COMPETITION BETWEEN BACTERIOPHAGE f2 RNA AND BACTERIOPHAGE T4 MESSENGER RNA

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Received April 7,1975

SUMMARY - In an Escherichia coli cell-free protein synthesis assay, mRNA isolated from cells late after infection by phage T4 out-competes bacteriophage f2 RNA. Addition of a saturating or subsaturating amount of T4 mRNA inhibits translation of f2 RNA, while even an excess of f2 RNA has no effect on translation of T4 mRNA. Peptide mapping of reaction products labeled with formyl-[^{35}S]-methionyl-tRNA was used to quantitate f2 and T4 protein products synthesized in the same reaction. We suggest that messenger RNA competition might be one mechanism by which T4 superinfection of cells infected with phage f2 blocks translation of f2 RNA and possibly host mRNA.

Infection of Escherichia coli cells by bacteriophage T4 results in cessation of host protein synthesis (1). Superinfection by T4 of cells infected with the RNA phage f2 results both in rapid inhibition of f2-directed protein synthesis and in degradation of intracellular f2 RNA (2-6). There is considerable disagreement in the literature about the effects of T4 phage infection on the protein synthesis apparatus. Most investigators have found that crude ribosomes from T4-infected cells have reduced ability to translate RNA phage RNAs and host mRNAs, but the magnitude effect is quite variable (reviewed in ref. 7). Steitz, et al., claimed that ribosomes from T4-infected cells differ from those of control cells in their ability to initiate translation of the three f2 phage genes (8). Of the three factors required for initiation of protein synthesis, only one — IF3 — is required specifically for translation of natural messen-

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ger RNAs (9-12). Lee-Huang and Ochoa reported that E. coli contains two species of IF3, one of which preferentially promotes translation of E. coli and f2 RNA while the other recognizes primarily late T4 mRNA (13). They suggested that T4 infection results in the inactivation of the IF3 factor which is preferential for f2 RNA and E. coli mRNA (14). By contrast, two other groups report only one IF3 factor in E. coli; they can detect no change in the specificity of factor IF3 after T4 infection (15, 16). Similarly, we and others could find no change in the specificity of the translational machinery after T4 infection (7, 17).

If there is no change in the specificity of ribosomes or initiation factors after T4 infection, how does inhibition of translation of f2 RNA or cellular mRNA by T4 occur? We showed previously that if two messenger RNAs differ in the rate constant for attachment to ribosomes and initiation of protein synthesis, then any nonspecific reduction in the rate of polypeptide chain initiation by the cell will result in preferential inhibition of translation of the mRNA with the lower rate constant (18). In this paper we show that, as measured by cell-free protein synthesis, bacteriophage T4 mRNA has a higher rate constant for polypeptide chain initiation than does phage f2 RNA. If these results can be extrapolated to the intact cell, they suggest a mechanism by which T4 infection can restrict translation of f2 RNA.

MATERIALS AND METHODS

A previous paper (7) detailed the isolation of f2 RNA and late T4 messenger RNA, the conditions for cell-free protein synthesis, and the peptide mapping techniques used for analysis of the products. In this study ribosomes, crude initiation factors, and S-100 supernatant from uninfected E. coli B were used and E. coli formyl-[^{35}S]-methionyl-tRNA$_f$ was the source of radioactivity.

RESULTS

Incorporation into protein of radioactivity from the initiator formyl-[^{35}S]-methionyl-tRNA$_f$ is a measure of the initiation of polypeptide chains.

Addition of equivalent amounts of f2 RNA or late T4 mRNA to E. coli ribosomes results in incorporation of the same amount of f[^{35}S]-met radioactivity (Fig. 1 and Table 1). The duration of the incubation used in this experiment was 10 min., and the rate of incorporation of f[^{35}S]-met into protein is linear for at least this time. The f2 RNA was the pure viral RNA, while the late T4 mRNA was contaminated with a large amount of host ribosomal RNA. Hence this experiment suggests that, per microgram of phage messenger RNA, each molecule of T4 mRNA initiates polypeptide synthesis at a greater rate than does f2 RNA; the magnitude of this effect, however, is very difficult to determine from this type of study.

