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Induction and Repression of L-Arabinose Isomerase in Bacteriophage-Infected *Salmonella typhimurium*

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The induction of L-arabinose isomerase in *Salmonella typhimurium* (LT2) is repressed on infection with clear plaque forming mutants (C_1 and C_2) of the temperate phage P22 (C^+). However, after infection with C^+ leading to lysogeny, there is a temporary repression. During this period, messenger ribonucleic acid (RNA) for L-arabinose isomerase accumulates. DNA-RNA hybridization data suggest that there is transcription of host DNA during the period of repression. Interference at the level of translation might be responsible for the cessation of induced enzyme synthesis.

Salmonella typhimurium LT2 on infection with the temperate phage P22 (C^+) produces phage deoxyribonucleic acid (DNA) before lysogeny is established (11, 12). Phage-specific DNA synthesis begins at about 4 min after phage infection, continues to increase for 2 to 4 min, and then decreases until complete cessation occurs at 16 min. Phage-specific protein synthesis under conditions of lysogeny as well as lysis was also studied (3). In infections leading to lysis, phage-specific protein synthesis was detected from the 4th min after infection and continues throughout the latent period, whereas, in the case of infections producing lysogeny, the synthesis continues from 4th to 8th min, after which the rate decreases and becomes practically zero after the 20th min. Studies on the synthesis of an inducible enzyme, L-arabinose isomerase, in *S. typhimurium* have already been done (A. K. Bhattacharya and M. Chakravorty, *unpublished data*). To understand the differences in the regulation of host protein synthesis after infections leading to lysis or lysogeny, the induction of L-arabinose isomerase in phage-infected *S. typhimurium* has been studied. The results corroborate the suggestion (11, 12) that the first phase after infection leading to lysogeny resembles that of a lytic infection. Evidence will also be presented to indicate that the interference in enzyme synthesis during this phase is most probably at the stage of translation and not at transcription. Similar results have already been obtained for β-galactosidase synthesis (1, 5, 7, 10).

MATERIALS AND METHODS

Bacteria and bacteriophage strains. *S. typhimurium* strain LT2 and the temperate phage P22 (C^+) were kindly provided by P. Margolin of the Cold Spring Harbor Laboratory of Quantitative Biology, Cold Spring Harbor, N.Y. The clear plaque mutants, C_1 and C_2, were generous gifts from M. Levine of the Department of Human Genetics, University of Michigan, Ann Arbor, Mich. The lysogenized strain of *S. typhimurium* carrying P22 as the prophage, LT2(C^+), was isolated and purified in this laboratory after infecting the host with P22 at a high multiplicity of infection (MOI).

Growth. The cells were normally grown at 35 C on a reciprocal shaker in minimal medium containing 0.2% glycerol. The composition of the minimal medium is as follows: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $(NH_4)_2SO_4$, 1.0 g; sodium citrate-$5H_2O$, 0.47 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; water, 1 liter. Growth was measured by following the optical density of the cell suspension at 640 nm in the Leitz colorimeter.

Induction of L-arabinose isomerase. Overnight cultures of LT2 were diluted 10 times in fresh medium and allowed to grow again. When the cultures had grown to an optical density of 0.6, they were again diluted to an optical density of 0.1. L-Arabinose (0.3%) was added as an inducer after the growing cell suspension reached an optical density of 0.3 (approximately 3×10^8 cells/ml). Samples were removed at intervals, and the enzyme induction was immediately stopped by adding chloramphenicol (50 μg/ml). Each sample was quickly cooled to 0 C, centrifuged, washed once with an equal volume of cold tris(hydroxymethyl)aminomethane (0.12 M; *p*H 8.0) and suspended in 1 ml of the same buffer. The cell suspensions were then treated with ethylenedia-

minetetraacetic acid (EDTA) at 37 C (6). The EDTA-treated cells, which were readily permeable to the substrates, were used for the assay of L-arabinose isomerase. EDTA-treated cells retained activity overnight at 0 C.

