THE BINDING OF [^{14}C] PHENYLALANYL-sRNA TO WHEAT GERM RIBOSOMES

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Introduction

Ribosomes from wheat germ do not incorporate amino acids in an in vitro system in the absence of exogenous messenger RNA. They are, however, able to incorporate phenylalanine into a polypeptide in the presence of poly U. (Marcus and Feeley, 1964). Several explanations can be offered to account for the inability of the ribosomes to incorporate amino acids in the absence of an exogenous messenger RNA:

1. The ribosomes are lacking in messenger RNA. 2. The ribosomes have bound messenger RNA but this messenger RNA cannot be "translated" into peptide because it is masked and inactive (Monroy et al., 1965) or because some special factor for protein synthesis initiation is lacking (Noll, 1966).

The specific binding of sRNA to ribosomes in the presence of oligo- or poly-nucleotides has been investigated in several laboratories (Kaji and Kaji, 1964; Kurland, 1966). In the present investigation we have studied the binding of [^{14}C] phenylalanyl-sRNA to ribosomes isolated from non-germinated wheat germ. Our results suggest that messenger RNA does exist in non-germinated wheat germ.

Materials

Preparation of wheat germ ribosomes: Two different preparations were used in this investigation. 1. "Washed ribosomes". These ribosomes were prepared essentially as described by Marcus and Feeley (1964). The crude ribosomal pellet was washed by suspending it in a Tris-HCl buffer, 0.01 M, pH 7.7 containing magnesium acetate, 10^{-3} M; mercaptoethanol, $5x10^{-3}$ M, and 2% sucrose. The suspension was centrifuged at 14,000 x g for 10 min. and at 100,000 x g for 1 hr. The washing was repeated and the final pellet was taken up in the same buffer. The ribosomes were frozen in liquid air and stored at -20° C. 2. "DOC treated ribosomes". These were prepared as follows: Deoxycholate was added to a "washed ribosomes" suspension to give a 0.5% concentration. 4.0 ml of this suspension was layered on a discontinuous sucrose gradient consisting of a 3.0 ml layer of 1.2 M sucrose and a 3.0 ml layer of 0.60 M sucrose. Both sucrose solutions contained Tris-HCl buffer, 0.05 M, pH 7.7; magnesium acetate 10^{-3} M; KCl, 0.025 M and mercaptoethanol $5x10^{-3}$ M. The gradient was centrifuged for 4 hrs at 100,000 x g. The pellet at the bottom of the tube was suspended in the same buffer as used for "washed ribosomes". Ultracentrifugal analysis in a Beckman Ultracentrifuge Model E did not reveal particles greater than 78 S in both ribosome preparations.

Wheat germ supernatant: The preparation of the supernatant used was identical to that described by Marcus and Feeley (1964) for "dialyzed supernatant". [^{14}C] Phenylalanyl-sRNA was prepared from yeast-sRNA (Calbiochem.) as described by Lapidot et al. (in press). Poly U, ammonium salt, was bought from Calbiochem. (Lot 63671). Wheat germ was generously supplied by the Hamashbir Hamerkazi Mills, Tel Aviv.

Results

Fig. 1 shows that [^{14}C] phenylalanyl-sRNA binding to wheat germ ribosomes is poly U dependent. Maximum binding is obtained at low poly U concentration and excess of poly U did not inhibit the binding reaction. Some [^{14}C] phenylalanyl-sRNA binding occurs even in the complete absence of poly U. But, as can be seen from Table I, [^{14}C] phenylalanyl-sRNA binding to "DOC treated ribosomes", in the absence of poly U, is greatly increased if wheat germ supernatant and GTP are added to the incubation mixture. This effect seems to be specific, both to GTP and to wheat germ supernatant, in so far that ATP cannot replace GTP, and E. coli supernatant and serum albumin cannot replace wheat germ supernatant. Heating the wheat germ supernatant at 60° C for 5 min. destroys its stimulating activity. This last observation makes it likely that the active agent (or one of the active agents)

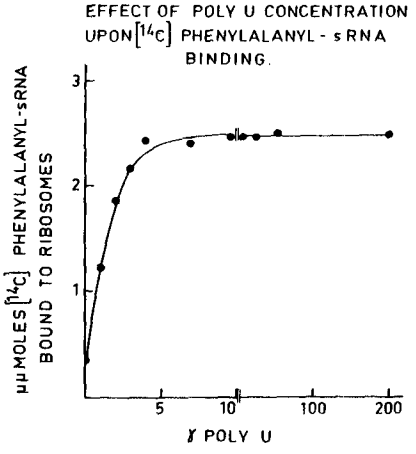

Fig. 1. Binding assays were performed by the method developed by Nirenberg and Leder (1964). Reaction mixtures in a final volume of 0.1 ml contained: Tris acetate, 1 μmole, pH 7.2; KCl, 5 μmoles; magnesium acetate, 2 μmoles; "washed ribosomes" 2.0A_{260} units; [^{14}C] phenylalanyl-sRNA, 11 μμmoles (phenylalanine), 4,000 cpm. Incubation 5 min. at 30° C. The dried Millipore filters were counted in a Packard liquid scintillation counter.

