Translation of RNA Coliphages by Amino Acid-incorporation Systems of the Enterobacteriaceae

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SUMMARY

Crude cell-free amino acid-incorporating systems derived from a variety of enterobacterial species are not uniform in their ability to translate RNA of the coliphages Q β or MS2. *Escherichia coli*, *Shigella alkalescens* and to a lesser extent *Citrobacter freundii* translate effectively under standard conditions, but *Salmonella typhimurium*, *S. pomona* and *Proteus morganii* do not. Ineffective species do not generally inactivate phage RNA faster than effective ones, and they are as effective as *E. coli* in their general capacity for mRNA translation. This was judged in representative species by the incorporation of phenylalanine and leucine labels as directed by polyuridylylate or mRNA of phage-infected *S. typhimurium*, and from the relative incorporation of methionine label into the normal initiating *N*-formyl-methionine residues of polypeptides. The ribosomal rather than the soluble components appear responsible for ineffectiveness in translation.

INTRODUCTION

Microbial amino acid-incorporating systems are sometimes selective in their translation of the RNAs of *Escherichia coli* RNA phages. Incompatibilities may be conferred by specific modifying factors (Hsu & Weiss, 1969; Dube & Rudland, 1970; Morrison & Malamy, 1971), or they may also normally exist in polypeptide incorporating systems of *Bacillus stearothermophilus* (Lodish, 1970) and a psychrophilic pseudomonad (Szer & Brenowitz, 1970). We have also observed that *Streptococcus faecium* preparations are unable to translate RNA of the Q β phage of *E. coli* at high or low Mg⁺² levels (Sarimo & Pine, 1971; and unpublished experiments). In reconstitution experiments with *E. coli* components, the soluble streptococcal components were competent in translating this RNA but the ribosomes were not. The latter also could not bind Q β RNA with [³⁵S]formylmethionyl-tRNA in initiation complexes (Sarimo & Pine, 1971). We now report incompatibilities in translation of *E. coli* RNA phages by even more closely related species of the Enterobacteriaceae.

METHODS

The procedure of Nirenberg (1963) was used with minor modification for the growth of bacteria, preparation of incubated, dialysed S30 extracts and assay of amino acid incorporation. [³⁵S]Methionine and a mixture of ³⁵S-labelled methionyl and *N*-formylmethionyl-tRNAs were prepared from *Escherichia coli* (Sarimo & Pine, 1971). Labelled *N*-formylmethionyl and internal methionyl residues in the proteins were determined from their electrophoretic peaks after pronase digestion (Pine, Gordon & Sarimo, 1969). Preparation of MS2 phage RNA was modified from a previous procedure (Davis & Sinsheimer, 1963). *N*-Acetylphenylalanine-containing tRNA was prepared by the method of Haenni & Chape-

Table 1. *Efficiency of S30 extracts of Enterobacteria in translating Q β RNA and polyU*

Organism	Polypeptide incorporation*						Relative† efficiency (%)
	[¹⁴ C]Leucine		[¹⁴ C]Phenylalanine				
	No RNA (pmol)	+ Q β mRNA (pmol)	No mRNA	+ Q β mRNA	+ polyU (pmol)	+ polyU + N-acetyl-phe-tRNA	
<i>Escherichia coli</i> B							
1	11.8	167	1.6	34.6	544	779	100
2	14.5	97	0.4	31	639	780	
<i>Shigella alkalescens</i>	11.2	76	6.2	17.2	632	558	48
<i>Salmonella typhimurium</i>	1.8	0.4	0.2	0.4	0.4	914	0.5
<i>Proteus morganii</i>	6.4	7.6	1.6	1.8	29	704	0.2

* Reaction mixtures contained per 0.25 ml: 10 mM-tris (hydroxymethyl) amino methane HCl, pH 7.8; 60 mM-KCl; 10 mM-NH₄Cl; 8 mM- β -mercaptoethanol; 5 mM-Mg acetate; preneutralized 6 mM-Na phosphoenol pyruvate; 4 mM-ATP; 0.4 mM-GTP; 1 μ g leucovorin; 1 μ g labelled amino acid containing 1.5×10^5 c.p.m.; 5 μ g of all other amino acids, 9.4 to 18.7 E_{260nm} of S30 extract; and 107 μ g polyU with N-acetyl phe-tRNA at 6.3 E_{260nm} units or 50 μ g Q β phage RNA. Components were incubated at 37 °C for 30 min.