Assay of L-arabinose isomerase. The enzyme was assayed by the method already described (2). One unit of enzyme is defined as the amount which produces ribulose, yielding the cysteine-carbazole color equivalent to an optical density of 0.1 at 520 nm as measured in the Leitz colorimeter.

Protein determination. Protein was determined by the Folin-Ciocalteau method (9). Optical density of the cell suspension (measured after dilution) was found to be proportional to the protein content of the cells. Therefore, the protein content was calculated from the optical density of the samples with reference to a calibration curve.

Incorporation of $^{35}SO_4^{2-}$. To follow $^{35}SO_4^{2-}$ incorporation by *S. typhimurium*, the cells were grown overnight in low-sulfur medium (LSM) described by Cohen and Levine (3) and diluted 20-fold in fresh LSM containing 0.2% glycerol. When the cell suspension reached an optical density of 0.7 to 0.8, it was again diluted to an optical density of 0.1 and allowed to grow. $^{35}SO_4^{2-}$ was added at a concentration of 0.3 mc/ml when the cells reached an optical density of 0.4. Samples (1 ml) were pipetted into chilled centrifuge tubes containing 1 ml of cell suspension as carrier and 2 ml of 10% trichloroacetic acid. The mixtures were kept in ice for 30 min to allow complete precipitation. The precipitates were then washed four times, each time with 10 ml of 1% trichloroacetic acid containing ammonium sulfate (2 g per liter), once with 10% thioglycolic acid, and then plated on steel planchets and counted in the Gas Flow Counter of Bhabha Atomic Research Centre, India.

Pulse labeling of ribonucleic acid (RNA). Cell suspensions (0.5 ml) of exponentially growing cells were quickly pipetted into tubes containing ^{3}H-uridine (0.1 μc). The tubes were kept shaking in a water bath at 37 C. The reaction was stopped after 24 sec by adding an equal volume of ice cold 10% trichloroacetic acid. The tubes are kept in ice for another 30 min to allow complete precipitation. The precipitate was then collected on a membrane filter (Millipore Corp., Bedford, Mass.), washed with 20 ml of cold 5% trichloroacetic acid, dried, and counted in a scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Denaturation of DNA. DNA in 0.01 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate) at a concentration less than 30 μg/ml was heated for 10 min at 100 C. The sample was chilled immediately in a dry-ice–acetone bath. DNA was denatured also by alkali treatment as described by Gillespie and Spiegelman (4).

DNA-RNA hybridization. DNA-RNA hybridizations were performed by the technique described by Gillespie and Spiegelman (4). Denatured DNA in 5 × SSC was filtered through membrane filters (Schleicher & Schull Co., Keene, N.H.), presoaked in 5 × SSC. DNA was passed through very slowly. Retention of DNA was 85 to 90%. The membranes were washed with 50 ml of 5 × SSC and dried overnight at room temperature and then at 80 C for 4 hr. Purified ^{3}H-labeled RNA was incubated with DNA fixed on filters, in 5 ml of 5 × SSC for 14 hr at 60 C. After incubation, both sides of the filters were washed with 2 × SSC (50 ml on each side) and treated with ribonuclease (20 μg/ml) for 1 hr at 37 C. Filters were again washed with 2 × SSC as before, dried, and counted in a Packard scintillation counter. The method of purification of RNA will be described in a later publication.

RESULTS

Induction of L-arabinose isomerase during infections leading to phage production. The enzyme can be induced in cells growing in minimal medium containing arabinose (A. K. Bhattacharya and M. Chakravorty, *unpublished data*). If the inoculum is glucose-grown, a lag period of 10 to 15 min is discernible before a measurable quantity of enzyme appears in the cell. On the other hand, if the inoculum is glycerol grown, the induction of the enzyme starts without any lag. Hence, for the experiments to be described below, the process of induction was studied in cells growing exponentially on glycerol from glycerol-grown inoculum. Upon infection by C mutants which cause lysis of the host cells, the enzyme L-arabinose isomerase cannot be induced in LT2 (Fig. 1). There is no indication of enzyme synthesis up to a period of 20 min after infection. In some experiments observations were made for longer periods as well. In no case was enzyme synthesis detectable. Induction is thus prevented by phage infection.

