfected cells. Thus, although R23 infection was unable to completely prevent the induction of β -galactosidase, it limited the capacity for enzyme synthesis. This effect was even more marked when inducer was added for a period of 3 min at 30 min after R23 infection. However, when R23 was added immediately after a 3-min exposure to IPTG and removal of inducer, the appearance and final level of enzyme activity were identical to that for inducer removal in uninfected cells. Thus, the final level of enzyme activity was reduced when R23 was present during the period of synthesis of enzyme-specific mRNA, but infection by R23 did not affect the enzyme-forming capacity once the enzyme-specific mRNA had been formed.

It has been proposed that the rate of decay of β -galactosidase-forming capacity after inducer removal is a reflection of the stability of enzyme-specific mRNA (25, 30). The effect of R23 infection on the rate of decay of enzyme-forming capacity was examined in cells induced for 3 min. The capacity for enzyme synthesis decayed at an exponential rate with $t_{1/2}$ of 1.6 min in uninfected cells (Fig. 5). The rate of decay of β -galactosidase-forming capacity after inducer removal was unaltered by the presence of R23 during the period

of induction, although the final level of enzyme synthesized was diminished, as already noted. Similarly, the rate of decay of enzyme-forming capacity was unaltered when bacterial cultures infected with R23 for 30 min were exposed to inducer for 3 min. Finally, the addition of R23 after the period of enzyme induction did not affect the rate of decay of enzyme-forming capacity. Thus, it appeared that R23 infection did not at any time affect the stability of enzyme specific mRNA.

Effect of ultraviolet irradiation of R23. To test if diminution of β -galactosidase-forming capacity in *E. coli* following R23 infection required a phage-specific genetic function, the ability of ultraviolet irradiated phage to inhibit β -galactosidase was tested. Ultraviolet irradiated phage was capable of inhibiting the synthesis of β -galactosidase during the first 25 to 30 min after infection, but did not inhibit enzyme synthesis after this time (Fig. 6). It thus appeared that the inhibition of synthesis of β -galactosidase could be separated into two phases—an "early" phase which did not appear to require the expression of the viral genome and a second or "late" phase which required the expression of the phage genome to maintain the inhibition of bacterial protein synthesis.

FIG. 3. β -Galactosidase induction and the effect of inducer removal and R23 infection. To cultures of *E. coli* K38, IPTG was added at 0 min. (A) Control (●); (B) infection with phage R23 at a multiplicity of 20 phage/cell at 0 min (○); (C) inducer removal at 3 min by filtration and resuspension 2 min later in inducer-free medium A (▲).

FIG. 4. β -Galactosidase induction and the effect of inducer removal. *E. coli* K38 was grown in medium A, and one-half of the culture (B) was infected with R23 at a multiplicity of 10 phage/cell. IPTG was added 3 min after infection. Inducer was removed from both uninfected (●) and R23-infected (○) cultures at 3 min by filtration and resuspension 3 min later in inducer-free medium A.

Effect of R23 on accumulation of inducer. The early inhibition noted after R23 infection did not appear to result from inhibition of bacterial ability to accumulate the inducer of β -galactosidase. The accumulation of ^{14}C -labeled IPTG by R23-infected cells was not diminished compared to the uninfected culture.

Gene responsible for "late" inhibition of bacterial protein synthesis. To determine the gene or genes required for the late inhibition of bacterial protein synthesis, we examined several amber mutants of R23. None of the mutants defective in the A protein required for phage "maturation" demonstrated a defect in the ability to inhibit bacterial protein synthesis either early or late after infection. On the other hand, a mutant defective in the viral RNA synthetase cistron lacked the ability to inhibit β -galactosidase late in infection. Like the ultraviolet irradiated phage, this mutant phage was capable of inhibiting the synthesis of β -galactosidase early after infection (Fig. 7). However, in the cultures infected by this mutant, in contrast to cultures infected by wild-type R23, inhibition of β -galactosidase synthesis was not maintained after the first 25 to 30 min of infection. It appeared, therefore, that expression of the RNA synthetase cistron was necessary for the inhibition of bacterial protein synthesis late in infection.

