Influence of Gene 55 on the Regulation of Synthesis of Some Early Enzymes in Bacteriophage T4-Infected *Escherichia coli*

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Received for publication 15 September 1972

In *Escherichia coli* B infected with phage T4 with mutations in gene 55 the shut-off of synthesis of two early enzymes was found to be delayed 10 to 15 min as compared to wild-type T4 infection. The kinetics of early enzyme formation was very similar to that obtained after DNA-negative mutant infection. The transcription of gene 1 (one of the genes coded for early enzymes) ceased at 9 to 12 min after infection whether the gene 55 product was active or not, as determined by the time dependence of fluorouracil rescuability of DNA synthesis in cells infected with a gene 1 amber mutant. The synthesis of the major part of the early RNA species, determined by pulse-labeling with uracil-5-³H, was furthermore found to be regulated independently of gene 55. An explanation for the extended early enzyme synthesis in DNA-negative and gene 55 mutant infection is offered: translation of early messengers is restricted after about 12 min in wild-type infection, whereas in cells infected with DNA-negative or gene 55 mutants, i.e., the absence of late protein synthesis, early messengers direct the formation of early enzymes for a longer time.

After infection of *Escherichia coli* with bacteriophage T4 the synthesis of early enzymes is abruptly shut off at 10 to 15 min (3, 9, 21). In nonpermissive bacteria infected with early amber mutants blocked in DNA replication and late gene transcription (1), the normal shut-off of early enzyme formation does not take place. This results in two- to threefold higher enzyme activities in the late period of infection compared to wild-type infection (3, 9, 21).

Regulation of the switch from early to late protein synthesis in wild-type infection was indicated by RNA-DNA hybridization studies to be at the level of transcription. Evidence was thus found for a substantial decrease in the abundance of early RNA species between the early and the late period of infection (16) whereas the amounts of late RNA species increased dramatically towards the latter part of infection (1).

In earlier work (1a, 2, 3) the mechanism for the shut-off of early enzyme synthesis was investigated. The period of synthesis of mRNA species coding for early enzymes was determined by the time dependence of fluorouracil (FU) rescue of early amber mutations (genes

1, 42, and 56). Synthesis of active enzyme corresponding to the mutated genes could be rescued by FU addition shortly after infection but not by addition late after infection (1a). Similarly, DNA synthesis could be rescued at early times but not at 12 min or later (2). This was interpreted to reflect the cessation of transcription of the studied early genes at 9 to 12 min after infection, since a block in the translation of the mutated messenger (1a) or a block in the uptake of FU into ribonucleotide pools (2) could be excluded. The rate of total early RNA synthesis, as determined by short-time uracil incorporation, was also found to decrease by more than 90% between the early and the late period during early amber mutant infection (2, 3).

If protein synthesis was arrested by chloramphenicol in the early period of infection, the shut-off of early mRNA synthesis was prevented, indicating that some early protein (or proteins) participates in the shut-off mechanism (3). The products of genes 33 and 55 might have such a function. Mutants in those genes have the so-called maturation-defective phenotype, characterized by failure to induce late pro-

tein synthesis (9) and late gene transcription (1) although DNA replication takes place. Participation of the gene-55 product in the shut-off of early transcription was indicated by studies by Sköld (18), demonstrating that in the absence of gene 55 function the transcription and translation of some early gene necessary for DNA synthesis continued into the late period of infection. Extended synthesis of several early proteins in cells infected with gene 33 and gene 55 mutants was reported by Hosoda and Levinthal (9).

The experiments to be presented here investigate the influence of gene 55 on early T4 functions. The results show that, although early mRNA synthesis was normally shut off, the synthesis of two early enzymes was extended in cells infected with gene 55 mutants compared to wild-type T4.

MATERIALS AND METHODS

Bacteria and bacteriophage. *E. coli* B was used as the nonpermissive host for amber mutants of T4. The suppressor strain *E. coli* CR63 was used for plating and preparation of stocks of amber mutants. Wild-type T4D (T4⁺) and mutants *amC42* (gene 1, deoxynucleotide kinase), *amN122* (gene 42, deoxycytidylate hydroxymethylase), and *amBL292* (gene 55) were kindly supplied by J. S. Wiberg. The temperature-sensitive (*ts*) mutants *tsL13* (gene 42) and *ts553* were obtained from W. Salser and E. P. Geiduschek, respectively. The procedure for preparation of phage stocks was described earlier (2).

