NONDISCRIMINATION OF RNA VIRAL MESSAGE IN BINDING TO 30S RIBOSOMES DERIVED FROM T4 PHAGE INFECTED ESCHERICHIA COLI

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Summary: The effect of T4 phage on ribosomes in terms of their ability to bind RNA viral template is examined. It is found that the 30S subunits of T4 ribosomes bind MS2 RNA as efficiently as do the subunits of uninfected E. coli ribosomes. On the other hand, analyses of the formation of 70S initiation complex, presumably from MS2 RNA-30S ribosome complex, using both labeled MS2 RNA and initiator tRNA, reveal that T4 ribosomes are only about half as active as E. coli ribosomes. The latter phenomenon has been reported previously. These results suggest that, following T4 infection, ribosomes are modified in such a way that the attachment of fMet-tRNA$_f$ to MS2 RNA-30S subunit complex is impaired.

INTRODUCTION: Ribosomes isolated from T4 phage infected Escherichia coli are restricted in their ability to translate MS2 viral and host messenger RNAs; translation of T4 messenger is unaffected.[1,2] This modification of ribosomes by T4 infection was found to be traceable to a change in a high-salt extractable fraction of the particles which contains initiation factors.[1,2] Subsequent reports[3,4] established that "T4 factors" exert their translational control at the level of initiation complex formation. These observations suggested that a change of IF3, which is responsible for initiation complex formation directed by natural mRNAs and possibly for recognition of messenger initiation signal,[5-9] may be involved. Further studies[10-12] showed that IF3 of E. coli is heterogeneous and that T4 infection alters the IF3 species specific for RNA viral template by a yet unknown mechanism.

In all of these studies, the effect of T4 infection on the initiation of translation was analyzed by determining the amount of labeled mRNAs or initiator tRNA that bound in the 70S initiation complex, and reduction in their binding was interpreted as a failure of the RNA viral message to interact with ribosomes from T4 infected cells. No attempt was made to determine the T4 ribosomal effect at the level of messenger-30S subunit

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interaction, which presumably precedes the formation of initiation complex. The present work represents such an effort.

MATERIALS AND METHODS: Uninfected and T4-infected cells - E. coli B cells, uninfected or infected with T4 (wild type), were prepared according to the methods already described.[1] The infection was carried out for 8 minutes at 37°C.

Ribosomes, labeled and unlabeled template RNAs and f[^{3}H]Met-tRNA$_f$ - These were prepared as previously described.[1,4]

Assays for binding of [^{3}H]template RNAs, and f[^{3}H]Met-tRNA$_f$ to ribosomes - The conditions of binding assays are as indicated. Binding of [^{3}H]mRNAs and [^{3}H]tRNA to ribosomes was assayed either by trapping of ribosomes on nitrocellulose filters (Millipore, HA) or by centrifugation on sucrose density gradient as described[4] with some modifications. For sucrose gradient analysis, 0.13 ml reaction system was set up and, following incubation as indicated, 0.10 ml was layered onto the top of a 5 ml-gradient and centrifuged for 75 minutes at 49,000 rpm. Gradient fractions were analyzed for O.D. at 260 nm and direct or non-filterable radioactivity. For non-filterable radioactivity, odd numbered fractions were diluted and passed through Millipore filters and the radioactivity of the filters was determined. The Millipore filters were not alkali-treated.

Radioactive compounds - The specific activities of [^{3}H]uracil and [^{3}H-CH_3]methionne were 25.4 and 3.3 C/mmole, respectively. These compounds were purchased from Schwarz/Mann, Orangeburg, New York.

RESULTS: Table 1 summarizes the results of binding assays of labeled initiator tRNA and mRNAs by the filtration technique. These experiments confirmed the previous finding that, whereas the two types of ribosomes are comparable in their ability to carry out T4 mRNA directed attachment of f[^{3}H]Met-tRNA$_f$, T4 ribosomes are only half as active as E. coli ribosomes in binding the initiator tRNA in the presence of MS2 RNA. On the other hand, when T4 ribosomes were compared to E. coli ribosomes with regard to their capacity to bind labeled MS2 RNA, there was no reduction in the binding by T4 ribosomes. The degree of binding of labeled T4 mRNA is about equal with both types of ribosomes. Neither fMet-tRNA$_f$ nor GTP was present in the [^{3}H]mRNA binding assays.

