A FACTOR REQUIRED FOR THE TRANSLATION OF BACTERIOPHAGE f2 RNA IN EXTRACTS OF T4-INFECTED CELLS

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Summary: Extracts prepared from cells infected with bacteriophage T4 are unable to translate f2 RNA in vitro unless they are supplemented with a fraction derived from the high-salt wash of ribosomes isolated from extracts of uninfected cells. Thus, it is likely that a translational control mechanism is responsible for the restriction of f2 phage replication in T4-infected cells.

Our attempts to analyze the regulation of bacteriophage protein synthesis have centered on studying the possible functions of those early proteins which are made in a cell-free system programmed by T4 DNA (Celis and Conway, 1968). Assuming that these proteins are concerned with the cessation of host protein synthesis (McCorquidale et al., 1967; Levinthal et al., 1967) and recognizing that several mechanisms might operate in arresting the synthesis of Escherichia coli proteins (Nomura et al., 1962), we chose as a model system the restriction of the production of coliphage f2 by T4 (Zinder, 1963). The unpublished observation of Salser and Gesteland, reported by Hattman and Hofschneider (1968), that extracts from T4-infected cells failed to translate M12 RNA in vitro was the basis for the assay of the translation factor.

It will be shown here that supplementation of extracts from T4-infected cells with protein-containing fractions derived from uninfected cells is required for the translation of f2 RNA but not for polyuridylate (polyU)-stimulated polyphenylalanine synthesis. The active material is found in the wash of ribosomes treated with 1 M NH_4Cl and it elutes from DEAE-cellulose at about 0.2 M NH_4Cl. Consequently, infection with bacteriophage

T4 must result in the modification of a host translation factor which is likely inactive for the initiation of protein synthesis on RNA-phage messengers.

Materials and Methods

Preparation of Extracts from Infected Cells: E. coli, strain B06, was grown to a density of 5 x 10^8 cells/ml in 14 l. of H broth (Steinberg and Edgar, 1962) when L-tryptophan (4 µg/ml) and T4 bacteriophage (multiplicity = 12) were added. After 3-1/2 minutes at 37°, the culture was poured onto 4 kg of super-cooled crushed ice (-70°) and the infected cells harvested in a refrigerated Sharples centrifuge. The cells were resuspended in an equal volume of buffer I (0.01 M Tris-Cl, pH 7.4 and 0.01 M $MgCl_2$) and broken in a cold French pressure cell. Dithiothreitol (DTT, 0.5 mM) and pancreatic DNase (2 µg/ml) were added, the suspension centrifuged first for 15 min at 15,000 x g and then for 30 min at 30,000 x g, and the supernatant solution (Inf S30) dialyzed overnight against 100 volumes of buffer I plus 0.01 M KCl and 0.5 mM DTT. Reaction mixtures were preincubated with the extract for 15 min at 37° without template RNA.

Preparation of the Translation Factor: Uninfected cells of E. coli B (200 g) from Grain Processing Corp., Muscatine, Iowa, were broken and centrifuged as described above. The ribosomes were pelleted at 145,000 x g for 2-1/2 hrs, resuspended in Buffer I and repelleted again. The translation factor was extracted from the washed pellet with 1 M NH_4Cl, 0.01 M Tris-Cl, pH 8.1, and 0.01 M $MgCl_2$; the ribosomes were removed by sedimentation and the protein in the supernatant fluid concentrated by ammonium sulfate precipitation (0.8 of saturation). The resulting precipitate was taken up in 30 ml of 0.01 M Tris, pH 7.6, and dialyzed overnight against the same buffer plus 0.1 mM DTT. This was applied to a DEAE-cellulose column (Whatman DE32,1 me/g; 1.7 x 18 cm). The development of the column was according to Iwasaki et al. (1968). Column fractions were made 20% in glycerol and stored at -76°; activity remained for three weeks.

Bacteriophage f2 was isolated and purified up to the CsCl step of Nathans (1968). Purification was continued at an analogous point in the method of Robinson et al. (1969), but phenol extraction of the RNA was after Nathans (1968).

Results

Initially we were unsuccessful in assaying for an inhibitory effect of a T4-induced repressor-like substance which would prevent f2 RNA translation by extracts from uninfected cells. Yet, our extracts from T4-infected cells, like those of Salser and Gesteland (Hattman and Hofschneider, 1968), could not translate f2 RNA when they were prepared from cultures where the infection was virtually complete (Table I). If the infection was not complete, extracts from such cells were still active for f2 RNA translation. It appeared that instead of containing an inhibitor, the T4-derived extracts lacked a component essential for the translation of f2 RNA but not for poly U-

Table I

Activity of Extracts from T4-infected Cells for the Translation of f2 RNA and Poly U

System	^{14}C-Lysine Incorporated/mg Protein c.p.m.
Experiment 1: f2 RNA Template	
Uninfected (Ribosomes, 0.09 mg + S105, 0.14 mg)	39,500
T4-Infected (Ribosomes, 0.06 mg + S105, 0.115 mg)	4,160
Experiment 2: Poly U Template	^{14}C-Polyphenylalanine/mg Protein c.p.m.
Uninfected (S30)	19,700
T4-Infected (S30)	22,200

Reaction mixtures contained 36 µg of f2 RNA (or 10 µg of poly U), 15 µg of E. coli B tRNA, 3 mM ATP, 0.4 mM GTP, 5 mM phosphoenolpyruvate, 2 µg of pyruvate kinase, 75 mM NH_4Cl, 10 mM 2-mercaptoethanol, 50 mM Tris-Cl, pH 7.8, 0.04 mM each of 19 unlabeled amino acids, 0.02 mM of ^{14}C-lysine (156 µc/µmole) (or 0.02 mM of ^{14}C-phenylalanine (100 µc/µmole) in a volume of 0.1 ml. After 30 min at 37°, the reactions were terminated and counted as previously described (Celis and Conway, 1968).

stimulated phenylalanine incorporation (Table I).