Media. The glycerol-Casamino Acids medium (5) was used for preparation of phage lysates and enzyme extracts. Low-phosphate mineral salts medium, dilution broth for phage, bottom agar, and top agar were described (2).

Chemicals. Thymidine-*methyl*-³H, 2,000 to 5,000 mCi/mmol and uracil-5-³H, 500 to 1,000 mCi/mmol were obtained from the Radiochemical Centre, Amersham, Great Britain, and deoxycytidine-2-¹⁴C 5'-triphosphate, (20–30 mCi/mmol) was from NEN Chemicals GmbH, Dreieichenhain, Germany. The uracil analogue 5-fluorouracil was generously supplied by Hoffman-La Roche, Switzerland. Chloramphenicol was supplied by Parke, Davis & Company, Detroit, Mich.

Infected cells. *E. coli* B was grown at 37°C to about 5×10^8 cells per ml in the mineral salts medium (generation time 45–50 min). The cells were harvested by filtration and resuspended at 2 to 3×10^9 cells per ml in the same medium and kept on ice. At infection (time zero) phage, 4 to 5 plaque-forming units (PFU) per bacterium, was added. After phage adsorption for two min at 37°C (unless otherwise mentioned) the culture was diluted 10-fold into warmed, aerated medium in growth tubes. Phage adsorption and killing of the bacteria always exceeded 99%. All experiments with FU were performed in the presence of thymidine (20 µg/ml) in order to neutralize the inhibitory effect of the FU-deoxyribonucleotide on thymidylate synthetase (4).

Determination of DNA and RNA synthesis. The synthesis of DNA was followed by the cumulative incorporation of ³H-thymidine as described earlier (2). RNA synthesis was determined by labeling with uracil-5-³H (a specific precursor for RNA due to the substitution in the 5'-position) (8) during 1-min pulses. At 1- or 2-min intervals 1-ml samples from infected cultures were pipetted into small tubes warmed at 37°C containing uracil-5-³H, 1 µCi, 1 µg in 0.05 ml of water. After 1 min of incorporation the pulse was terminated by the addition of 2 ml of cold 10% trichloroacetic acid. The precipitates were collected on glass fiber filters (Whatman GF/C) and counted in a scintillation counter.

Enzyme assays. Extracts were prepared, and deoxycytidine triphosphatase was assayed as previously described (1a). Deoxynucleotide kinase was assayed as described by Wiberg et al. (21).

Phage crosses. *E. coli* B was grown in the glycerol-Casamino Acids medium to 3×10^8 cells per ml. The two phage strains were added simultaneously, each at a multiplicity of infection (MOI) of 3 to 6. After incubation for 60 min at 30°C, chloroform was added to complete lysis and the phage progeny was plated under permissive conditions. Plaques were picked and identified by complementation tests or, in the case of *ts* × *am* recombinants, by their plating characteristics (growth only on plates with CR63 as indicator and incubated at 30°C).

Complementation tests. Drops of the two phage suspensions (10^8 PFU per ml) were placed on top of each other on plates seeded with *E. coli* B, and the plates were incubated at the nonpermissive temperature (for *ts* mutants).

RESULTS

Purification and characterization of a *ts* mutation in gene 55. The mutant *ts553* has been reported to carry a *ts* mutation in gene 55 and to be temperature-sensitive with respect to the initiation and continuation of the synthesis of late RNA species (7, 13, 14). Unexpectedly it was found, however, that *ts553* did not synthesize DNA at 43°C (Fig. 1a), whereas DNA synthesis took place in *E. coli* B infected with *amBL292* (gene 55) both at high and low temperature (Fig. 1b). After a shift from 30 to 43°C at 20 min after infection with *ts553*, DNA synthesis leveled off with a lag of about 15 min (Fig. 1a). These results suggest that *ts553* has a *ts* mutation in a gene necessary for DNA replication. It also carries a mutation in gene 55 since *amBL292* and *ts553* do not complement each other at 43°C.

