Ribosomes from Escherichia coli: Lack of Specificity for Viral RNA

Abstract. *The class of ribosomes from* Escherichia coli *that associate with plant virus RNA in vitro can be separated from the bulk of ribosomes by gradient centrifugation. The relative activity for subsequent incorporation of amino acid into polypeptides directed by the viral RNA in comparison to that directed by polyuridylic acid is the same for that class as for the bulk of ribosomes.*

As a consequence of the initial formulation and experimental verification of the messenger RNA hypothesis for protein synthesis (*1*), it has been widely assumed that ribosomes are nonspecific machines for translating polynucleotide messages. The finding that the site of action of the streptomycin-dependent suppressor is the ribosome (*2*) makes modification of this assumption necessary. Our own previous work indicated that messengers compete for ribosomes when the latter are limiting in an in vitro system (*3*). This observation led us to ask whether ribosomes from *Escherichia coli* can be divided into subclasses on the basis of their ability to distinguish between messengers. The evidence we now present indicates that ribosomes from *E. coli*, prepared by conventional procedures (*4, 5*), do not make this distinction at the first step, that is, at the time of attachment of the messenger.

The RNA from turnip-yellow-mosaic virus (TYMV) associates with *E. coli* ribosomes in vitro to form complexes, termed monosomes, containing one molecule of RNA and one 70*S* ribosome (*4, 5*). Monosomes have sedimentation coefficients in the range from 80 to 100*S*, and are therefore separable from single ribosomes by zone centrifugation. We may then ask whether the monosome fraction is enriched in ribosomes that utilize this viral RNA in preference to another messenger. Accordingly, a saturating amount of the viral RNA was incubated for 5 minutes at 26°C with *E. coli B* ribosomes and supernatant to promote irreversible attachment of the RNA (*6*) and then centrifuged at 35,000 rev/min (Spinco SW 39 rotor) for 60 minutes at 10°C on a 10 to 30 percent (by volume) glycerol gradient containing 0.05*M* tris buffer at *p*H 7.6, 0.05*M* KCl, and 0.01*M* $MgCl_2$ (*7*). After centrifugation, the gradients were separated by puncturing the bottom of the tube and withdrawing fractions which were immediately chilled. Each fraction was divided into four portions which were supplemented with supernatant and cofactors and assayed (*5*) for incorporation of (i) C^{14}-phenylalanine, (ii) C^{14}-proline, (iii) C^{14}-phenylalanine with polyuridylic acid (polyU) added, and (iv) C^{14}-proline with TYMV-RNA added. Series i is