In order to determine more reliably the relative rates of initiation for T4 mRNA and f2 RNA, we added the RNAs together, at varying concentrations, to the same cell-free reaction. In such a study it is necessary to differentiate the products of translation of the two mRNA preparations. To this end, the products of the reaction were digested with a mixture of trypsin and chymotrypsin, and the resultant labeled peptides, each derived from the N-terminus of a protein, were resolved by paper ionophoresis at pH 3.5 (Fig. 2). Comparing columns 2, 3, and 13 with columns 4, 5, and 6, it is clear that one can use this technique to differentiate f2 from T4-directed initiation events. Addition of f2 RNA re-

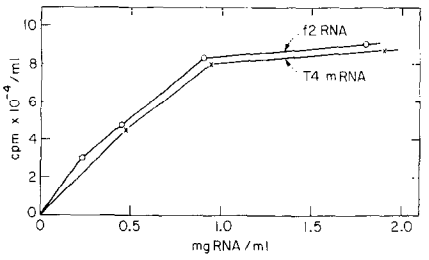


Fig. 1 Protein synthesis directed by f2 and T4 messenger RNA. Reactions (0.15 ml) contained, per ml: 43 A_{260} units of salt-washed ribosomes; 310 μg of crude initiation factors; 0.2 ml E. coli S-100; about 5 x 10^6 cpm N-formyl-[^{35}S]-methionyl-tRNA (5000 mCi/mmole); and other components as detailed previously (7, 28). The entire reaction was taken for measurement of protein radioactivity after 10 minutes incubation at 37°C; incorporation into protein was linear for at least this time. The background from reactions without RNA was not subtracted.