Enzyme induction during infections leading to lysogeny. When cells are infected with C^+ at a MOI of 20, more than 90% of the cells become lysogenic. Under this condition, the synthesis of L-arabinose isomerase also stops but only for a short period, about 10 min after phage infection, and finally the rate becomes almost the same as that of uninfected cells (Fig. 2). When the host was infected with C_1 and C_2 mutant phages under conditions which give complementation leading to high frequency lysogenization, there is a longer lag, about 20 min, before the induction of the enzyme is initiated (Fig. 2). However, LT2 lysogenic for phage C^+ can be induced for the enzyme L-arabinose isomerase synthesis as well as uninfected LT2 (Fig. 3). The above results indicate that, when the invading virus establishes a lysogenic relationship, the induction of the enzyme within the host takes place normally, though in the initial stages of infection the situation is similar to that of lytic infection. These findings are in conformity with the observations of Levine and his co-workers (3, 12) in DNA and protein synthesis during establishment of lysogeny. For some unknown reasons, the process of enzyme induction is inhibited for a longer period

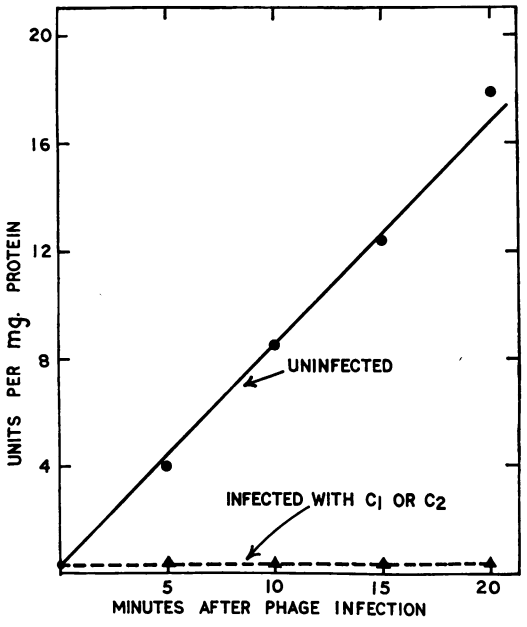

FIG. 1. *Induction of L-arabinose isomerase in Salmonella typhimurium infected with C_1 or C_2 leading to lysis. The exponentially growing culture of LT2 was infected with C_1 or C_2 mutant at an MOI of 5. The inducer was added at the time of addition of the phage.*

in case of mixed infection with C_1 and C_2 than in case of single infection with C^+.

Enzyme synthesis in lysogenized cells treated with mitomycin C. It has already been shown that phage multiplication results in the inhibition of enzyme induction. Therefore L-arabinose isomerase synthesis was followed in lysogenic cells induced to phage production by mitomycin C. Mitomycin C is known to be an excellent lytic inducer of lysogenic cells including *S. typhimurium* (8). In the mitomycin C-treated cells, enzyme induction continues at the same rate as control up to about 1 hr; after that there is no further synthesis (Fig. 4). Phage production is observed 60 min after the addition of the antibiotic. This probably accounts for the similar delay in inhibition of L-arabinose isomerase synthesis.