Because *ts553* has been widely studied it was of interest to isolate the pure gene 55 *ts* mutation and to characterize the contaminating mutations to some extent. The purification procedure involved as a first step the isolation of the unknown mutations outside gene 55. Among the progeny from *E. coli* B simultaneously

FIG. 1. DNA synthesis in *E. coli* B infected with *ts553*, *amBL292*, and *ts554*. Cells were grown and harvested as described in the experimental section and infected at 43 C. Two minutes after infection the cells were diluted 10-fold into medium containing ³H-thymidine (1 μ Ci/ml, 10 μ g/ml) and the incorporation was followed in one culture kept at 43 C and another shifted to 30 C at 2 min. Symbols: a, *ts553*; ●, 30 C; ○, 43 C; ×, shift from 30 to 43 C at 20 min after infection; b, *amBL292*; ●, 30 C; ○, 43 C; c, *ts554*; ●, 30 C; ○, 43 C.

infected with *ts553* and *amBL292*, *ts* × *am* recombinants were found at a frequency of about 5/1,000. The amber mutation was eliminated by picking spontaneous revertants, able to grow on *E. coli* B at 30 C. Of the *ts* strains found in this way 11 were found to be complemented by *amBL292* but not by *ts553* (Table 1). These strains were expected to carry the *ts* mutations outside gene 55 contaminating *ts553*. When those 11 *ts* strains were tested for complementation with each other they were found to fall into three complementation groups (Table 1). Four strains (group A), which were unable to complement each other, were found to complement each of two other strains (group B). None of the group A or B mutants could complement the five strains of group C. This complementation pattern indicates that the group A and B mutants, respectively, have mutations each in one gene, whereas the group C mutants have mutations in both these genes. The group B and C mutants did not induce DNA synthesis at the nonpermissive temperature, whereas DNA formation was temperature independent with the group A mutants (Table 1). This indicates that one of the *ts* mutations is located in an early gene, involved in DNA replication and that the gene carrying the second contaminating mutation is not necessary for DNA synthesis.

The *ts* mutants found in this way should be able to complement *ts* mutants in gene 55 but not *ts553*. This was used to identify purified *ts*(55) mutants in the progeny from a cross between *ts553* and T4⁺. Five *ts* isolates were found from such a cross, which were complemented by the group A, B and C *ts* mutants but not by *ts553* or *amBL292* (group III, Table 2).

TABLE 1. Characteristics of the *ts* mutations outside gene 55 of *ts553*

Complementation group	No. of strains isolated	Complementation ^a with					DNA synthesis ^b	
		<i>ts553</i>	<i>amBL292</i>	A	B	C	30 C	43 C
A	4	—	+	—	+	—	+	—
B	2	—	+	+	—	—	+	—
C	5	—	+	—	—	—	+	—

^a Complementation was tested by spotting on plates seeded with *E. coli* B and incubated at 43 C.

^b DNA synthesis was determined by cumulative incorporation of ³H-thymidine, as described in Materials and Methods. When DNA synthesis was observed (+) it was at a rate comparable to that of T4⁺. (—) Denotes ³H-thymidine incorporation at a rate similar to that of *tsL13* (gene 42) at 43 C.

As expected these purified *ts*(55) mutants could induce DNA synthesis both at 30 and 43 C (Fig. 1c). The lack of other recombinants than those expected from a cross between T4⁺ and a mutant carrying the three identified *ts* mutations (one in gene 55 and the two of the complementation groups A and B) indicates that no more mutations can be expected in *ts553* (Table 2). The purified gene 55 *ts* mutants were somewhat leaky. Thus, at 90 min after infection at 43 C, 2 to 4 PFU per infected cell were produced. Under our conditions *amBL292* showed a similar degree of leakiness in *E. coli* B at 37 C.

One of the *ts*(55) isolates was chosen for further study and was given the preliminary

TABLE 2. Characteristics of the recombinants from the cross *ts553* × *T4*⁺

Complementation group	No. of strains isolated	Complementation ^a with					DNA synthesis ^b	
		<i>ts553</i>	<i>amBL292</i>	A	B	C	30 C	43 C
I	1	-	-	-	-	-	+	+
II	7	-	-	+	-	-	+	-
III	5	-	-	+	+	-	+	+
IV	6	-	+	-	+	-	+	+
V	4	-	+	+	-	-	+	-
VI	1	-	+	-	-	-	+	-

^a A, B, and C denote the three types of *ts* strains, carrying the *ts* mutations outside gene 55 from *ts553*, described in Table 1.

^b See legend to Table 1.

designation *ts554*. From a cross between *amC42* and *ts554* the double mutant *amC42* × *ts554* (gene 1 × gene 55) was obtained.