Table 1

Quantitation of Competition Between f2 and T4 Messenger RNAs in vitro

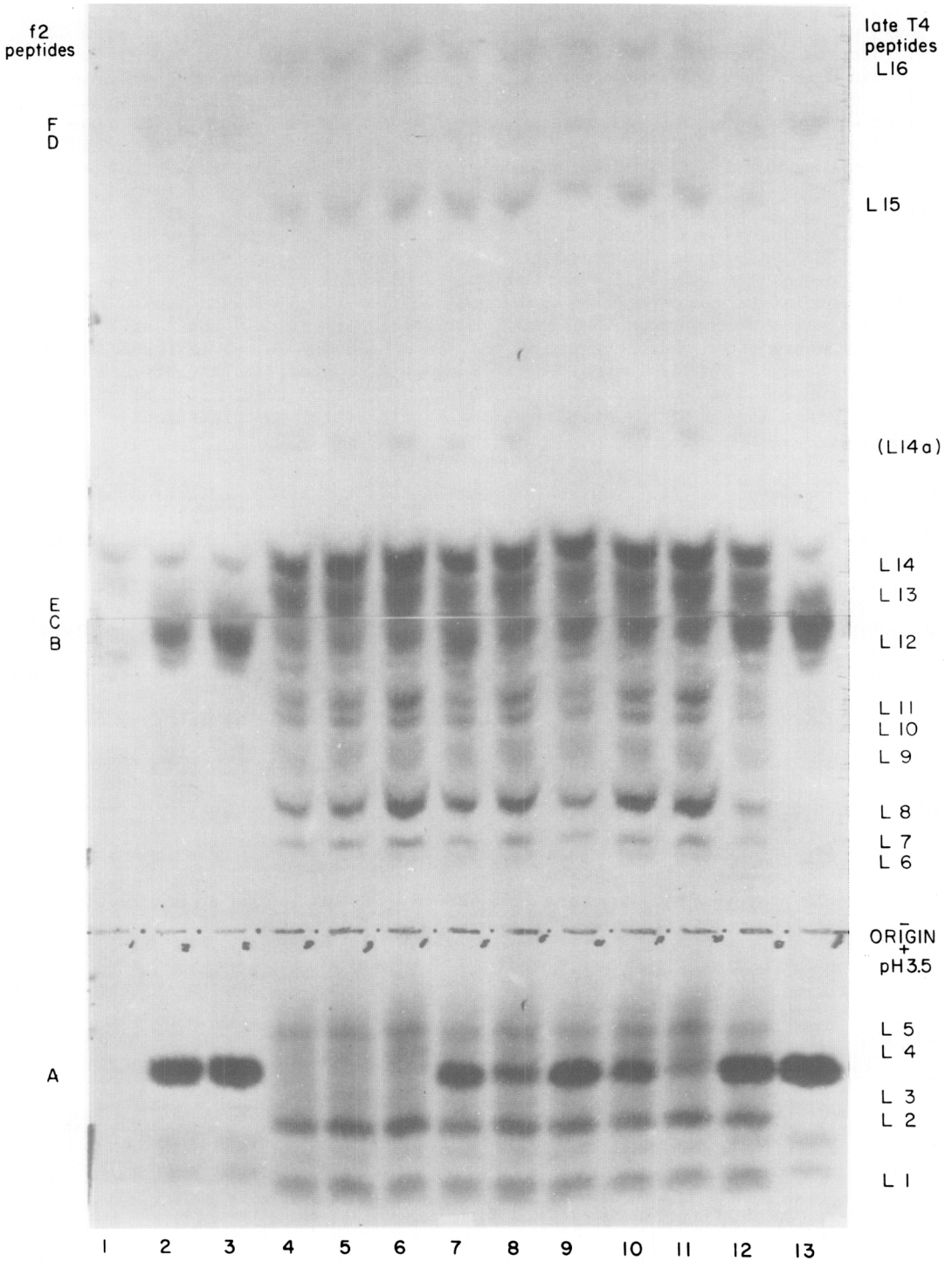
Sample	f2 RNA, mg/ml	T4 RNA, mg/ml	Initial incorporation x 10^{-3}	Percent of incorporation when no competing RNA added: f2 peptides: A	f2 peptides: D and F
2	0.23	-	30.3	100 (13700)	100 (453)
7	0.23	0.47	64.0	44.7	52.3
8	0.23	0.94	84.3	19.3	40.8
3	0.45	-	46.7	100 (20600)	100 (641)
9	0.45	0.47	75.5	42.9	55.2
10	0.45	0.94	94.7	19.2	38.1
11	0.45	1.9	92.3	5.2	15.3
13	0.90	-	83.1	100 (35500)	100 (1090)
12	0.90	0.47	103.8	46.1	55.7
				T4 peptides (sum):	
4	-	0.47	45.1	100 (19200)	
7	0.23	0.47	64.0	108	
9	0.45	0.47	75.5	108	
12	0.90	0.47	103.8	95.7	
5	-	0.94	79.6	100 (28800)	
8	0.23	0.94	84.3	106	
10	0.45	0.94	94.7	114	
6	-	1.9	86.0	100 (38000)	
11	0.45	1.9	92.3	99.4	

TABLE 1 - Peptide bands from Figure 2 were cut out and counted for radioactivity. As discussed in the test, the sum of radioactivity in peptides A, D, and F is taken as the measure of f2-specific synthesis, and the sum of radioactivities in peptides L2, L5, L7, L8, L9, L10, L11, L14, L14a, L15, and L16 were used to quantitate T4-specific synthesis. In the case of samples which received no com-