Protein synthesis during infections leading to lysogeny. The synthesis of the inducible enzyme L-arabinose isomerase is delayed for a few minutes after phage infections which lead to lysogeny (Fig. 2). The question arises whether this is true for the synthesis of all the proteins or only for the inducible enzymes. Overall protein synthesis in LT2, infected with C^+ or mixedly with C_1 and C_2, was measured by using $^{35}SO_4^{2-}$. The results presented (Fig. 5) indicate that, for the first 2 min after phage infection, there is some protein synthesis. Very soon, however, the rate of protein synthesis is slowed down and then starts to increase after 8 min. The average rate of protein synthesis after 8 min in case of infections leading to lysogeny (as measured by the extent of in-

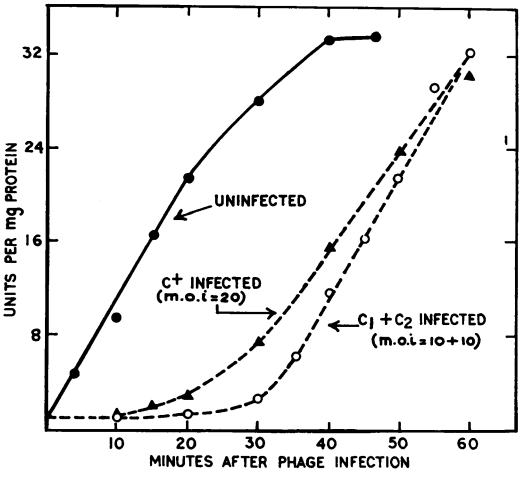

FIG. 2. *Induction of L-arabinose isomerase in Salmonella typhimurium after phage infection leading to lysogeny. The inducer and the phage were added simultaneously. In case of mixed infection with C_1 and C_2, both the mutants were added simultaneously, each at MOI of 10. Sampling and assay of the enzyme were as described under Materials and Methods.*

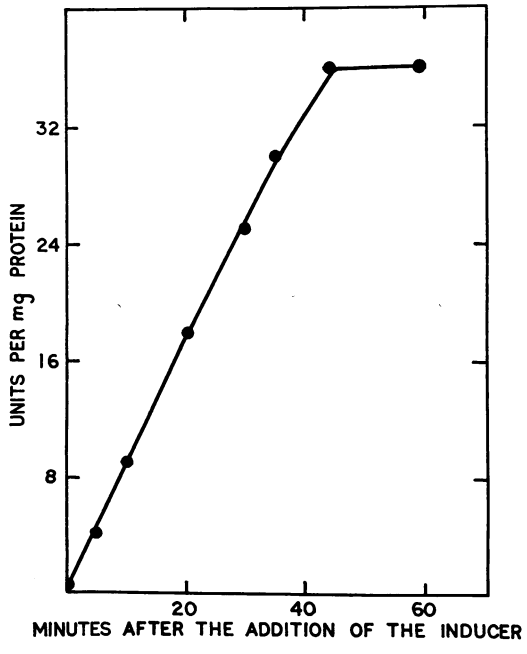

FIG. 3. *Induction of L-arabinose isomerase in the lysogenized cells of Salmonella typhimurium.*

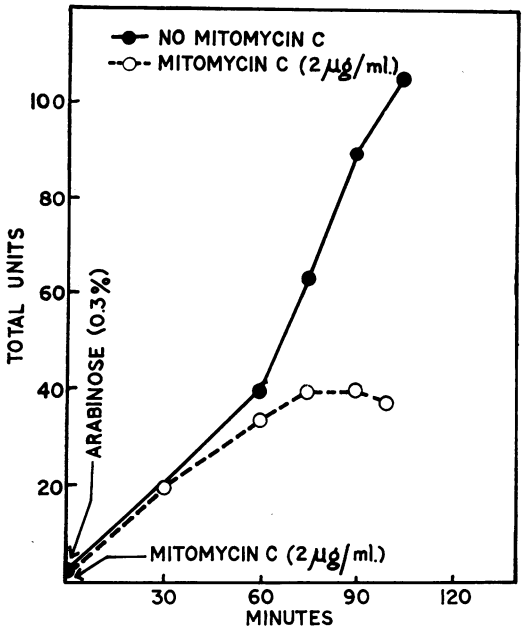

FIG. 4. *Enzyme induction in lysogenized cells of Salmonella typhimurium induced to lysis by mitomycin C. LT2(C+) cells were grown in minimal medium containing glycerol, 0.2%. When exponentially growing culture attained an optical density of 0.3 at 640 nm, it was divided into two batches. One batch was treated with mitomycin C, 2 μg/ml. Inducer was added immediately to both the batches. Samples taken at indicated periods were assayed as before.*

