TRANSLATION OF ONCOGENIC VIRAL RNA AND EUKARYOTIC MESSENGER RNA IN THE *E. COLI* CELL-FREE SYSTEM

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Received 26 October 1972

1. Introduction

Recently several studies have been published which describe the faithful translation of animal messenger and viral-RNA in eukaryotic cross systems [1–12].

Although stimulation of amino acid incorporation by viral RNA or eukaryotic messengers in bacterial cell-free systems has been reported, reliable translation has only been described incidentally [13–19].

We compared the translation of eukaryotic- and oncogenic viral-RNA's in the cell-free system of *E. coli* with that of the reticulocyte lysate. Our results provide evidence that in the bacterial system the viral RNA's are translated into several distinct polypeptides, whereas no indication for the translation of eukaryotic messengers could be obtained. Moreover, addition of the viral RNA's to the mammalian systems described, appeared not to result in translation.

2. Materials and methods

Rauscher leukemia virus (RLV) was isolated from the plasma of leukemic mice as described [20]. Purified mouse mammary tumor virus (MTV) from mammary tumors of (C3H/HeA × 020)F_1 mice was a generous gift of Dr. P. Hageman. After incubation of the virus with pronase in the presence of SDS the viral RNA's were extracted with chloroform–phenol [21, 22]. The 60 S component of RLV-RNA was isolated by centrifugation in isokinetic glycerol gradients (15.0–30.2%, w/v). Denaturation of RNA was performed in 3 mM KCl for 3 min at 65° [23]. After heating and rapid cooling the RNA preparations were immediately tested for their messenger activity. Globin 9 S and α-crystallin 14 S mRNA were a gift of Dr. A. Berns. Monolayers of mouse spleen- and thymus-cells (JLS-V5) were used for the propagation of radioactive-labeled RLV. The JLS-V5 cell line was a generous gift of Dr. W. Schäfer. The preparation of phage M12 RNA and of the cell-free system of *E. coli* has previously been described [24]. The incubation mixture contained per ml: 50 nmoles of each of the added unlabeled amino acids, 10 μmoles magnesium acetate, 50 μmoles Tris-HCl, pH 7.8, 70 μmoles NH_4Cl, 8 μmoles 2-mercaptoethanol, 0.25 mg *E. coli* tRNA, 5 μmoles ATP, 0.3 μmoles GTP, 5 μmoles PEP, 8 μg pyruvate kinase, and 0.24 ml of pre-incubated S-30. The amounts of RNA and radioactive label added to the system are given in the legend of table 1. After 30 min of incubation at 37°, 10 μl aliquots from the incubation mixture were removed for the estimation of total protein synthesis by precipitation with TCA. The remainder was treated with pancreatic RNAase and analyzed by electrophoresis on 12.5% SDS–polyacrylamide gels [25].

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3. Results and discussion

3.1. Effect of viral RNA and eukaryotic mRNA on amino acid incorporation

Addition of either RLV-RNA or MTV-RNA to the cell-free system of *E. coli* resulted in a significant stimulation of the amino acid incorporation (table 1). When equal amounts of RLV- and MTV-RNA were tested, the stimulatory effect of the latter was always more pronounced. An even higher stimulation could be obtained if instead of total RLV-RNA the purified 60 S RNA component was added to the system. This is probably due to an inhibitory effect of the low molecular weight viral RNA (4–5 S), present in the virion, on the translation of 60 S RLV-RNA. Evidence for this assumption was derived from the fact that this low molecular weight RNA fraction also inhibited the translation of phage M12 RNA (cf. [26]). Denaturation of RLV-RNA consistently resulted in an additional stimulation of the amino acid incorporation, while denaturation of MTV-RNA was much less effective. For comparison the effect of several distinct eukaryotic mRNA preparations on the amino acid incorporation was studied. Addition of globin 9 S mRNA resulted in a stimulation of the amino acid incorporation which could be enhanced by denaturation. The effect of lens 14 S mRNA, coding for the A_2-chain of α-crystallin, was almost negligible.

No significant stimulation could be observed after addition of either 18 S or 28 S ribosomal RNA to the cell-free system.

Table 1
Stimulation of amino acid incorporation by viral RNA and eukaryotic RNA preparations in the cell-free system of *E. coli*.