DISCUSSION

Infection by R23 reduced both the rate of incorporation of radioactive amino acids (Fig. 8)

and the synthesis of β -galactosidase in *E. coli*. Although the inhibition of β -galactosidase synthesis was noted shortly after infection, the maximal effect occurred after the first 20 min of infection. This was in keeping with the large amount

FIG. 5. Decay of β -galactosidase synthesis as a function of time following inducer removal in uninfected and R23 infected cultures. Cells of *E. coli* K38 infected with R23 for 3 min were induced for 3 min and transferred to inducer-free medium (from Fig. 4). Uninfected culture (●); R23-infected culture (○). The points represent $(E_{\max} - E_t)/E_{\max}$ at any given time, t ; $E_{\max}$ is the final level of enzyme activity reached after inducer removal.

FIG. 6. β -Galactosidase synthesis and the effect of ultraviolet irradiated R23. *E. coli* K38 was grown in medium A, and one-third of the culture was infected with R23 at a multiplicity of 10 phage/cell (C). A second culture was infected with the same number of phage particles which had been previously treated with ultraviolet light (B). IPTG was added to these cultures and to an uninfected control culture (A) 4 min after the addition of phage.

of phage coat protein being synthesized late in infection. After the first 30 min of infection, about 75% of the protein synthesized was characterized as phage coat protein, and between 45 and 75 min, coat protein accounted for all of the protein being synthesized (49).

The inhibition of β -galactosidase after R23 infection differed from that observed with T-even phage infection. Infection of *E. coli* by T-even phages at the time of inducer addition completely prevented the synthesis of β -galactosidase (4, 23). R23, on the other hand, limited the synthesis of β -galactosidase, but it was unable to completely inhibit enzyme-forming capacity.

The diminution in β -galactosidase-synthesizing capacity could result from (i) an effect of R23 on the rate of overall protein synthesis, (ii) interference with the synthesis of enzyme-specific mRNA, (iii) suppression of expression of this message, (iv) alteration of the stability of enzyme-specific mRNA, or a combination of these four factors. It was unlikely that the diminution in enzyme synthesis was due to an effect of R23 on the rate of overall protein synthesis, since the latter did not appear to be greatly different in R23-infected cultures during the first 20 min compared

FIG. 7. β -Galactosidase synthesis and the effect of an RNA synthetase mutant of R23. The experiment was performed as outlined in Fig. 6, except that curve B represents a culture infected with an RNA synthetase mutant of R23 at a multiplicity of 10 phage/cell.

to uninfected cultures (Fig. 8). Furthermore, infection by R23 affected the final level of enzyme activity when R23 was present during the period of synthesis of enzyme-specific mRNA, but did not affect the enzyme-forming capacity once the enzyme-specific mRNA had been formed. It appeared unlikely that R23 infection altered the enzyme-synthesizing capacity by affecting overall protein synthesis or by suppressing the expression of enzyme-specific mRNA. Finally, unlike T-even phage infection (23), R23 infection did not at any time affect the stability of enzyme-specific mRNA. Thus, it appeared that R23 reduced the β -galactosidase-synthesizing capacity by interfering with the synthesis of enzyme-specific mRNA.

Inhibition of enzyme synthesis by R23 appeared to have two distinct phases. Inhibition of enzyme synthesis during the first 20 to 25 min of infection did not require the expression of a viral gene. The inhibitory process during this period was unaffected by ultraviolet irradiation of phage suspension. Whether this effect is due to action on cell membranes, as postulated for T2 (23), remains to be clarified. A second function appeared to determine the inhibition of β -galactosidase after the first 20 to 25 min of infection. This late