corporation per minute) is about one-fifth that of uninfected cells (*unpublished data*). The infected cells regain the normal rate of protein synthesis after about 20 to 30 min. The incorporation of $^{35}SO_4^{2-}$ into protein (Fig. 5) indicates that not only the synthesis of the inducible enzyme L-arabinose isomerase but also total protein synthesis is slowed down to a very low level after infections leading to lysogeny. The cells start overcoming this inhibition after 8 min.

Rate of messenger RNA (mRNA) synthesis. The rate of mRNA synthesis after phage infection leading to lysis or lysogeny was followed by giving a pulse of ^{3}H-uridine for 24 sec at different intervals after phage infection (Fig. 6). Phage infection causes an immediate decrease in the rate of mRNA synthesis. In the case of infections leading to lysogeny, there is a sudden increase in the mRNA synthesis at 4 min, reaching a rate equal to that of the uninfected cells. The rate drops again until 8 min when it begins to increase. The peak of mRNA synthesis around 4 min is very reproducible. After 20 min, the cells synthesize mRNA at the rate for uninfected cells. On the other hand, after infections leading to lysis, the rate of mRNA synthesis decreases. After 5 min the rate starts increasing and attains the maximum value within a short period. This maximum rate in the infected host is, however, much less than that in the noninfected host.

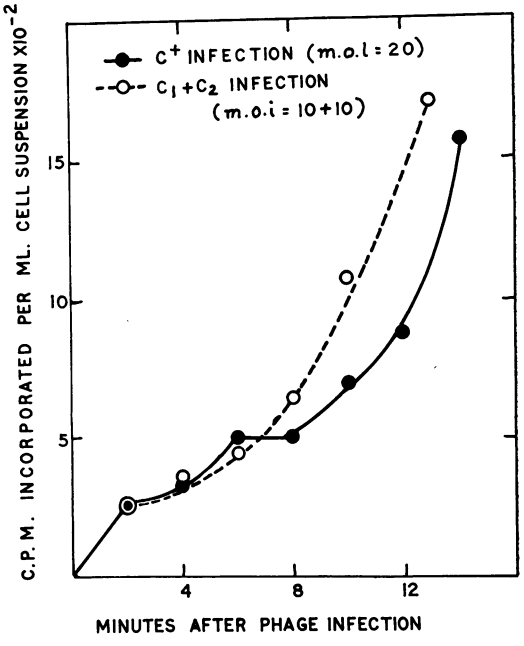

FIG. 5. *Incorporation of $^{35}SO_4^{2-}$ by S. typhimurium after phage infection leading to lysogeny. Cells were grown in low sulfur medium. Phage and $^{35}SO_4^{2-}$ were added simultaneously. Samples (1 ml) were pipetted at indicated times. Precipitation and washing of the samples were as described under Materials and Methods. The 0-min sample showed 5 to 10 counts above the background. All the counts were corrected for 0-min control.*