Exp. I		Exp. II	
RNA added	[^{14}C]amino acid (cpm incorporated)	RNA added	[^{35}S]methionine (cpm incorporated)
None	558	None	5820
Phage M12	12,693	Phage M12	138,866
RLV	1058 (2010)*	RLV	8784 (14,398)
RLV 60 S	1928 (2749)	Globin 9 S	8960 (10,102)
MTV	2033 (2386)	α-crystallin 14 S	6506 (7290)
Ribosomal 18 S	604		
Ribosomal 28 S	613		

To each 60 μl incubation mixture, in experiment I, 4 μg RNA and 5 μCi [^{14}C]amino acid mixture (54 Ci/mg-atom of carbon) was added. In experiment II, to each 60 μl incubation mixture 5 μg RNA and 8 μCi [^{35}S]methionine (32 Ci/mmole) was added. After 30 min of incubation at 37° the reaction was terminated by the addition of 60 μl of 0.025 M EDTA and TCA precipitable radioactivity was determined in 10 μl aliquots of the incubation mixture.

* Values obtained after denaturation of the RNA are given in parentheses.

No significant stimulation could be observed after addition of either 18 S or 28 S ribosomal RNA to the cell-free system.

3.2. Analysis of the in vitro products by SDS polyacrylamide gel electrophoresis

From the results presented in fig. 1 A it can be concluded that addition of native RLV-RNA to the cell-free system gives rise to the synthesis of a number of distinct polypeptides. Co-electrophoresis of these polypeptides with the proteins derived from the virion indicated that at least those migrating in the low molecular weight range (slice no. 52–71) coincide with the native viral proteins (fig. 1 B). Furthermore additional polypeptides are synthesized which migrate in the 30,000 dalton region (slice no. 32–40). The effect of denaturation of RLV-RNA on the translation is striking. This resulted in the appearance of a high molecular weight polypeptide (slice no. 24–27) which is only present in trace amounts in the control experiment. In order to obtain more accurate data about the size and number of the synthesized polypeptides, autoradiographic analysis on dried gels was performed. In fig. 2 A, the autoradiogram of the polypeptides synthesized under the direction of denatured RLV-RNA is shown (b). For comparison, the gel pattern of labeled viral proteins is also depicted (c). It may be concluded that denatured RLV-RNA mainly gives rise to the synthesis of two polypeptides, RE_0 and RE_2, with molecular weights of approx. 45,000 and 15,000 daltons, respectively. The electrophoretic mobility of the polypeptide RE_2 is identical to that of the native protein R_2. It might be possible that the polypeptide RE_0 is identical to one of the minor viral proteins present in the 45,000 daltons region. No clear indication could be obtained whether a polypeptide of the same size as the viral protein R_1 (gs 1) was synthesized. Furthermore several faint bands are visible of which at least some seem to have no corresponding polypeptide syn-