FIG. 8. Incorporation of ^{14}C -methionine and the effect of R23 infection. To cultures of *E. coli* K38, grown in tryptone medium, ^{14}C -methionine was added to a final concentration of $1\text{ }\mu\text{C}/\text{ml}$. R23 was added to one-half of the culture at a multiplicity of 10 phage/cell. Samples of both cultures were removed at various intervals, and the protein was determined as described (49). Control (●); R23-infected culture (○).

inhibition disappeared when phage was treated with ultraviolet irradiation. The late inhibition was also absent with mutant phage defective in the RNA synthetase cistron, but was present with phage defective in the "maturation protein." It thus appeared that expression of the viral RNA synthetase cistron was a requirement for the maintenance of inhibition of bacterial protein late in infection and, in its absence, bacterial protein synthesis was able to resume after the early inhibition. The exact mechanism of this inhibitory effect, however, requires further elucidation. Several possible factors must be considered: (i) The primary gene product, the viral RNA synthetase, may function in some way as a "repressor" of bacterial RNA and protein synthesis. (ii) Viral specific RNA whose synthesis requires the induction of the viral RNA synthetase may be responsible for the inhibitory process. (iii) Viral coat protein may function as repressor. The synthesis of R23 coat protein appears to be dependent upon the synthesis of progeny RNA (49). (iv) An unidentified protein may be responsible for inhibition, and the synthesis of this protein, like the coat protein, may be dependent upon the synthesis of progeny RNA. It is possible that the viral RNA synthetase cistron is only one of several cistrons whose expression is required for the inhibition of bacterial protein synthesis late in infection. It is also possible that the same factor, be it RNA or protein, may be responsible for both early and late inhibition of host processes, the two

phases then representing "parental" and "progeny" effects.

ACKNOWLEDGMENTS

This investigation was supported by grants ME-2851 and MA-2822 from the Medical Research Council of Canada.