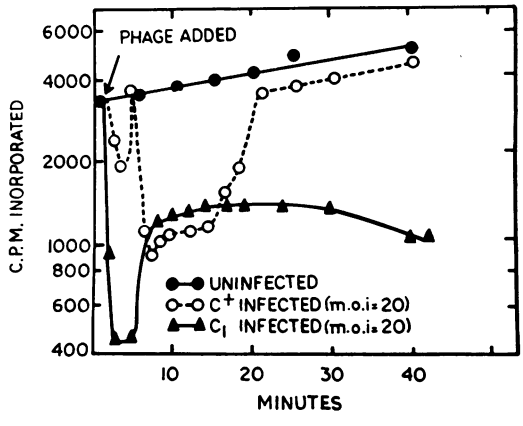

FIG. 6. *Kinetics of mRNA synthesis in phage-infected Salmonella typhimurium.*

Accumulation of mRNA after phage infection. After infection with the temperate phage C^+, the induction of L-arabinose isomerase is inhibited for 10 min and then recovers (Fig. 2), mimicking the more permanent inhibition of induction seen in infections leading to lysis. The observed recovery offers the possibility of studying the mechanism of the temporary inhibition of isomerase induction. Absence of enzyme induction during this period may be due to one of the following reasons. (i) Phage infection stops host mRNA synthesis. (ii) Phage infection blocks translation of host mRNA. (iii) Subunits of the enzyme are made, but phage infection prevents association of the subunits into an enzymatically active protein. To check the above three possibilities, an experiment was done the following way. Actively growing cells of LT2 were infected with the phage P22 C^+ (MOI = 20). Inducer was simultaneously added. Samples were collected at 5 and 10 min after phage infections. Each sample was divided into three parts. To one, chloramphenicol (50 μg/ml) was added to stop further enzyme synthesis. This part (Fig. 7, IIa) will measure the amount of enzyme synthesized at the time of sampling. The other two parts were immediately diluted with inducer-free media, and the inducer was removed by quick filtration. The cells were then suspended in fresh media (containing 0.2% glycerol) without any inducer and incubated at 37 C for 30 min, one part in presence and the other in absence of chloramphenicol. The experimental procedure has been summarized in Fig. 7.

If no host mRNA synthesis takes place during the first 10 min of the infection, one would expect no increase in enzyme activity when the cells are incubated for another 30 min in absence of the inducer (Fig. 7, IIb, c). If, however, messenger synthesis does occur, but translation is blocked, an increase in enzyme activity would be expected in the sample incubated without inducer and without chloramphenicol, but not in the presence of the chloramphenicol. Any difference in enzyme content between these samples after 30 min of incubation might be due to the mRNA made during the first 10 min of the infection in the presence of the inducer. An equal increase in enzyme activity in both the samples, in the presence and absence of chloramphenicol, would indicate that both transcription and translation of the host proceeded during the early infections but that assembly of subunits into an active enzyme was inhibited.

As a control, the experiment was done with uninfected cells to find out whether mRNA of L-arabinose isomerase is stable enough to be expressed. Uninfected cells show 20 and 42 units of enzyme at 5 and 10 min, respectively, after the addition of the inducer. The enzyme level does not increase by further incubation in the presence of chloramphenicol. This indicates that in uninfected cells, there are no free subunits of the enzyme which have to undergo assembly to make active enzyme molecules. But the enzyme level increases from 20 to 33 units and 42 to 57 units in case of 5-min and 10-min samples, respectively, in absence of inducer, as a result of further incubation in absence of chloramphenicol (Table 1, Ia and b). This indicates that mRNA for L-arabinose isomerase is stable enough to be translated into protein.

In case of infected cells, no enzyme synthesis takes place during the 30-min incubation period