In order to examine in more detail the discrepancy between the effect of T4 infection on the ribosomal binding of MS2 RNA and that of fMet-tRNA$_f$, we performed a sucrose gradient analysis; the results are illustrated in Figure 1. In the absence of initiator tRNA and GTP, labeled MS2 RNA binds exclusively to 30S particles; T4 ribosomes are equally, if not more, efficient as control ribosomes in forming the MS2 RNA-30S subunit complex

TABLE 1

COMPARISON OF E. COLI AND T4 RIBOSOMES IN THE BINDING OF MESSENGER RNAs AND OF fMet-tRNA$_f$ DIRECTED BY THE mRNAs

Exp. No.	RNA template	Label	Ribosome source	cpm bound	Per cent relative to E. coli
1	none	f[^{3}H]Met-tRNA$_f$	E. coli	2,578	-
	none		T4	3,891	-
	T4		E. coli	35,626	100
	T4		T4	38,275	104
	MS2		E. coli	16,626	100
	MS2		T4	9,976	43
2	T4	[^{3}H]T4mRNA	none	726	-
	T4		E. coli	4,869	100
	T4		T4	4,578	93
	MS2	[^{3}H]MS2 RNA	none	36	-
	MS2		E. coli	3,657	100
	MS2		T4	4,099	123

Conditions for labeled initiator tRNA binding were as follows: Reaction mixture (0.065 ml) contained 40 mM Tris (pH 7.5), 80 mM NH_4Cl, 5 mM Mg acetate, 4 mM β-mercaptoethanol, 2 mM GTP, 43 μμmoles of f[^{3}H]Met-tRNA$_f$ (9.4 x 10^4 cpm), 200μg of E. coli or T4 ribosomes, and a template RNA or H_2O. The template was either T4 mRNA or MS2 RNA, and was present in amounts of 150μg or 15μg, respectively. The binding was carried out for 15 minutes at 37°C. The reaction mixture (0.065 ml) for [^{3}H]template RNAs contained, besides Tris, NH_4Cl, Mg acetate, βMe, and ribosomes at the same concentrations as above, 100μg of [^{3}H]T4 mRNA (3.4 x 10^4 cpm), or 15μg of [^{3}H]MS2 RNA (1.4 x 10^4 cpm). Incubation was for 2 minutes at 4°C. The radioactive RNAs bound by ribosomes were assayed by trapping of the radioactivity on Millipore filters as described.[4]

(Fig. 1A). It was necessary to pass gradient fractions through nitrocellulose filters in order to distinguish ribosome-bound from free labeled mRNA which sediments as 27S. The second peak sedimenting behind the 30S ribosomal complex is unidentified. In the presence of fMet-tRNA$_f$ and GTP and at a higher temperature, although with uninfected E. coli ribosomes a large part of the [^{3}H]MS2 RNA is still associated with 30S subunits,

another large part of the label is apparently converted into 70S ribosomal initiation complex (Fig. 1B). With T4 ribosomes, more labeled MS2 RNA is associated with 30S ribosomes and less with 70S ribosomes as compared with the control ribosomes. Here also, the unidentified slow sedimenting peak is present. A similar experiment in which f[^{3}H]Met-tRNA$_f$ served as the label (Fig. 1C) also shows that T4 ribosomes are much less efficient than E. coli ribosomes in forming 70S initiation complex. No label was found associated with 30S ribosomes.

DISCUSSION: The results presented above indicate that the discrimination in translation of RNA viral and, possibly, host messengers by ribosomes derived from T4 infected E. coli is not at the level of MS2 RNA-30S ribosome interaction, but at the subsequent step which involves attachment of initiator tRNA to the mRNA-ribosome complex. That the 30S subunit of T4 ribosomes bind MS2 RNA as efficiently as the subunit of uninfected E. coli ribosomes had escaped previous detection because the effect of T4 viral infection had been studied by observation of the reduction of labeled RNA viral message in the 70S ribosomal region.

The possibility that MS2 RNA-30S ribosome-fMet-tRNA$_f$ complex is formed normally, but prevented from being converted into 70S initiation complex is not likely since such interference would result in the piling up of the former complex under the experimental conditions shown in Figure 1C.