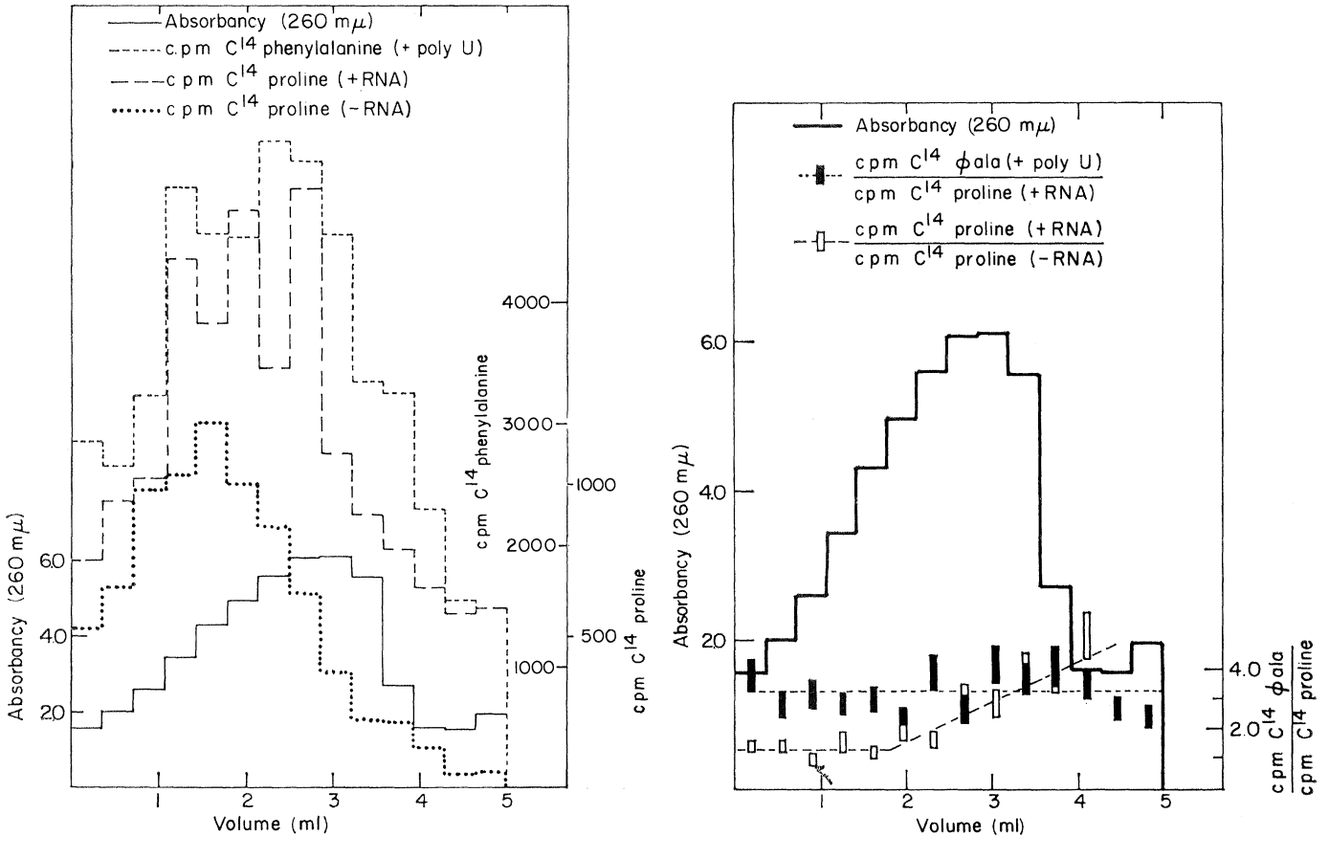

Fig. 1 (left). Activity for amino acid incorporation in ribosome fractions after centrifugation in glycerol gradients. TYMV-RNA (350 μg) and *Escherichia coli* ribosomes (2.6 mg) were incubated with *E. coli* supernatant and centrifuged as described. After centrifugation, 0.07-ml portions were incubated for 30 minutes at 26°C with a saturating amount of supernatant, 0.4 μmole of adenosine triphosphate, 0.08 μmole of guanosine triphosphate, 0.8 μmole of phosphoenol pyruvate, 8 μg of pyruvate kinase, 2 μmole of mercaptoethanol, 10 μmole of tris *p*H 7.5, 10 μmole KCl, and 2 μmole $MgCl_2$ in a final volume of 0.20 ml. To each tube from series iii 34 μg polyU was added; to each tube from series iv 150 μg TYMV-RNA was added. Each tube contained 0.10 μmole each of 19 amino acids; those in series i and iii contained 0.01 μmole C^{14}-phenylalanine (specific activity 369 mc/mmole), those in series ii and iv contained 0.01 μmole C^{14}-proline (specific activity 205 mc/mmole). Fig. 2 (right). Relative activity for amino acid incorporation in ribosome fractions. Data from Fig. 1.

essentially a blank for series iii. Series ii locates those ribosomes that bound the viral RNA during the 5-minute incubation period and remained associated during zone centrifugation. Series iii locates those ribosomes that can respond to polyU. Series iv locates those ribosomes that can respond to the viral RNA, including those in series ii. Series i gave negligible incorporation; the results of series ii, iii, and iv are found in Fig. 1. We show in Fig. 2 the ratio of phenylalanine incorporation to that of proline (iii to iv, filled rectangles) compared with the same absorbancy profile. The ribosomes responding to added viral RNA are not separated by zone centrifugation from those responding to polyU.