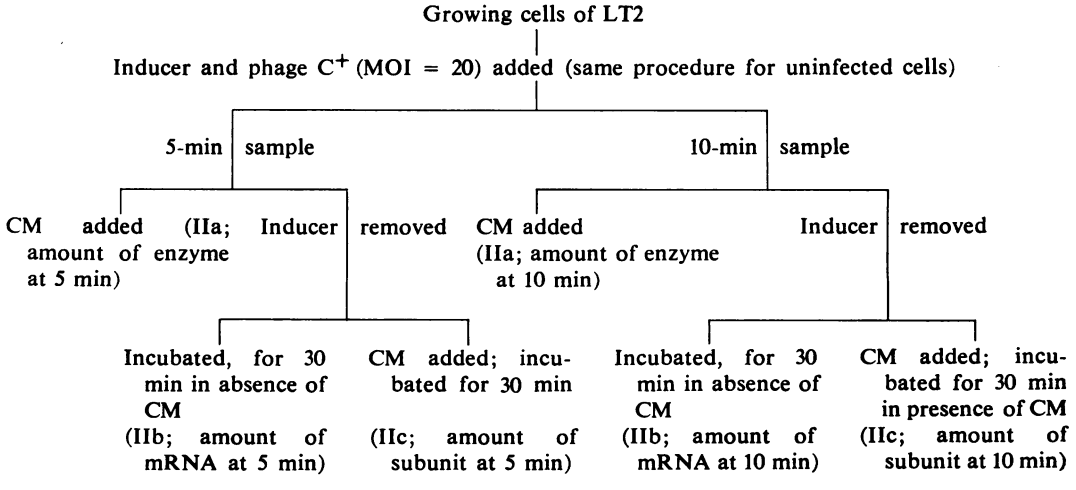

FIG. 7. *Plan of the experiment, the results of which are presented in Table 1. CM, choramphenicol.*

TABLE 1. *Accumulation of mRNA of L-arabinose isomerase*[a]

Conditions	Total units of L-arabinose isomerase/time of sampling after addition of inducer	
	5 min	10 min
I. Uninfected cells		
a. Induction stopped by chloramphenicol	20	42
b. Inducer removed and then incubated for 30 min in absence of chloramphenicol	33	57
c. Inducer removed and then incubated for 30 min in presence of chloramphenicol	21	40
II. Infected cells		
a. Induction stopped by chloramphenicol	3	3
b. Inducer removed and then incubated for 30 min in absence of chloramphenicol	7	13
c. Inducer removed and then incubated for 30 min in presence of chloramphenicol	4	4

[a] Growing cells (3×10^8/ml) of LT2 were infected with C^+ (MOI = 20). Inducer (0.3%) was simultaneously added. Samples (60 ml) were collected at 5 and 10 min after phage infection. Each sample was divided into three equal parts. To one, chloramphenicol (50 μg/ml) was immediately added to stop further enzyme synthesis. The other two batches were immediately diluted with inducer-free media; the inducer was removed by quick filtration. The cells were then suspended in fresh media (containing 0.2% glycerol) in the absence of the inducer and incubated at 37 C for 30 min, one batch in presence and the other in absence of chloramphenicol.

in presence of chloramphenicol. This rules out the possibility that absence of enzyme synthesis is due to a defect in subunit assembly (Table 1, cf. IIa and c). On the other hand, when the infected cells are washed free of inducer and incubated in the absence of chloramphenicol, the enzyme level increases from 3 units to 7 and 13 units in the 5- and 10-min samples, respectively (Table 1, IIa and b). The small amount of enzyme formed during the postincubation period is taken as a rough estimate of accumulated mRNA. These results can be explained if we assume that enzyme synthesis is stopped at the translation level rather than at the transcription stage.

DNA-RNA hybridization. To find out whether the RNA synthesized during the first 10 min after infection leading to lysogeny is host specific or phage specific, mRNA synthesized during this period was hybridized with phage as well as host DNA. The mRNA types synthesized at 5 and 10 min after infection leading to lysogeny, and at 10 min after infection leading to lysis, were hybridized with both phage and host DNA (Table 2). At 10 min after infection leading to lysis, 90% of the RNA is phage specific. At 5 min after infection leading to lysogeny, about 70% of the RNA transcribed is phage specific and 30%, is host specific. At 10 min, more host-specific messengers are synthesized and the ratio of host and phage specific messengers become 1 :1.

DISCUSSION

It is well known that, after infection with lytic phages, host-specific protein synthesis is inhibited but the mechanism is obscure. Repression of the inducible enzyme l-arabinose isomerase in *S. typhimurium* is an example of this phenomenon. This type of inhibition has already been observed for β-galactosidase (1, 7, 10).