peting RNA, the total recovered radioactivity in f2-specific peptides (samples 2, 3, and 13) or T4 peptides (samples 4, 5, and 6) are given in parentheses. These values are set equal to 100% of incorporation for each mRNA concentration. All backgrounds were subtracted before tabulating the data. Backgrounds for reactions which contained a constant amount of f2 RNA and increasing amounts of T4 mRNA (top part of table) were obtained from the corresponding regions of paper which received the same amount of T4 messenger RNA without f2 RNA. For peptide A (coat), backgrounds were between 384 and 3318 counts/min (mean 2340); for peptides D and F (deformylated polymerase and maturation), backgrounds were between 38 and 367 (mean 253). Backgrounds for reactions which contained a constant amount of T4 mRNA and increasing amounts of f2 RNA (bottom half of table) were obtained from the corresponding regions of paper which received the same amount of f2 RNA without T4 messenger RNA. For the 11 T4 peptides counted, the sums of the background regions were between 2302 and 5544 counts/min (mean 3826). Values for initial incorporation were obtained from 15 μl aliquots taken from the original reaction tubes in Figure 2. The recovery of only about half the initial incorporated radioactivity in the final peptides is due to three factors: (a) only some of the virus-specific peptides were counted; (b) the efficiency of counting the final peptides is only about 80% that of the initial reaction; and (c) minor losses of the protein during precipitation and digestion.

sults in appearance of six new labeled peptides A - F. A, C, and E are the peptides containing f[^{35}S]-methionine from the N-termini of the f2 coat, replicase, and A proteins, respectively, while peptides B, D, and F are the respective deformylated derivatives (19). From reactions containing f[^{35}S]-met-tRNA and late T4 mRNA, one can identify at least 16 radioactive peptides (L1 - L16, columns 4 - 6 of Fig. 1). Several groups have shown that late T4 mRNA directs the synthesis of several late T4 proteins in cell-free extracts from uninfected E. coli (20-22). Although we do not know the identity of any of the proteins which yield f-met peptides L1 - L16, it is reasonable to assume that they are derived from bona fide late T4 proteins. f2 peptides A, D, and F (the latter two do not resolve at pH 3.5) do not co-migrate with T4 peptides or background peptides and can be used to quantitate f2-specific protein; likewise, T4 peptides L2, L5, L7, L8, L9, L10, L11, L14, L14a, L15, and L16 yield a measure of T4-specific synthesis.

The results of the mRNA competition are shown in Fig. 2; the data is quantitated in Table 1. When even a subsaturating amount of T4 mRNA (0.47 mg/ml) is added to the cell-free extract, addition of a large excess of f2 RNA (0.9 mg/ml) has no effect on the amount of nature of the T4-specific translation products (compare samples 4, 7, 9, and 12). By contrast, even when reactions con-


f2 peptides
F
D
E
C
B
A
late T4 peptides
L16
L15
(L14a)
L14
L13
L12
L11
L10
L9
L8
L7
L6
ORIGIN
−
+
pH3.5
L5
L4
L3
L2
L1
1 2 3 4 5 6 7 8 9 10 11 12 13

tain a high concentration of f2 RNA (0.9 mg/ml, sample 13), addition of a subsaturating amount of T4 mRNA (0.47 mg/ml) results in two-fold inhibition of f2 translation (sample 12). When a subsaturating amount of f2 RNA is used (0.47 mg/ml, sample 3), addition of increasing amounts of T4 mRNA results in almost complete inhibition of f2 RNA translation (samples 3, 9, 10, and 11).

DISCUSSION

Under conditions where the concentration of messenger RNA is in excess of that required to saturate a cell-free protein synthesis reaction, those mRNAs with the greatest affinity for the saml1 ribosome subunit should be

Fig. 2 Competition between f2 and T4 messenger RNAs *in vitro*: Autoradiogram of digests of proteins labeled by N-formyl-[^{35}S]-methionyl-tRNA.