Kinetics of early enzyme synthesis in gene 55 mutants. It has been well established that the synthesis of early enzymes, which in wild-type *T4*-infected cells stops between 10 and 15 min, continues beyond this time in nonpermissive bacteria infected with DNA-negative amber mutants. This is confirmed by the measurements of the two early enzymes deoxycytidine triphosphatase and deoxynucleotide kinase shown in Fig. 2. In *E. coli* B infected with amber mutants in genes 1 or 42 the enzyme activities leveled off at 20 to 30 min after infection, whereas after 15 min no rise in enzyme activities occurred in the culture infected with *T4*⁺. In *E. coli* B infected with *amBL292* (gene 55) the two enzyme activities appeared with the same kinetics as in cells infected with the DNA-negative mutants, i.e., the shut-off of the synthesis of the enzymes was delayed by 10 to 15 min leading to two to three times the *T4*⁺-induced activities (Fig. 2).

Similar results were obtained when the gene 55 product was inactivated at high temperature in cells infected with a gene 55 *ts* mutant. This mutant, called *ts554*, was obtained from *ts553* by the elimination of two *ts* mutations outside gene 55. At the permissive temperature (30 C) the shut-off of deoxynucleotide kinase synthesis took place at 20 to 25 min after infection both with *ts554* and *T4*⁺ (Fig. 3a). At 43 C no increase in enzyme activity was found after 15 min in wild-type-infected cells, whereas in *ts554*-infected cells the activity increased until 30 min (Fig. 3b). The delay of the onset and shut-off of deoxynucleotide kinase synthesis at

30 C, compared to 37 C (Fig. 2) and 43 C, is presumably an effect of the slowing down of the infection at the lower temperature. The deoxynucleotide kinase activity in *ts554*-infected cells 30 to 40 min after infection was two to three times higher at 43 C than at 30 C. In *T4*⁺-infected cells the final enzyme activity was similar at both temperatures. Thus, the same type of distortion of the regulation of early enzyme synthesis was found here for gene 55 mutants as for early, DNA-negative, amber mutants. Hosoda and Levinthal (9) have reported that the pattern of synthesis of a few other early enzymes in cells infected with gene 33 and 55 amber mutants was intermediate in type between the pattern seen for *T4*⁺ and DNA-negative amber mutants.

FIG. 2. Early enzyme activities in *E. coli* B infected with *T4*⁺ or amber mutants in genes 1, 42, and 55. Cells were grown and infected in the glycerol-Casamino Acids medium at 37 C. At different times extracts were prepared and assayed as described in Materials and Methods. The counts per minute values along the ordinate represent the radioactivity in the product formed in assays of extracts from 0.4×10^8 cells. a, Deoxynucleotide kinase. b, Deoxycytidine triphosphatase. Symbols: Δ , *T4*⁺; $\circ$, *amBL292*; $\otimes$, *amC42*; $\times$, *amN122*.

Influence of gene 55 on the regulation of transcription of gene 1. It was previously shown that the time of cessation of transcription of individual early genes can be determined by FU rescue of DNA synthesis of early amber mutants (2). The rescuing effect of FU is ascribed to its incorporation in the place of uracil into the amber triplet, UAG, in mRNA. FUAG is occasionally misread as CAG resulting in insertion of glutamine at the mutated site (15) and partial restoration of the mutated enzyme activity (1a, 15). The rate of DNA synthesis in response to FU addition is a measure of the activity of the rescued early enzyme, which in turn is proportional to the amount of FU-corrected messenger synthesized between the time of FU addition and the cessation of transcription of the mutated gene. FU was shown to be taken up into the ribonucleoside triphosphate pool also after addition at late times after infection (2, 3).

FIG. 3. Deoxynucleotide kinase synthesis in *E. coli* B infected with T4⁺ or ts554 at 30 or 43 C. Cells were grown in the glycerol-Casamino Acids medium and infected at 30 or 43 C. Extracts were prepared and assayed as described in Materials and Methods. The counts per minute values along the ordinate represent the radioactivity in the product formed in assays of extracts from 0.4×10^8 cells. a, T4⁺, b, ts554. Symbols: x, 30 C; o, 43 C.

