AMBIGUITIES OF TRANSLATION OF POLY U IN THE RABBIT RETICULOCYTE SYSTEM

Hildegard Lamfrom* and Marianne Grunberg-Manago
Institut de Biologie Physico-Chimique
13 rue Pierre Curie
Paris, France

Received February 3, 1967

Ambiguities of translation of the genetic code have been observed in cell-free extracts from Escherichia coli and other bacteria, and have been particularly well studied for the UUU codon (Bretscher and Grunberg-Manago, 1962; Matthaei, Jones, Martin and Nirenberg, 1962; Weisblum, Benzer and Holley, 1962; Sager, Weinstein and Ashkenazi, 1963; Friedman and Weinstein, 1964; Protass, Speyer and Lengyel, 1964). Variations of Mg^{++} concentration, sRNA concentration, pH and temperature, as well as the addition of polyamines, ethanol and other agents have been reported to influence the accuracy of translation. In contrast, mammalian cell-free systems derived from rat liver and rabbit reticulocytes have been reported to show high fidelity of translation of poly U under a variety of conditions (Weinstein, Friedman and Ochoa, Jr., 1966). Since, however, these mammalian studies were carried out with crude preparations of ribosomes we have reexamined the coding properties of poly U in the reticulocyte system, using purified ribosomes. We have found that purified reticulocyte ribosomes show a lack of fidelity with poly U similar to that observed in bacterial systems.

EXPERIMENTAL

The preparation of rabbit reticulocytes and cell fractions (1:2 lysate and supernatant S_1) followed the procedure previously described (Lamfrom and Knopf, 1965). High salt treated ribosomes were used in all experiments, except where otherwise stated; they were prepared as follows: the 1:2 lysate was brought to 0.2 M KCl and 0.05 M tris pH 7.8 and incubated at 37°C for 15 min. The lysate was then readjusted to the usual buffer concentrations

* Present address: Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403, U.S.A.

(0.02 M KCl, 0.01 M tris pH 7.8, 0.0015 M $MgCl_2$) and the ribosomes isolated by a 2 hr. centrifugation at 150,000 x g. Unwashed ribosomes were isolated from a 1:6 lysate by a 2 hr. centrifugation at 150,000 x g. Pellets of treated and untreated ribosomes, as well as supernatant S_1 were stored at -90°C

The complete reaction mixture was incubated for 60 min. at 37°C and contained in 0.1 ml: 0.2 mg ribosomes, 0.05 ml supernatant S_1, 30 µmoles ammonium acetate, 1 µmole tris pH 7.8, 0.6 µmole mercaptoethanol, 0.1 µmole ATP, 0.5 µmole phosphoenolpyruvate, 5 µgm pyruvate kinase, 0.75 µmole $MgCl_2$ unless otherwise stated, 4 mµmoles of all amino acids omitting the corresponding radioactive amino acid. Reaction mixtures also contained, as indicated, 0.25 µC of either ^{14}C-labeled phenylalanine (specific activity 98.5), or ^{14}C-labeled leucine (specific activity 94), or ^{14}C-labeled isoleucine (specific activity 96), all uniformly labeled L-isomers obtained from the Radiochemical Center, Amersham. Where indicated 25 µgm poly U was added to the reaction mixtures. Poly U was prepared according to Grunberg-Manago and Michelson (1964). All samples of poly U used in these experiments were tested and found to be pure in an assay which could detect non-uridine containing residues at a level of 0.5%. The poly U samples were analysed after alkaline hydrolysis by paper electrophoresis in 0.5 M ammonium formate (pH 3.5) and by paper chromatography in n-propanol-water-conc. ammonia (55 : 35 : 10).

Samples (0.04 ml) were removed from the incubation mixture at t = 0 and t = 60 min., precipitated with TCA containing 3% casamino acids and heated for 20 min. at 90°C. Samples were plated on millipore filters, washed with additional TCA and counted on a thin end window gas flow counter (25% efficiency).

The incorporation, as given in the tables, represents the difference between the incorporation at 0 and 60 min. Ambiguity is defined as the molar ratio of incorporation of leucine or isoleucine to that of phenylalanine, expressed as percentage.