† $100 \times (\text{Q}\beta \text{ RNA-directed enterobacterial incorporation} / \text{Q}\beta \text{ RNA-directed } E. coli \text{ incorporation})$: (polyU + N-acetyl phe-tRNA-directed enterobacterial incorporation / polyU + N-acetyl phe-tRNA-directed *E. coli* incorporation).

ville (1966). Crude RNA enriched with phage P22 mRNA was prepared from lysogenic *Salmonella typhimurium* treated for 60 min with 8 μ g/ml of mitomycin C and extracted with phenol (Bolle, Epstein & Salzer, 1968). Purified Q β phage RNA was obtained from Dr B. A. Bassel, State University of New York Upstate Medical Center.

RESULTS AND DISCUSSION

Initially, translation of Q β RNA was examined in four enterobacterial species with uniformly labelled [¹⁴C]leucine and phenylalanine (Table 1). Only *Escherichia coli* and the closely related *Shigella alkalescens* translate Q β RNA effectively, but *Salmonella typhimurium* and *Proteus morganii* do not. The same relative results were also obtained at incorporation times of 10 to 60 min and at the higher Mg²⁺ concentrations of 10 to 14 mM. Incorporation was generally linear with time or began after a slight lag, and was maximal at 30 min. All species translate polyU, however, and, at the low Mg²⁺ concentrations used, they are variably dependent on N-acetylphenylalanyl-tRNA as an initiator (Table 1). In other experiments they also incorporated similar amounts of labelled [³⁵S]methionine into normal initiator N-formylmethionyl-tRNA.

To determine whether the destruction of mRNA was responsible for poor translation activity, phage Q β RNA was added to one-fifth the normal amount of S30 extract under the standard conditions of polypeptide incorporation. The labelled leucine was added 30 min afterwards together with a normal amount of Q β -translating *Escherichia coli* extract that minimized the contribution of the first extract. [¹⁴C]Leucine incorporation was reduced to varying extents by each pre-incubation, but the extent of reduction did not correlate with competence of the extracts in translating Q β RNA. Thus the incompatibilities in translation do not appear to be determined by mRNA destruction.

Enterobacterial translation was further investigated with RNA of the coliphage MS2

Table 2. Polypeptide incorporations directed by RNAs of MS2 phage, *Escherichia coli* ribosomes and phage P22-infected *Salmonella* cells

Extract	[¹⁴ C]Phenylalanine incorporation* net pmol/30 min/tube			[³⁵ S]Methionine incorporation† N-formylmethi- onine:methionine P 22-Salmonella
	PolyU + N- acetylphenyl- alanine-tRNA	MS 2 RNA	P 22-Salmonella RNA	
<i>Citrobacter freundii</i>	697	13	—	—
<i>Escherichia coli</i>	276	142	56	1:5.4
<i>Salmonella pomona</i>	111	2	34	1:5.3
<i>Escherichia coli</i> : <i>Salmonella</i> <i>pomona</i> 1:1	204	25.2	20.2	—
<i>Escherichia coli</i> ribosomes (2 ×) + <i>Salmonella pomona</i> supernate	724	82	29	—
<i>Salmonella pomona</i> ribosomes (2 ×) + <i>Escherichia coli</i> supernate	178	0.9	7.2	—

* Components and conditions as in Table 1, with the following modifications per 0.25 ml volume: 5.6 mM-Mg acetate; 5 mM-spermidine 3 HCl; 10 mM-putrescine 2HCl; plus mRNAs as follows: 1242 µg RNA extracted from purified *Escherichia coli* ribosomes; 80 µg polyU with 120 µg N-acetylphenylalanine-tRNA (unfractionated); 200 µg phage MS 2 RNA or 736 µg RNA of phage P 22-infected *Salmonella typhimurium*. Ribosomes in reconstituted mixtures were at twice the S₃₀ level.