H. Watanabe is a recipient of the Research Fellowship of the Damon Runyon Memorial Fund for Cancer Research.

LITERATURE CITED

- Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
- Argetsinger, J. E., and G. N. Gussin. 1966. Intact ribonucleic acid from defective particles of bacteriophage R17. *J. Mol. Biol.* **21**:421-434.
- August, J. T., S. Cooper, L. Shapiro, and N. D. Zinder. 1963. RNA phage induced RNA polymerase. Cold Spring Harbor Symp. Quant. Biol. **28**:95-97.
- Benzer, S. 1953. Induced synthesis of enzymes in bacteria analyzed at the cellular level. *Biochim. Biophys. Acta* **11**:383-395.
- Bishop, D. H. L. 1965. Ribonucleic acid synthesis by *Escherichia coli* C3000/L after infection by the ribonucleic acid coliphage ZIK/1, and properties of coliphage ZIK/1 ribonucleic acid. *Biochem. J.* **97**:17-26.
- Bishop, D. H. L. 1966. Ribonucleic acid synthesis by *Escherichia coli* C3000/L after infection by the ribonucleic acid coliphage ZIK/1, and properties of the coliphage-induced double-stranded ribonucleic acid. *Biochem. J.* **100**:601-613.
- Brenner, S., F. Jacob, and M. Meselson. 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **190**:576-581.
- Capecchi, M. R. 1966. Cell-free protein synthesis programmed with R17 RNA: identification of two phage proteins. *J. Mol. Biol.* **21**:173-193.
- Cohen, S. S. 1948. The synthesis of bacterial viruses. I. The synthesis of nucleic acid and protein in *Escherichia coli* B infected with T2^{r+} bacteriophage. *J. Biol. Chem.* **174**:281-293.
- Davern, C. I. 1964. The isolation and characterization of an RNA bacteriophage. *Australian J. Biol. Sci.* **17**:719-725.
- Ellis, D. B., and W. Paranchych. 1963. Synthesis of ribonucleic acid and protein in bacteria infected with an RNA bacteriophage. *J. Cellular Comp. Physiol.* **62**:207-213.
- French, R. C., and L. Siminovitch. 1955. The action of T2 bacteriophage ghosts on *Escherichia coli* B. *Can. J. Microbiol.* **1**:757-774.
- Granboulan, N., and R. M. Franklin. 1966. High resolution autoradiography of *Escherichia coli* cells infected with bacteriophage R17. *J. Bacteriol.* **91**:849-857.
- Gussin, G. N. 1966. Three complementation groups in bacteriophage R17. *J. Mol. Biol.* **21**:435-453.
- Haruna, I., K. Nozu, Y. Ohtaka, and S. Spiegelman. 1963. An RNA "replicase" induced by and selective for a viral RNA: isolation and properties. *Proc. Natl. Acad. Sci. U.S.* **50**:905-911.
- Heisenberg, M. 1966. Formation of defective bacteriophage particles by fr amber mutants. *J. Mol. Biol.* **17**:136-144.
- Hershey, A. D., J. Dixon, and M. Chase. 1953. Nucleic acid economy in bacteria infected with bacteriophage T2. I. Purine and pyrimidine composition. *J. Gen. Physiol.* **36**:777-789.
- Hotham-Iglewski, B., and R. M. Franklin. 1967. Replication of bacteriophage ribonucleic acid: alterations in polyribosome patterns and association of double-stranded RNA with polyribosomes in *Escherichia coli* infected with bacteriophage R17. *Proc. Natl. Acad. Sci. U.S.* **58**:743-749.
- Horiuchi, K., H. F. Lodish, and N. D. Zinder. 1966. Mutants of the bacteriophage f2. VI. Homology of temperature-sensitive and host-dependent mutants. *Virology* **28**:438-447.
- Howes, W. V. 1965. Protein synthesis in *Escherichia coli*: a phage-mediated interruption of translation. *Biochim. Biophys. Acta* **103**:711-713.
- Hudson, J. B., and W. Paranchych. 1967. Effect of bacteriophage R17 infection on host-directed synthesis of ribosomal ribonucleates. *J. Virol.* **1**:529-537.
- Jacob, F., S. Brenner, and F. Cuzin. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329-348.
- Kaempfer, R. O. R., and B. Magasanik. 1967. Effect of infection with T-even phage on the inducible synthesis of β -galactosidase in *Escherichia coli*. *J. Mol. Biol.* **27**:453-468.
- Kaempfer, R. O. R., and B. Magasanik. 1967. Mechanism of β -galactosidase induction in *Escherichia coli*. *J. Mol. Biol.* **27**:475-494.
- Kepes, A. 1963. Kinetics of induced enzyme synthesis. Determination of the mean life of galactosidase-specific messenger RNA. *Biochim. Biophys. Acta* **76**:293-309.
- Levin, A. P., and K. Burton. 1961. Inhibition of enzyme formation following infection of *Escherichia coli* with T2^{r+}. *J. Gen. Microbiol.* **25**:307-314.
- Lindqvist, B. H., and R. L. Sinsheimer. 1967. Process of infection with bacteriophage ϕ_{X174} . XIV. Studies on macromolecular synthesis during infection with a lysis-defective mutant. *J. Mol. Biol.* **28**:87-94.
- Lodish, H. F., K. Horiuchi, and N. D. Zinder. 1965. Mutants of the bacteriophage f2. V. On the production of non-infectious phage particles. *Virology* **27**:139-155.
- Monod, J., and E. Wollman. 1947. L'inhibition de la croissance et de l'adaptation enzymatique chez les bactériens infectés par le bacteriophage. *Ann. Inst. Pasteur* **73**:937-956.
- Nakada, D., and B. Magasanik. 1964. The roles of inducer and catabolite repressor in the syn-