RESULTS

Table 1 shows that ribosomes prepared from a lysate previously incubated at 0.2 M KCl have a lower endogenous messenger activity than ribosomes derived from untreated lysate. Moreover, such treated ribosomes are more sensitive to poly U induced phenylalanine incorporation. Under optimal conditions (0.0075 M $MgCl_2$, 0.3 M ammonium acetate, 0.01 M tris pH 7.8) poly U directed phenylalanine incorporation proceeds linearly for 60 min. at 37°C

and exceeds background incorporation 40 to 110 fold.

Table 1

Enhancement of poly U directed phenylalanine incorporation with treated ribosomes

	μμmoles phenylalanine / mg ribosomes		
	- poly U	+ poly U	stimulation
Ribosomes : unwashed	305	2260	7.4 fold
Ribosomes : from KCl-treated lysate	24	2520	105 fold

The conditions of incubation and treatment of samples are described in the experimental section. The Mg^{++} concentration during incubation was 0.0075 M.

Under certain conditions, which will be described below, poly U also stimulates the incorporation of substantial amounts of leucine; the incorporation of isoleucine was found to be very low in all instances in this system and is therefore of questionable significance.

Isotopic dilution experiments were carried out for each sample of ^{14}C-phenylalanine, ^{14}C-leucine and ^{14}C-isoleucine in order to rule out that any of the observed ambiguities could be due to the incorporation of radioactive amino acids present as contaminants in the ^{14}C amino acid preparations.

Effect of Mg^{++} concentration

Table 2 shows that the background incorporation (minus poly U) for phenylalanine and leucine is not appreciably changed between 0.0075 and 0.015 M Mg^{++}. However, with increasing Mg^{++} concentration the poly U induced phenylalanine incorporation is depressed, while the leucine and isoleucine incorporations are greatly enhanced. The overall effect of raising the Mg^{++} concentration is to increase the ambiguity of translating poly U.

Preheating the ribosomes for 2 min. at 50°C did not alter the coding ambiguities of poly U at any of these Mg^{++} concentrations. The low fidelity of translation of poly U was also neither a function of the age of ribosomes or supernatant, nor was it dependent on a particular preparation of poly U. The same level of misreading was noted with 4 different samples of poly U. With 9 different preparations of ribosomes stored from 5 days to 17 weeks at -90°C the level of misreading at 0.0075 M Mg^{++} was always very low (2.7 - 10%), while at 0.015 M Mg^{++} poly U coded for leucine in amounts corresponding

to 20 - 56% of the incorporated phenylalanine.

Table 2

Effect of Mg^{++} concentration on poly U ambiguity

Mg^{++}	Poly U	μμmoles AA/mg ribosomes						Ambiguity	
		Phe		Leu		Ileu		Leu	Ileu
M			net		net		net		
0.0075	-	26		71		19			
	+	2630	2604	125	54	15	-	2.1%	-
0.010	-	36		59		19			
	+	2380	2344	234	175	33	14	7.5%	0.6%
0.0125	-	44		67		24			
	+	1580	1536	344	277	39	15	18%	1.0%
0.015	-	21		68		14			
	+	1475	1454	624	556	42	28	39%	1.9%

The conditions of incubation and treatment of samples are described in the experimental section.

Effect of pH

The effect of pH was studied at a Mg^{++} concentration (0.0125 M) at which some degree of misreading occurs at pH 7.8. Table 3 illustrates that even though the phenylalanine and leucine incorporations are greatly depressed by lowering the pH, proportionately more leucine is incorporated at pH 7.0 and 6.5 than at pH 7.8. Thus the leucine ambiguity of poly U is enhanced below pH 7.8 in the reticulocyte cell-free system.

Table 3

Effect of pH on poly U ambiguity

pH	Poly U	μμmoles AA/mg ribosomes						Ambiguity	
		Phe		Leu		Ileu		Leu	Ileu
			net		net		net		
7.8	-	52		101		38			
	+	1390	1338	382	281	34	-	21%	-
7.0	-	24		33		12			
	+	372	348	155	122	32	20	35%	0.6%
6.5	-	16		23		20			
	+	270	254	110	87	13	-	34%	-

The conditions of incubation and treatment of samples are described in the experimental section. The Mg^{++} concentration during incubation was 0.0125 M.