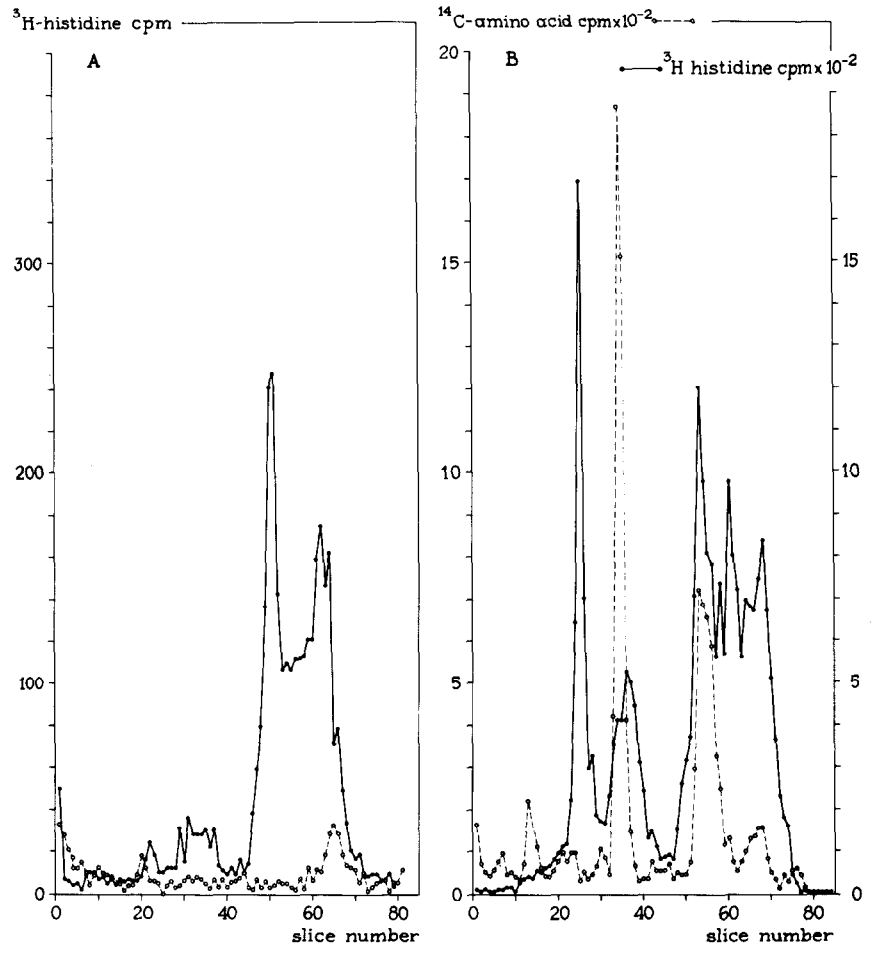

Fig. 1. SDS gel analysis of the polypeptides synthesized under direction of RLV-RNA. A) Polypeptides synthesized in the absence (o - - o - - o) and presence (•—•—•) of RLV-RNA. B) Comparison by co-electrophoresis of the polypeptides synthesized *in vitro* under the direction of denatured RLV-RNA (•—•—•) with the viral proteins labeled with [^{14}C] amino acids (o - - o - - o). Incubation in the presence of [^{3}H]histidine. After electrophoresis the gels were sliced in 1 mm sections. To measure the radioactive labels separately the dried gel slices were processed with the aid of the Tri-Carb Sample Oxidizer (Packard Model 305).

thesized in the endogenous system.

As the RNA present in the RLV virion consists of several RNA species, experiments were performed to investigate whether there exists a difference between the polypeptides synthesized under the direction of total RNA and the purified 60 S RNA component. Analysis of the *in vitro* products showed that both RNA preparations directed the synthesis of the same polypeptides (fig. 2 B, a–c). From these results it may be concluded that the RNA component sedimenting in the 60 S region contains all the genetic information necessary to code for the polypeptides synthesized in the *in vitro* system.

Evidence for the reliability of the *in vitro* translation of RLV-RNA can be derived from the observation that under direction of RNA isolated from the non-related mouse mammary tumor virus (MTV) quite different polypeptides are synthesized. MTV-RNA directs the synthesis of at least one polypeptide ME_1 which coincides with one of the native MTV proteins (P_6), (fig. 3 A, a–c). This polypeptide migrates slightly faster than the RLV-RNA directed polypeptide RE_2.