The induction of the enzyme L-arabinose isomerase is repressed after infections leading to lysis (Fig. 1). In case of infections leading to lysogeny, synthesis of the inducible enzyme is inhibited for 10 min after infection, after which the inhibition is overcome and the enzyme is induced (Fig. 2). This is in conformity with the results obtained by

TABLE 2. *Hybridization of pulse-labeled RNA with phage or host DNA*[a]

Pulse-labeled RNA	Counts/min of input	DNA (30 μg)	Counts/min of hybridized	Per cent hybridized
At 5 min after C^+ infection leading to lysogeny	9,600	Phage	2,571	26.78
		Host	1,190	12.4
At 10 min after C^+ infection leading to lysogeny	16,120	Phage	2,841	17.60
		Host	2,776	17.22
At 10 min after C_1 infection leading to lysis	16,800	Phage	1,086	6.46
		Host	149	0.88
Of uninfected host	17,640	Phage	130	0.74
		Host	3,950	22.00

[a] mRNA was isolated from infected cells exposed to ^{3}H-uridine for 24 sec. Methods of isolation of RNA will be published later. DNA-RNA hybridization was carried out on a nitrocellulose membrane filter by the method of Gillespie and Spiegelman (4).

Levine and his co-workers (3, 12), suggesting a lytic condition at the initial phase. If lysogeny is already established, there is no indication of such repression (Fig. 3). Studies recorded with mitomycin C (Fig. 4) are also in agreement with these observations. Enzyme synthesis is repressed as soon as mitomycin C induces the phage. The induction and repression of L-arabinose isomerase in a phage-infected host may not be isolated phenomena but are expressions of the general situations in terms of mRNA production or protein synthesis in the host. This is also evident from the rate of overall protein synthesis (Fig. 5), which is slowed down within a short time after phage infection. The rate then tends to increase and resumes the normal rate around the 20th minute.

The basic question that arises is whether the mRNA production is inhibited because of phage infection or its translation is interfered with. An attempt was made to answer this question by using a temperate phage. During the early phase after infection leading to lysogeny, when there is no induction of L-arabinose isomerase, the cells were tested for their ability to synthesize the enzyme in the absence of the inducer. Experiments done with uninfected cells showed an increase in the level of enzyme as a result of further incubation in absence of chloramphenicol (Table 1; 1a and b), indicating that mRNA for L-arabinose isomerase is stable enough to be translated later. In the case of infected cells, no enzyme synthesis takes place during 30 min of incubation in presence of chloramphenicol (Table 1; IIa and c). Yet, in the absence of chloramphenicol, the enzyme level increases to a small but significant extent. This small amount of enzyme formation is taken as an indication of the accumulation of mRNA for L-arabinose isomerase during the early phase. This indicates that, after infection leading to lysogeny, there is transcription of the mRNA for L-arabinose isomerase. Interference at the level of translation is perhaps responsible for the inhibition of enzyme synthesis.

The decrease in the rate of mRNA synthesis after phage infection (Fig. 6) indicates that transcription of the host genome is continued at a very reduced rate after phage infection. In case of infections leading to lysogeny, there is a sudden burst of mRNA synthesis around 4 min, which is followed by an immediate drop in the rate of mRNA synthesis. The rate of mRNA synthesis starts increasing around the 5th min and attains the normal rate, i.e., the rate of uninfected cells, 20 min after infection.

DNA-RNA hybridization between mRNA and different DNA types (phage or host) indicated that, unlike the infections leading to lysis, there is transcription of host-specific messengers in case of infections leading to lysogeny.

The hybridization data, along with the evidence for the accumulation of L-arabinose messenger, indicate that in case of infections leading to lysogeny some host specific messengers are synthesized. The translation of this message is most probably interferred with, as was also concluded by Howes et al. for β-galactosidase (5).

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