Series ii, in which the activity for proline incorporation runs ahead of most of the 70*S* ribosomes, shows that monosomes were indeed formed prior to centrifugation. Comparison with series iv indicates that not all the ribosomes that can respond to the viral RNA are in the monosome region, despite the fact that the first 5-minute incubation was with a saturating amount of RNA. This finding is in agreement with our previous assertion that the initial attachment of TYMV-RNA to ribosomes is reversible but can lead to an enrichment of active ribosomes (*5*). Finally, the other comparison in Fig. 2 (iv to ii, open rectangles) shows that the methods used are sensitive enough to reveal differences between mixtures such as iii and iv, if such differences existed.

The viral RNA and polyU compete for ribosomes when the latter are limiting (*3*). We may then use a protocol similar to the one just described to ask whether TYMV-RNA selects a class of ribosomes which respond distinguishably from the bulk of ribosomes when presented with a mixture of the viral RNA and polyU. Therefore the viral RNA and ribosomes were mixed at 0°C (*6*) and then subjected to zone centrifugation as before, except that the time of centrifugation was decreased to 40 minutes. The following assays for incorporation were performed on the fractions: (i) C^{14}-proline with added TYMV-RNA, (ii) C^{14}-phenylalanine with added polyU, (iii) C^{14}-proline with both viral RNA and polyU added, and (iv) C^{14}-phenylalanine with both polynucleotides added. Both i and ii are repeats of the first experiment, but the first 5-minute incubation was omitted; these give the same result (not shown). Series iii and iv (Figs. 3 and 4) are the competition assays; the ribosome fractions are indistinguishable on this basis as well.

These results may be summarized by the following statements: *Escherichia coli* ribosomes that associate with TYMV-RNA in vitro can be separated from the bulk of ribosomes by centrifugation in glycerol gradients. When the two classes of ribosomes separated in this way are tested for their ability to incorporate amino acids, the relative response to two messengers, TYMV-

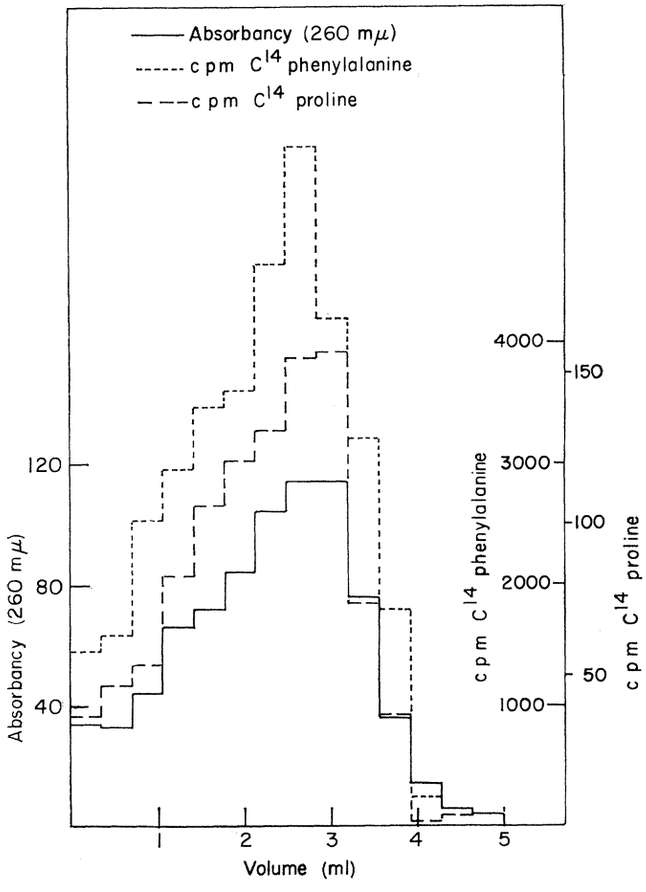

Fig. 3 (left). Activity for amino acid incorporation in ribosome fractions after glycerol gradient centrifugation, with TYMV-RNA (200 μg) added to 19 mg of ribosomes just prior to centrifugation; to each of two tubes, both 34 μg of polyU and 150 μg of TYMV-RNA were added. Under these conditions ribosomes are limiting, and the relative incorporation of phenylalanine and proline depends upon competition of the two messengers for ribosomes (*4*). Conditions of incubation in assay after centrifugation were identical to those for series iii and iv (Fig. 1).