- thesis of β -galactosidase by *Escherichia coli*. J. Mol. Biol. 8:105-127.
31. Nathans, D. 1965. Cell-free protein synthesis directed by coliphage MS2 RNA: synthesis of intact viral coat protein and other products. J. Mol. Biol. 13:521-531.
 32. Nathans, D., G. Notani, J. H. Schwartz, and N. D. Zinder. 1962. Biosynthesis of the coat protein of coliphage f2 by *E. coli* extracts. Proc. Natl. Acad. Sci. U.S. 48:1424-1431.
 33. Nathans, D., M. P. Oeschger, K. Eggen, and Y. Shimura. 1966. Bacteriophage-specific proteins in *E. coli* infected with an RNA bacteriophage. Proc. Natl. Acad. Sci. U.S. 56:1844-1851.
 34. Nomura, M., B. D. Hall, and S. Spiegelman. 1960. Characterization of RNA synthesized in *Escherichia coli* after bacteriophage T2 infection. J. Mol. Biol. 2:306-320.
 35. Nomura, M., K. Matsubara, K. Okamoto, and R. Fujimura. 1962. Inhibition of host nucleic acid and protein synthesis by bacteriophage T4: its relation to the physical and functional integrity of host chromosome. J. Mol. Biol. 5:535-549.
 36. Nomura, M., C. Witten, N. Mantei, and H. Echols. 1966. Inhibition of host nucleic acid synthesis by bacteriophage T4: effect of chloramphenicol at various multiplicities of infection. J. Mol. Biol. 17:273-278.
 37. Nonoyama, M., A. Yuki, and Y. Ikeda. 1963. On some properties of phage β , a new RNA containing phage. J. Gen. Appl. Microbiol. 9:299-305.
 38. Notani, G. W., D. L. Engelhardt, W. Konigsberg, and N. D. Zinder. 1965. Suppression of a coat protein mutant of the bacteriophage f2. J. Mol. Biol. 12:439-447.
 39. Ohtaka, Y., and S. Spiegelman. 1963. Translational control of protein synthesis in a cell-free system directed by polycistronic viral RNA. Science 142:493-497.
 40. Pardee, A. B., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β -galactosidase by *E. coli*. J. Mol. Biol. 1:165-178.
 41. Pardee, A. B., and L. S. Prestidge. 1961. The initial kinetics of enzyme induction. Biochim. Biophys. Acta 49:77-88.
 42. Siminovitch, L., and F. Jacob. 1952. Biosynthèse induite d'un enzyme pendant le développement des bactériophages chez *Escherichia coli* K12. Ann. Inst. Pasteur 83:745-754.
 43. Steitz, J. A. 1968. Identification of the A protein as a structural component of bacteriophage R17. J. Mol. Biol. 33:923-936.
 44. Terzi, M. 1967. Studies on the mechanism of bacteriophage T4 interference with host metabolism. J. Mol. Biol. 28:37-44.
 45. Terzi, M., and C. Levinthal. 1967. Effects of λ phage infection on bacterial synthesis. J. Mol. Biol. 26:525-535.
 46. Tooze, J., and K. Weber. 1967. Isolation and characterization of amber mutants of bacteriophage R17. J. Mol. Biol. 28:311-330.
 47. Vinuela, E., I. D. Algranati, G. Feix, D. Garwes, C. Weissmann, and S. Ochoa. 1968. Virus-specific proteins in *Escherichia coli* infected with some amber mutants of phage MS2. Biochim. Biophys. Acta 155:558-565.
 48. Waites, W. M., and B. A. Fry. 1964. Effect of infection with phage λ on the synthesis of protein, RNA and DNA in *Escherichia coli*. J. Gen. Microbiol. 34:413-426.
 49. Watanabe, M., H. Watanabe, and J. T. August. 1968. Replication of RNA bacteriophage R23. I. Quantitative aspects of phage RNA and protein synthesis. J. Mol. Biol. 33:1-20.
 50. Weissmann, C., L. Simon, and S. Ochoa. 1963. Induction by an RNA phage of an enzyme catalyzing incorporation of ribonucleotides into ribonucleic acid. Proc. Natl. Acad. Sci. U.S. 49:407-414.
 51. Zinder, N. D., and S. Cooper. 1964. Host-dependent mutants of bacteriophage f2. I. Isolation and preliminary classification. Virology 23:152-158.