In the experiments shown in Fig. 4 the time pattern of FU rescue of DNA synthesis was compared for *E. coli* B infected with amC42 (gene 1) and amC42 $\times$ amBL292 (gene 1 $\times$ gene 55). It can be seen that the rate of ³H-thymidine incorporation decreased when FU was added at increasing times after infection and that addition at 12 min or later could not rescue any DNA synthesis. If gene 55 function were necessary for the shut-off of gene 1 transcription, DNA synthesis was expected to be rescuable by FU addition later for the double mutant than for the single gene 1 mutant (3, 18). The results were quite similar for the two mutants (Fig. 4), which shows that whether the gene 55 product was functional or not the transcription of gene 1 ceased between 9 and 12 min after infection. It cannot be excluded, however, that the gene 55 product of amBL292 was rapidly rescued by FU added at late times and could then immediately shut off the synthesis of gene 1 messengers. In order to meet this objection the FU rescue experiments were repeated with the gene 55 ts mutation ts554, which was found not to be revertible by FU. The influence of the gene 55 product on the regulation of gene 1 transcription was studied by comparing the time dependence of FU rescue of DNA synthesis during infection at 43 C with amC42 and amC42 $\times$ ts554, respectively. The results were quite similar to those presented in Fig. 4. Thus, also when the gene 55 product was inactive throughout infection (at 43 C) transcription of gene 1 ceased at 9 to 12 min after infection.

Influence of gene 55 on the availability of an early messenger for translation at different times. It was thus found that the shut-off of gene 1 transcription is unaffected by the absence or presence of gene 55 function in amC42-infected cells. It can be asked if the gene 55 product affects the functional stability of early messengers or the capacity to translate early messengers in the late period of infection. This can be approached by studying the rescue of DNA synthesis by temperature-shift in cells infected with DNA-negative, temperature-sensitive mutants (3, 11).

After infection with tsL13 (gene 42) DNA synthesis is blocked at 43 C (3, 11) due to the irreversible inactivation of the deoxycytidylate hydroxymethylase (20). A shift to permissive temperature (30 C) shortly after infection restored DNA synthesis to a rate comparable to that in T4⁺-infected cells (Fig. 5). Shifts at later times resulted in lower rates of thymidine incorporation and a shift later than 30 min after infection was ineffective in this respect (Fig. 5). Reactivation of preformed deoxycytidylate hy-

FIG. 4. Rates of DNA synthesis rescued by FU addition at different times after infection of *E. coli* B with amC42 and amC42 $\times$ amBL292. Cells, infected as described in Materials and Methods, were diluted 2 min after infection into mineral salts medium containing ^3H -thymidine (1 $\mu\text{Ci}/\text{ml}$, 20 $\mu\text{g}/\text{ml}$) at 37 C. At the times indicated, FU (10 $\mu\text{g}/\text{ml}$) was added to different portions of the cultures. The rate of cumulative incorporation of ^3H -thymidine into DNA at 60 to 90 min after FU addition was expressed as percentage of the value obtained after addition of FU at 3 min. The rate of ^3H -thymidine incorporation after addition of FU at 3 min was about 14% of that in wild-type-infected cells. Symbols: O, amC42; $\times$, amC42 $\times$ amBL292.

droxymethylase plays a negligible part in the generation of this time-dependent pattern of rescue as indicated by shift experiments in the presence of chloramphenicol (data not shown; 11). The rate of rescued DNA synthesis in these experiments is a measure of the amount of gene 42 messenger which upon a shift to 30 C can be translated into active enzyme. The FU rescue experiments, on the other hand, determine the capacity for synthesis of individual early mRNA species. This explains why the decrease in rescuability is slower in the temperature-shift experiments.

Insertion of an amber mutation in gene 55 (amBL292) into the genome of tsL13 did not influence the rate of the decrease in temperature-shift rescuability of DNA synthesis (Fig. 5). This indicates that the presence or absence of gene 55 product does not influence the availability of the gene 42 messenger for translation.

Influence of gene 55 on total early RNA synthesis. It was earlier shown by short-time incorporation of uracil-5- ^3H that total early RNA synthesis decreased dramatically at the end of the early period in cells infected with DNA-negative amber mutants (2, 3). Uracil was shown to be taken up into the nucleotide pools throughout the infection cycle (2, 3). In order to study more closely the changing rates of RNA synthesis the incorporation of uracil-5- ^3H during 1-min pulses at close intervals was determined (Fig. 6). After infection of *E. coli* B with amC42 (gene 1) late genes are not transcribed due to the block in DNA replication (1). The rate of incorporation of uracil-5- ^3H in such cells was seen to increase rapidly between min 2 and min 4 (Fig. 6). From 6 min until about min 12 the rate of incorporation decreased dramatically and was thereafter maintained at a level less than 10% of the maximal rate (min 5). The pattern of uracil incorporation in T4 $^+$ -infected cells (Fig. 6) was very similar until about min 8, but thereafter the rate of incorporation in-