in the supernatant is of protein nature. Under the conditions of the binding assay no radioactive material is incorporated in hot 5% TCA insoluble material (Table I). But still the possibility exists that small oligopeptides are formed which are not precipitated by 5% TCA. In order to exclude this possibility we analyzed in greater detail the exact nature of the ribosomal bound radioactive material. From two binding reaction mixtures without

Table I

Effect of 100,000 x g supernatant and GTP upon [^{14}C] phenylalanyl sRNA binding in the absence of poly U

Additions	cpm in material bound to the ribosomes	cpm in hot 5% TCA insoluble material[a)]
None	150	0[b)]
GTP	140	
Supernatant	170	
GTP + supernatant	550	0[b)]
GTP + supernatant (ribosomes omitted)	0[b)]	
ATP + supernatant	170	
GTP + serum albumin	130	
GTP + E. coli supernatant	90	
GTP + heated supernatant[c)]	140	
Poly U (10 γ)	1,600	0[b)]

Assays were performed (in the absence of poly U) with 1.5 A_{260} "DOC treated ribosomes" as described in Fig. 1. Additions: GTP or ATP, 0.1 μmole; supernatant (wheat germ or E. coli) or serum albumin, 100 γ protein. E. coli supernatant (S-100) was prepared according to Nirenberg, 1963.

a) Binding reactions were terminated by adding 5% TCA and the hot 5% TCA insoluble material counted.
b) Not higher than the average background.
c) Wheat germ supernatant kept at 60° C for 5 min.

poly U, (final volume 0.25 ml, but concentrations of the incubation components were identical to those given in Table I) one containing supernatant and GTP and one without them, we isolated the ribosomes on Millipore filters as usual. The Millipore filters were soaked in a small volume of NaOH 0.25 N, shaken in a Vortex test tube shaker for 1 hr. The NaOH solutions were evaporated to near dryness at room temperature. Their contents were analyzed by paper chromatography. (The solvent used was n-butanol:acetic acid:water 78:5:17 v/v). Only one radioactive peak could be detected, a peak which had an R_f value identical to that of phenylalanine. With "washed ribosomes" supernatant and GTP added separately or simultaneously had no effect on the binding of [^{14}C] phenylalanyl-sRNA in the absence of poly U. The addition of phosphoenolpyruvate (PEP) and PEP kinase (which can regenerate GTP from GDP) to a binding mixture with "washed ribosomes" containing GTP and supernatant, failed to cause any increase in binding.

Discussion

The best criterion for the existence of messenger RNA on ribosomes is the capability of the ribosomes to incorporate amino acids into polypeptide in an appropriate medium. Wheat germ ribosomes do not fulfill this condition but they can bind phenylalanyl-sRNA under certain circumstances without addition of a synthetic polynucleotide. The reason for the binding of [^{14}C] phenylalanyl-sRNA in the absence of poly U, supernatant and GTP is not clear. But it is the additional binding which takes place in the presence of supernatant and GTP which arouses our interest. Arlinghaus et al. (1964) also found an increase in phenylalanyl-sRNA binding to reticulocyte ribosomes, by supernatant and GTP. They conclude that the translocase, the enzyme which moves messenger RNA and

sRNA in relation to ribosomes, moves the sRNA from one binding site to the second sRNA binding site on the ribosomes and as a result of this movement more binding sites become available. As GTP and a heat labile supernatant factor are involved in [^{14}C] phenylalanyl-sRNA binding to wheat germ ribosomes we suggest that at least part of these ribosomes contains messenger RNA and that a sRNA molecule is bound to those ribosomes. In order that [^{14}C] phenylalanyl-sRNA can be bound to those ribosomes, the first sRNA has to be moved to a second ribosomal sRNA binding site. We have still to explain the observation of Marcus and Feeley (1964) namely, the incapability of the ribosomes to incorporate [^{14}C] phenylalanine into a peptide without the addition of a messenger RNA. (This observation was confirmed by us in our laboratory). An attractive explanation may be that the ribosomal bound sRNA is a kind of "nonsense"-sRNA which cannot be charged with an amino acid and therefore cannot participate in the polymerization step.

Supernatant and GTP had no influence upon the [^{14}C] phenylalanyl-sRNA binding by "washed ribosomes". Until now we cannot offer an experimentally based explanation for this observation.

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