Sample	f2 RNA (mg/ml)	late T4 RNA (mg/ml)
1	-	-
2	0.23	-
3	0.45	-
4	-	0.47
5	-	0.94
6	-	1.9
7	0.23	0.47
8	0.23	0.94
9	0.45	0.47
10	0.45	0.94
11	0.45	1.9
12	0.90	0.47
13	0.90	-

Reactions (0.15 ml) contained the indicated amounts of phage RNA and the other components described in the legend to Figure 1. Incubation was at 37°C for 10 min. A 15 µl aliquot was taken for determination of total acid-precipitable radioactivity in the 150 µl reaction (see Table 1). Protein was precipitated from the remainder of the reactions and digested with trypsin and chymotrypsin as detailed previously (19, 29). Paper ionophoresis at pH 3.5 (40 volts per cm, 2 hr) was also detailed in reference 29. The paper was exposed to Kodak Royal Blue X-ray film for 7 days.

L1 - L16 peptide bands directed by late T4 mRNA (7)

A - F peptides directed by f2 RNA (19)

translated preferentially (18, 23). Our results show clearly that when late T4 mRNA and phage f2 RNA are added, in excess, to a cell-free reaction, T4 mRNA is translated preferentially. Presumably this is because T4 mRNA competes more effectively for ribosomes in vitro. Although it is difficult to quantitate the relative rate constants for ribosome attachment and chain initiation from this type of data, from samples 3, 9, 10, and 11 of Fig. 2 and Table 1 we estimate that the ratio of these constants for T4 mRNA relative to that of f2 RNA is at least 2 to 4.

For several reasons, however, this experiment must be interpreted with caution. First, the T4 mRNA preparation used contained all of the RNA found in infected cells. It is not certain that the specific inhibition of f2 protein synthesis demonstrated in Fig. 2 and Table 1 is due to T4 mRNA, and not to some other RNA present in the preparation. Similar RNA preparations from uninfected E. coli cells do not, however, inhibit translation of f2 RNA (Lodish, unpublished data). Second, the T4 message was obtained late in infection, while shutoff occurs in vivo at early times. Third, the secondary structure of the T4 mRNA or f2 RNA might not be equivalent to their structures in situ. The secondary and tertiary structures of mRNA are of great importance in translation (24), and extrapolation of this result to the situation in the infected cells depends on the assumption that the structure of the mRNAs are preserved during mRNA extraction and cell-free translation. Finally, the f2 replicative intermediate RNA also appears to be a competent messenger species (25, 26); the relationship of T4 mRNA to the replicative intermediate may be different from that observed with f2 single strands.

Our results do suggest a simple explanation of how T4 infection might block translation of pre-existing f2 RNA (and possibly cellular mRNAs). Inhibition of f2 translation in vivo requires synthesis of T4 mRNA, and is essentially complete by 4 min. after T4 infection. T4 infection might also result in a reduction in the amount of mRNA-binding initiation factor IF3, although the existing published data is equivocal on this point (7, 8, 14-16).

In any case, T4 infection might result in the presence of a large excess of mRNA, relative to ribosomes and other factors required for chain initiation. Under conditions where the amount of ribosome subunits or other factors required for translation of all mRNAs is limiting, translation of those mRNAs with the highest rate constant for ribosome attachment and chain initiation will occur preferentially; the present study indicates that, under such conditions, T4 mRNA should compete away f2 mRNA. This explanation does not require any change in the specificity of the translational apparatus after T4 infection.

The degradation of f2 single-stranded RNA and replicative intermediate RNA observed after T4 superinfection (6) could be a result of the inhibition of mRNA translation; f2 RNA, unprotected by ribosomes, might be a better substrate for a host ribonuclease than RNAs engaged in mRNA translation. Alternatively, a specific endoribonuclease induced by T4 could be responsible for the observed degradation of f2 RNA (6, 27).

ACKNOWLEDGEMENT

This work was supported by grant AI-08814 from the U.S. National Institutes of Health. H.F.L. was recipient of Research Career Development Award GM-50179 also from the N.I.H.

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