† Components and conditions as above, but proportionately in 1 ml volume and with label as [³⁵S]-methionine, approximately 1 mc/mmol, 1.5×10^6 c.p.m. added as the free amino acid and 109000 c.p.m. charged to 1.8 mg *Escherichia coli* B tRNA. Individual electrophoretic peaks of N-formylmethionine and methionine ranged from 308 to 2689 c.p.m. Backgrounds without mRNA were negligible.

(Table 2). In these assays, spermidine and putrescine, which slightly stimulated translation generally, were added with an optimal amount of Mg⁺². *Escherichia coli* is effective, *Citrobacter freundii* marginally effective and *Salmonella pomona* ineffective in translating MS 2 RNA, as judged by phenylalanine incorporation. In contrast, all are effective in translating polyU. *Salmonella pomona* and *E. coli* are also about equally effective in translating mRNA prepared from phage P 22-infected *S. typhimurium*. With [³⁵S]methionine as the label, approximately one of six to seven residues incorporated into polypeptides is N-formylated in both *E. coli* and *S. pomona* preparations (Table 2). The normal removal of the initiating formyl residues is inhibited under these conditions (Adams, 1968). Judging from the N-formylmethionine content of the synthesized polypeptides, translation of the crude mRNA must frequently begin at the initiating AUG codon. The incompatibility of *S. pomona* in translating RNA of the MS2 phage therefore cannot be due to a lowered general capacity for initiating translation of normal mRNAs. Ribosomal RNA has no mRNA activity in any of the preparations. Treating the host system used for preparing P 22 mRNA with 30 µg/ml of rifampicin for 20 min also markedly reduces mRNA activity. These characteristics further attest that authentic mRNAs are being translated. When the ribosomes and supernatant factors of *E. coli* and *S. pomona* are interchanged, the incompatibility of *S. pomona* in MS 2 RNA translation appears due to the ribosomal and not the soluble component (Table 2). In an additional experiment crude initiation factors prepared from *E. coli* ribosomes did not clearly improve MS2 RNA translation by S₃₀ extracts or ribosomal components of *S. pomona*.

In conclusion, effectiveness in translating the RNAs of RNA-containing coliphages appears to vary among enterobacterial preparations that otherwise effectively translate polyU or a mixture of mRNAs of combined bacterial and DNA phage origin, and have similar

ability to initiate translation normally with *N*-formylmethionine residues. *Escherichia coli* and *Shigella alkalescens* tribally allied in the Eschericheae, and to a limited extent *Citrobacter freundii* (Salmonelleae) can translate the RNA phages of *E. coli*, while other representatives outside of the Eschericheae (*Salmonella typhimurium*, *S. pomona* and *Proteus morganii*) did not effectively do so. Comparisons have been based on translation of very different mRNAs under standard conditions and hence are not absolute. The ribosomes, which are likely responsible for the poor compatibilities, could be modified by phages or episomes (Hsu & Weiss, 1969; Dube & Rudland, 1970; Morrison & Malamy, 1971), but as the incidence of poor compatibility is high the differences are more likely inherent. Since the genome of RNA-containing coliphage can sometimes be effectively translated by phylogenetically remote systems (Schwartz, Eisenstadt, Brawerman & Zinder, 1965), translational variations may in part reflect random differences among ribosomes.

The incompatibility of one of the species tested here, *Salmonella typhimurium* in translating Q β RNA has recently been confirmed in another laboratory (B. A. Bassel, private communication). In preliminary studies deficiencies appeared in ribosomal attachment of the RNA and the extent of translation.

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