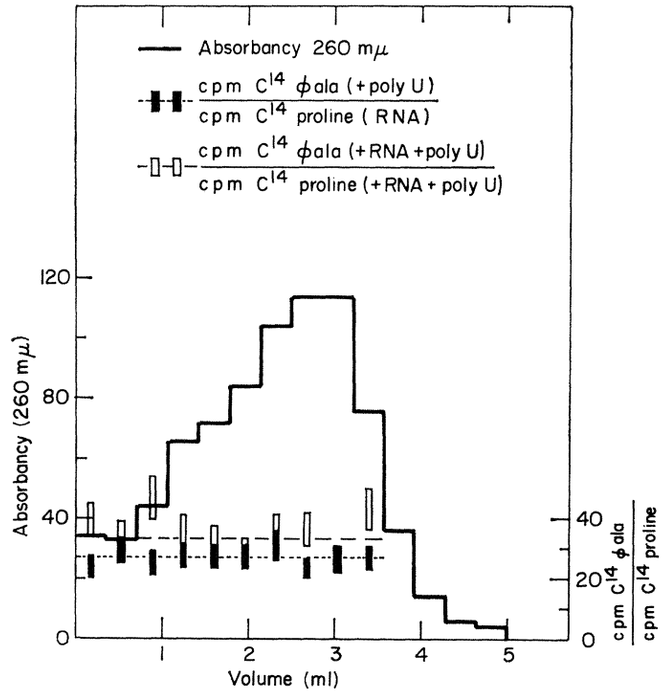

Fig. 4 (right). Relative activity for amino acid incorporation of ribosome fractions; data from Fig. 3.

RNA and polyU, is the same for both classes. Thus, while association with the viral RNA in vitro distinguished "active" from "inactive" ribosomes, it did not distinguish a class particularly responsive to that messenger. If these messengers are representative, then ribosomes are nonspecific with respect to the attachment of messengers.

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References and Notes

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6. Omission of supernatant and the 5-minute incubation period prior to centrifugation is a significant difference in the two experiments. The initial attachment of TYMV-RNA to ribosomes is immediately reversed by polyU, whereas, after incubation of the viral RNA with ribosomes in the presence of supernatant, the RNA is displaced less effectively, and only after a lag of several minutes.
7. The substitution of glycerol for sucrose in the gradients gave greater recovery of activity. In separate runs containing P^{32}-labeled TYMV ($S_{20,w} = 116S$) as a marker, the peak in the absorbancy profile corresponded with $S_{20,w} = 70S$.
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Alcohol Dehydrogenase in Drosophila melanogaster: Isozymes and Genetic Variants

Abstract. *Alcohol dehydrogenase, in* Drosophila melanogaster *homozygous for the alleles* Adh^F *or* Adh^S, *is found in three electrophoretically different forms.* Adh^F *differs from* Adh^S *in that isozymes with faster electrophoretic mobilities are present. In* Adh^F/Adh^S *heterozygotes, hybrid isozymes as well as the parental isozymes are present, indicating that the dehydrogenase may exist as a dimer of two polypeptide subunits. The gene,* Adh, *is located on the second chromosome, with a map position of 50.1 and a cytological position between 34E3 and 35D1.*

In multicellular organisms an enzyme sometimes occurs in several separable forms called isozymes. In the case of lactate dehydrogenase the enzyme is considered to be a tetramer composed of either or both of two individual polypeptide subunits. Presumably each subunit is a gene product, but evidence for two genes has not been obtained. Although lactate dehydrogenase is virtually absent in *Drosophila melanogaster*, another isozyme system was discovered which is under genetic control and for which the genetic locus has been identified. This enzyme is alcohol dehydrogenase (ADH), and the existence of isozymes has been independently discovered and reported by Johnson and Denniston (*1*). Instead of the two isozymes of ADH reported, we find that there are three. Other electrophoretic variants of enzymes and proteins have also been reported to occur in *Drosophila melanogaster* (*2*).