To test this possibility, we used as the basic assay an amino acid incorporating system derived from T4-infected cells and then tried to stimulate the inactive system with components of extracts from uninfected cells. This assay worked well, and the activating component was traced to the ribosomal fraction (Table II). Furthermore, the translation factor could be washed free of the ribosomes with 1 M ammonium chloride (Table III).

Table II

Stimulation of f2 RNA Translation by the Ribosome Fraction

Components	^{14}C-Lysine Incorporated/ mg Protein c.p.m.
Complete (Inf S30)	180
+ S105, 0.08 mg	289
+ Ribosomes, 0.09 mg	1,550
+ S105 + Ribosomes	1,817

The assay was the same as in Table I. The complete system contained 1.41 mg of S30 from T4-infected cells (Inf S30). The supplements of supernatant enzymes and ribosomes were from uninfected cells and were inactive when assayed separately and in the absence of S30 from infected cells.

Table III

Stimulation of f2 RNA Translation by the Ribosomal Wash

System	^{14}C-Lysine Incorporated/mg Protein c.p.m.
Complete	1,135
+ 1 M NH_4Cl-washed Ribosomes, .090 mg	1,305
+ Ribosomal Wash, 0.1 mg	2,990

The assay was as described in Table I except the complete system contained unwashed ribosomes (.061 mg) from T4-infected cells and S105 (0.28 mg) from uninfected cells instead of the usual S30 from infected cells. See Methods for the conditions used for washing the ribosomes and concentrating the wash.

At this point, it appeared that what might be missing from the T4-derived extract was a translation initiation factor. Consequently, we fractionated the concentrated ribosomal wash using the chromatographic method of Iwasaki *et al*. (1968) which resolves the three initiation factors, F_1, F_2 and F_3.

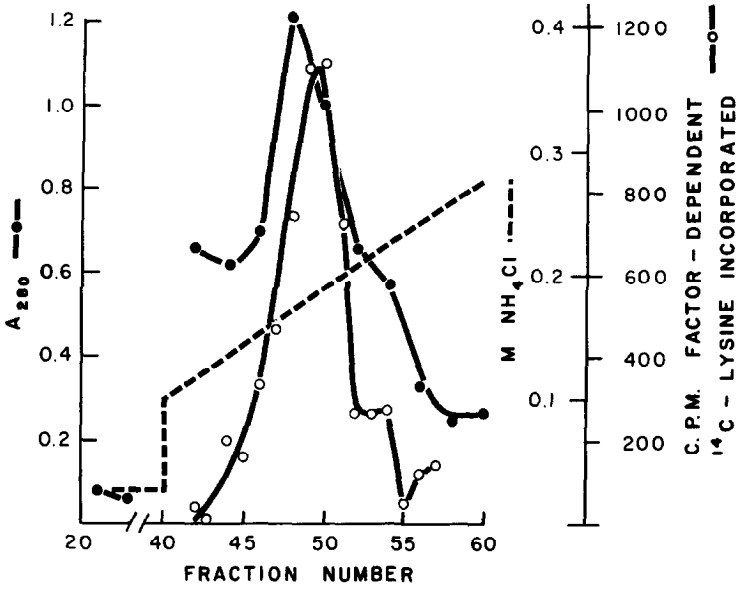

Figure 1. DEAE-cellulose elution pattern of the translation factor. Column fractions (0.005 ml) were assayed using the conditions of Table III. Each point was corrected for the activity in the absence of added column fractions, 450 c.p.m.

Figure 1 shows the results of such an experiment. Most of the activity for stimulating f2 RNA translation appears in one major peak which elutes at a position similar to initiation factor F_2.

Discussion

The mechanism by which T4 interferes with RNA phages has been somewhat in doubt. Yarosh and Levinthal (1967) suggest several possibilities which result in specific interruption of RNA-phage RNA synthesis while Hattman and Hofschneider (1968) propose that T4 blocks the functioning of RNA-phage RNA as messenger. There is no question, however, that a part of the T4 genome must be expressed for restriction to occur.

The simplest interpretation of our results is that an E. coli translation factor is modified by a T4-induced enzyme so that f2 RNA can no longer be recognized. The question can then be raised as to whether such a modified factor could still recognize T4 mRNA. After this work was completed, we learned

that Hsu and Weiss (1969) had isolated a few months earlier from T4-infected cells a factor which allows the translation of T4 mRNA but which is inactive with MS2 RNA.

It is also possible that T4 might induce the synthesis of a new factor rather than modify an existing one; however, if this were so, there must be an additional mechanism for inactivating the existing E. coli factor. Such inactivation must be complete since the E. coli factor seems to be dominant to the T4 factor, in respect to f2 RNA translation, when both are offered in vitro.

The translation factor described here has many features in common with initiation factor F_2 (C) which apparently can participate in the binding of T4 mRNA's to the ribosome (Herzberg et al., 1969). Moreover, our factor also bears a resemblance to the factor of Brown and Doty (1968), the M factor of Davison et al. (1969), and the T4-transcription factor of Travers (1969) in its association with the ribosomes and in the salt concentration required to elute it from DEAE-cellulose. All three of these factors stimulate transcription rather than translation, the Brown and Doty (1968) factor requiring ribosomes for its stimulatory effect. These factors might link transcription to translation by binding to specific intiating regions on messenger RNA's. Perhaps modification of one such factor by a T4-induced enzyme might greatly change the specificity of the factor for RNA-phage messengers and thus control their translation.

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