FIG. 5. Rates of DNA synthesis rescued by temperature-shift at different times after infection of *E. coli* B with tsL13 or tsL13 $\times$ amBL292. *E. coli* B was harvested and infected as described in Materials and Methods; adsorption, however, took place at 43 C. At 2 min after infection the cells were diluted 10-fold into medium containing ^3H -thymidine (1 $\mu\text{Ci}/\text{ml}$, 20 $\mu\text{g}/\text{ml}$) and the culture was divided into subcultures. At the times indicated the subcultures were shifted from 43 C to an ice bath for 10 s and then to 30 C. The rate of ^3H -thymidine incorporation between 60 and 90 min after infection was determined and expressed as percentage of the rate obtained after the shift to low temperature at 3 min. Symbols: O, tsL13; $\times$, tsL13 $\times$ amBL292.

FIG. 6. One minute pulse incorporation of uracil-5-³H in *E. coli* B infected with T4⁺ or amber mutants in genes 1 and 55. Cells were grown and harvested as described in Materials and Methods. Fifty seconds after infection the cells were diluted 10-fold and aerated at 37 C. The incorporation of uracil-5-³H into RNA during 1-min pulses was determined as described in Materials and Methods. The counts per minute incorporated was plotted versus the middle of the pulse after subtracting the background (obtained by mixing uracil-5-³H with trichloroacetic acid and then adding infected bacteria). Symbols: ○, *amC42*; ×, *amBL292*; ●, T4⁺.

creased to a second broad peak. This late peak, which was absent in *amC42*-infected cells, reflects the synthesis of late species of RNA. The time of onset of the late peak of incorporation is consistent with the time of the first appearance of late structural proteins at 7 to 8 min (17). Careful studies of RNA synthesis by cumulative uracil incorporation curves made by Haselkorn et al. (8) regularly showed a shoulder at 8 to 10 min after infection (at 30 C) with T4⁺. This conforms well with the decrease in the rate of total RNA synthesis observed here at the switch from early to late RNA synthesis in T4⁺-infected cells.

The material labeled in the early peak of incorporation (1-12 min) in *amC42*-infected cells is presumably made up of the two early

classes of RNA described by Salser et al. (16), whereas the residual incorporation seen after 12 min may correspond to the synthesis of quasi-late RNA species (7, 16).

In cells infected with *amBL292*, where transcription of late genes does not take place due to the lack of gene 55 product (1), the pattern of uracil incorporation was quite similar to that of *amC42* (Fig. 6). This suggests that early RNA synthesis is similarly shut off in cells infected with the gene 1 and the gene 55 mutants. Thus, a functional gene 55 product is not necessary for the shut-off of the synthesis of the major part of early RNA species. Similar results were obtained in incorporation studies with the gene 55 *ts* mutant (data not shown): early RNA synthesis was shut-off as well when the gene 55 product was inactive (at 43 C) throughout infection as when it was inactivated by temperature-shift-up in the late period of infection with *amC42* × *ts554*.

DISCUSSION

The experiments reported here investigate how the gene 55 function affects the regulation of early enzyme synthesis and the transcription of early genes.

Experiments recently presented by Stevens (19) indicate that genes 33 and 55 code for polypeptides which are associated with the RNA polymerase in the late period of infection. It is well known that undisturbed function of genes 33 and 55 is necessary for the onset of late protein synthesis (9) and synthesis of late species of RNA (1, 7). A requirement for continuous activity of the gene 55 product for transcription of late genes was suggested from temperature-shift experiments with the mutant *ts553* (7, 13, 14).

In the course of our experiments it was found that the mutant *ts553* does not carry a single gene 55 mutation but contains two additional *ts* mutations, one of which is located in a gene necessary for DNA synthesis. In the light of the complexity of the genotype of *ts553*, experiments with this mutant are difficult to interpret. The gene 55 mutant *ts554*, derived from *ts553* by elimination of the two contaminating mutations, was used for the experiments described here.