Although the specific role in translational initiation of mRNA-30S ribosome interaction, as measured under the present experimental conditions, has not been firmly established, the following observations suggest that the interaction is a prerequisite for the formation of the 70S initiation complex: (1) It is initiation factor dependent,[13] and various synthetic polynucleotides and aurintricarboxylic acid which interfere with this interaction also inhibit the 70S initiation complex formation (unpublished data). (2) The formation of 70S initiation complex appears to be accompanied by a corresponding decrease in the amount of MS2 RNA-30S ribosome complex (Figs. 1A and 1B). Kinetic studies to determine the precise relationships between the two complexes, however, were difficult due to the degradation of labeled viral message during binding reaction (unpublished observation).

The relatively undramatic reduction in the ability of T4 ribosomes to direct MS2 RNA dependent formation of 70S initiation complex does not reflect a poor infection; under similar experimental conditions, bacterial killing was 99.8% in 5 minutes of infection, and phage production after an additional 3 hour incubation was greater than 30 infective particles per infected cell. It appears, therefore, that the interference of MS2 RNA

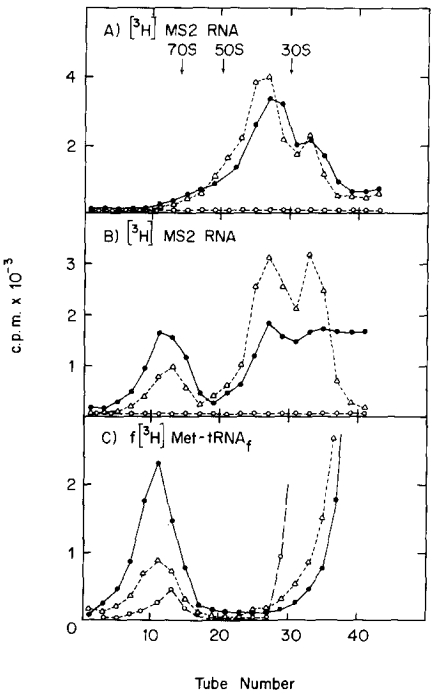

Figure 1. Sucrose gradient analysis of the ribosomal binding of labeled RNA viral message and initiator tRNA. Analysis was made as described in Methods.
1A: Binding of [^{3}H]MS2 RNA in the absence of fMet-tRNA$_f$ and GTP. Reaction and incubation conditions were the same as described for experiment 2 of Table 1 for the binding of [^{3}H]MS2 RNA, except that the specific activity of [^{3}H]-labeled MS2 RNA is 1,300 cpm/µg. Counts represent non-filterable radioactivity.
1B: Binding of [^{3}H]MS2 RNA in the presence of fMet-tRNA$_f$ and GTP. Reaction conditions were the same as described in Table 1 for labeled initiator tRNA binding, except that equivalent amounts of [^{3}H]MS2 RNA (sp. act., 1,300 cpm/µg) and unlabeled fMet-tRNA$_f$ replaced unlabeled MS2 RNA and f[^{3}H]Met-tRNA$_f$, respectively. Time of binding was 5 minutes at 37°C. Counts again represent non-filterable radioactivity.
1C: MS2 RNA directed binding of f[^{3}H]Met-tRNA$_f$. Conditions of binding were the same as above, except that MS2 RNA was unlabeled and present at a higher level (100 µg in a 0.13 ml system), and f[^{3}H]Met-tRNA$_f$ (sp. act., 2,180 cpm/µµmole) served as the label and that time of reaction was 3 minutes at 37°C. Radioactivity represents direct counts. •——•, uninfected E. coli ribosomes; Δ——Δ, T4 ribosomes; o——o' minus ribosomes in Figures 1A and 1B, minus MS2 RNA in Figure 1C.

translation is only partial, at least in vitro, although greater effects (>80%) have been observed.[3,4]

In recent years, a number of reports[10,12] mainly from the laboratories of Revel and Ochoa, described a separation of factor IF3 into cistron specific species and a change in RNA viral cistron specific species following T4 infection. More recently, isolation of interference factors that speci-

fically block the activity of certain IF3 has been reported.[14,15] The relationship of the present observation to these findings is not immediately clear. However, the following hypothetical scheme is consistent with the results of the present experiments: Following T4 phage infection, ribosome associated "inhibitor(s)" is produced that allows RNA viral message to attach normally to 30S ribosomal subunits. This inhibitor, by virtue of its ability to recognize and interact with certain initiation sequences, leads to the blocking of initiator tRNA attachment to the mRNA-30S ribosome complex. Although it is known that the initiation of protein synthesis directed by RNA phage template requires the formation of 30S ribosomal complex involving the template RNA and initiator tRNA,[16,17] the sequences leading to the 30S initiation complex are still unclear.

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