Effect of sRNA and streptomycin

Table 4 shows that in the reticulocyte system the miscoding of poly U for leucine is only slightly stimulated by either E. coli sRNA or streptomycin or by their simultaneous addition.

Table 4

Effect of sRNA and streptomycin on poly U ambiguity

	Net incorporations : μμmoles AA/mg ribosomes			Ambiguity	
	phe	leu	ileu	leu	ileu
complete system	1980	244	-	12%	-
+ sRNA	1900	315	68	17%	3.6%
+ streptomycin	1700	300	26	18%	0.15%
+ sRNA + streptomycin	1570	342	28	22%	1.8%

Conditions of incubation and treatment of samples are given in the experimental section. The Mg^{++} concentration during incubation was 0.0125 M. Where indicated the reaction mixtures also contained 0.05 mg coli sRNA and/or 0.03 mg streptomycin. Net incorporation represents incorporation during 60 min. in the presence of poly U minus incorporation during 60 min. in the absence of poly U.

CONCLUSION

Purified reticulocyte ribosomes, unlike crude reticulocyte ribosomes, show ambiguity in the translation of poly U under a variety of conditions. As in E. coli (Davies, Gilbert and Gorini, 1964; Szer and Ochoa, 1964) miscoding for leucine increases as the Mg^{++} concentration is raised. Whereas in E. coli lowering the pH below pH 8 minimizes the leucine ambiguity (Grunberg-Manago and Dondon, 1965), in reticulocytes the level of misreading in fact increases. Streptomycin has been shown to induce substantial misreading in E. coli, both in vitro (Davies, Gilbert and Gorini, 1964) and in vivo (Gorini and Kataji, 1964); with purified reticulocyte ribosomes streptomycin has only a slight effect on the fidelity of translating poly U.

Acknowledgement

It is a pleasure to thank Dr. Calvin S. McLaughlin for many helpful discussions and for the preparation and analysis of the poly U samples. We are grateful to Madame Lili Dondon for her capable assistance, and the Philippe Foundation for the support of one of us (HL). This work was aided by grants from the Centre Nationale de la Recherche Scientifique, National Institutes of Health (CA 04850), D.G.R.S.T. (no. 61 FR-087), L.N.F.C.C. (Comité de la Seine), F.R.M.F. and C.E.A.

REFERENCES

Bretscher, M.S. & Grunberg-Manago, M. (1962). Nature 195, 283.
Davies, J., Gilbert, W. & Gorini, L. (1964). Proc. Nat. Acad. Sci., Wash. 51, 883.
Friedman, S.M. & Weinstein, I.B. (1964). Proc. Nat. Acad. Sci., Wash. 52, 988.
Gorini, L. & Kataji, E. (1964). Proc. Nat. Acad. Sci., Wash. 51, 487.
Grunberg-Manago, M. & Michelson, A.M. (1964). Biochim. Biophys. Acta 86, 431.
Grunberg-Manago, M. & Dondon, J. (1965). Biochem. Biophys. Res. Comm. 18, 517.
Lamfrom, H. & Knopf, P.M. (1965). J. Mol. Biol. 11, 589.
Matthaei, J.H., Jones, O.W., Martin, R.G. & Nirenberg, M.W. (1962). Proc. Nat. Acad. Sci., Wash. 48, 666.
Protass, J.J., Speyer, J.F. & Lengyel, P. (1964). Science 143, 1174.
Sager, R., Weinstein, I.B. & Ashkenazi, Y. (1963). Science 140, 304.
Szer, W. & Ochoa, S. (1964). J. Mol. Biol. 8, 823.
Weinstein, I.B., Friedman, S.M. & Ochoa, Jr., M. (1966). Cold Spring Harbor Symp. Quant. Biol. 31,
Weisblum, B., Benzer, S. & Holley, R.W. (1962). Proc. Nat. Acad. Sci., Wash. 48, 1449.