The kinetics of the formation of two early enzymes in cells infected with phage carrying either an amber or a *ts* mutation in gene 55 was similar to that observed with DNA-negative amber mutants. Thus, the enzyme activities continued to increase for about 10 min longer than in T4⁺-infected cells and the cessation of the increase was less abrupt. This indicates that

gene 55 must be expressed in order to obtain normal regulation of early enzyme synthesis. Either the gene 55 product itself or a late function dependent on gene 55 expression may be involved in the regulation of transcription of early genes or influence the translation of early messengers.

By studying the rescuability of DNA synthesis by FU addition at different times after infection with a gene 1 amber mutant or a gene 1-gene 55 double mutant, it could be tested whether the gene 55 product (in the absence of late transcription) could influence the time pattern of transcription of gene 1. It was found that the shut-off of transcription of gene 1 does not require gene 55 function at least in the absence of DNA synthesis.

When total early RNA synthesis was studied (by pulse-labeling with uracil-5-³H) similar patterns were observed for gene 1 and gene 55 mutants, with a marked decline in early RNA synthesis between 6 and 12 min after infection. In T4⁺-infected cultures the pattern of incorporation was very similar to that seen in the mutant-infected cells until the onset of late RNA synthesis. This suggests that the transcription of early genes is similarly regulated in cells infected by T4⁺ and gene 1 and gene 55 mutants. No requirement of the gene 55 product could, thus, be detected for the shut-off of synthesis of early RNA either when a defined early transcript was studied or when total early RNA synthesis was determined. This does not, however, exclude that the gene 55 product is involved in the regulation of synthesis of a few early messengers as was reported by Sköld (18) and Wilhelm and Haselkorn (22). However, the results presented here on uracil-5-³H incorporation in cells infected with the gene 55 amber mutant stand in apparent conflict with results presented by Sköld (18). There, a gene 1 × gene 55 double mutant showed prolonged incorporation of labeled FU into RNA compared with the single gene 1 mutant. The reason for this discrepancy is not clear at present.

Rescue by temperature-shift of DNA synthesis in cells infected with early *ts* mutants offered a method to study the amounts of the messenger corresponding to the mutated gene available for translation at different times after infection. A comparison between the time patterns of rescue of DNA synthesis by FU (Fig. 4) (2) and temperature-shift (Fig. 5) makes a rough calculation of the survival times of early messengers possible. (It must be pointed out, however, that the FU experiments were made at 37 C, while the initial temperature in the temperature-shift

experiments was 43 C.) The gene 42 messenger remained active from 12 min, when the cessation of FU rescue of DNA synthesis of a gene 42 amber mutant was observed (2), until 30 min, which was the last time when a temperature-shift could rescue DNA synthesis in *ts*L13-infected cells (Fig. 5). This maximal survival time of roughly 20 min is consistent with determinations of the rate of decay of T4 mRNA in vivo (6, 23).

Insertion of a gene 55 amber mutation into the genome of *ts*L13 did not change the pattern of temperature-shift rescue of DNA synthesis. Thus, compared to the conditions in early *ts* mutant-infected cells, where early mRNA remains translatable until 30 min at nonpermissive temperature, the lack of gene 55 function did not affect the availability of the gene 42 messenger for translation. Young and Van Houwe (23) tested for the presence of an early messenger by its ability to direct synthesis of α -glucosyl transferase in vitro in RNA harvested at different times after infection. The presence or absence of the gene 55 product was found not to affect the kinetics of accumulation of the glucosyl transferase messenger (23).

The results of this and earlier papers (1a, 2, 3) show that transcription of some early genes is shut off before 12 min after infection with early, DNA-negative mutants and gene 55 mutants, whereas extended synthesis of some early enzymes is observed under the same conditions. This suggests that the abnormal regulation of early enzyme synthesis in early mutant infection is due to the absence of late protein synthesis rather than to the block in DNA synthesis. The extended early enzyme synthesis can be explained by either of the following mechanisms: (i) due to the block in late gene transcription there is no competition between early and late messengers for translation; (ii) in the absence of late gene transcription the stability of early messengers is increased; (iii) the mutant-infected cells fail to synthesize a regulation factor switching translation specificity from early to late mRNA sequences. Indeed, recent reports have indicated a role of T4-induced initiation factors in the switch between early and late protein synthesis (10, 12). The results, thus, provide evidence for some mechanism for translational restriction of early enzyme synthesis in wild-type infection which is dependent on the expression of late genes. In the absence of late transcription (in *E. coli* B infected with early DNA-negative or gene 55 mutants) early messengers direct the synthesis of early enzymes for a prolonged time.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

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