For electrophoresis, polyacrylamide gels (5 percent acrylamide) were prepared according to the procedure of Raymond and Wang (*3*). The gels were stored in 0.05*M* tris buffer adjusted with H_3PO_4 to *p*H 8.5 at 25°C. The gels were cut, and filter paper, containing the sample, was inserted between the cut ends. In general, a sample consisted of one or more *Drosophila* crushed on the filter paper with a glass rod. A potential of 18 to 27 volt/cm was applied. The gels were cooled on top and bottom to at least 20°C during electrophoresis. A complete separation of ADH isozymes was obtained in 8 hours; however, most isozymes are separated within 2 hours. The ADH activity on the gels was detected by a modified staining technique for lactate dehydrogenase (*4*). The developing solution contained 90 ml of tris buffer (0.05*M*, *p*H 8.5), 4 ml of nicotinamide adenine dinucleotide solution (10 mg/ml), 4 ml of phenazine methosulfate solution (2 mg/ml), 2 ml of nitro blue tetrazolium (10 mg/ml), and 0.75 ml of ethanol (95 percent) or *sec*-butanol. Purple spots of the reduced formazan were usually visible within 1 hour after start of incubation at 37°C, but longer incubation was necessary to observe areas of low ADH activity. To demonstrate that the assay is dependent on added ethanol it was necessary to evaporate the two indicator dyes to dryness before preparation of the above developing solution. *Iso*-propanol and *sec*-butanol are more effective substrates than ethanol for the gel assay.

Inbred strains of *Drosophila melanogaster* regularly have three ADH isozymes in either of two types of patterns. One pattern is a slow type, is found in Canton-S wild type (Fig. 1*a*), and consists of bands 1, 4, and 7; the other pattern is a fast type, and is found in Samarkand, Swedish-b, and many other wild types, and consists of bands 3, 6, and 9 (Fig. 1*b*). In their respective patterns the slowest band is most active enzymatically, and the fastest band is least active. Ordinarily, adult flies were examined, but the isozymes are also all present in the fully grown larvae and in the pupae. Larvae were actually the best source of bands 7 and 9.

Examination of over 100 laboratory strains has not revealed further variety among inbreds. Of course, it may not be assumed that enzymes of two stocks are identical merely because they have the same electrophoretic mobility. In fact, many differences would not be revealed by this technique.

A cross of a strain containing bands 1, 4, and 7 with a strain containing bands 3, 6, and 9 yields hybrid F_1 progeny (Fig. 1*c*) that contain the isozymes of both parents plus three additional isozymes. One band unique to hybrids is band 2. It belongs in the group of what may be termed primary ADH isozymes and has a mobility intermediate between the two parental primaries, bands 1 and 3. Band 5 is

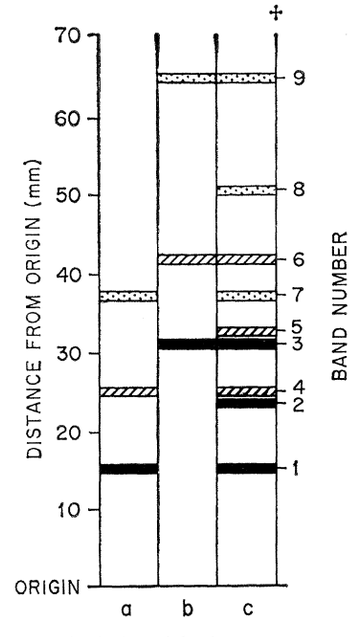

Fig. 1. Alcohol dehydrogenase zymograms of Canton-S (*a*), Samarkand (*b*), and F_1 hybrid between the two (*c*).