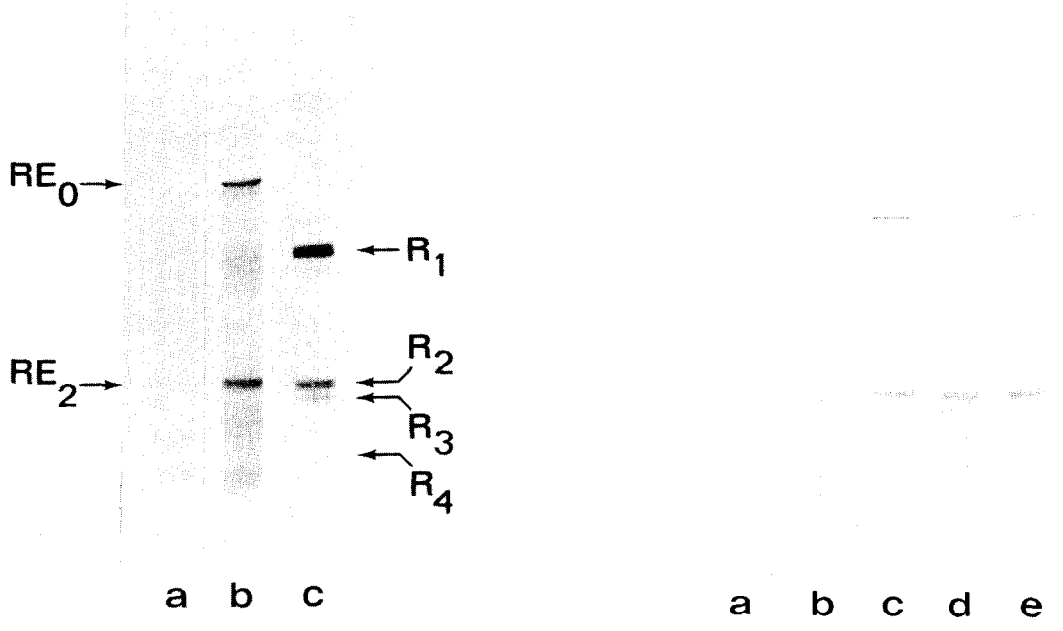

Fig. 2. Autoradiograms of the polypeptides synthesized under the direction of RLV-RNA and separated by SDS gel electrophoresis. A) Products of the endogenous *E. coli* system (a); with denatured RLV-RNA (b); RLV proteins labeled with [^{14}C] amino acids (c). B) Products of the endogenous *E. coli* system (a); with RLV-RNA (b); with denatured RLV-RNA (c); with the 60 S RLV-RNA component (d); with the denatured 60 S RLV-RNA component (e). Incubation in the presence of [^{14}C] amino acids.

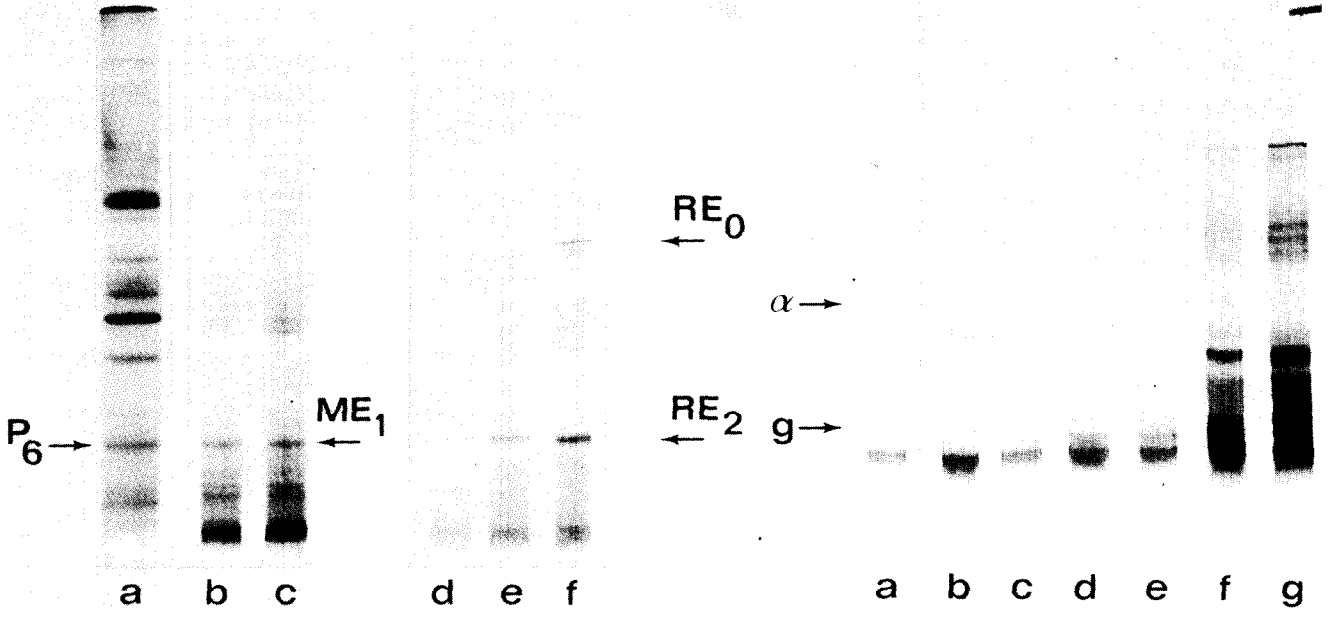

Fig. 3. A) Autoradiograms of the polypeptides synthesized under the direction of MTV- or RLV-RNA and separated by SDS gel electrophoresis. Native MTV proteins stained with Coomassie blue (a); *in vitro* system supplemented with MTV-RNA (b); with denatured MTV-RNA (c); without RNA (d); with RLV-RNA (e); with denatured RLV-RNA (f). Incubation in the presence of [^{14}C] amino acids. B) Autoradiograms of the polypeptides synthesized under the direction of globin 9 S and α-crystallin 14 S mRNA. Products of the endogenous *E. coli* system (a); with globin 9 S mRNA (b); with denatured globin 9 S mRNA (c); with α-crystallin 14 S mRNA (d); with denatured α- crystallin 14 S mRNA (e); with native RLV-RNA (f); with denatured RLV-RNA (g). α stands for α-crystallin, g stands for globin. Incubation in the presence of [^{35}S]methionine.

Denaturation of MTV-RNA does not result in the synthesis of an additional polypeptide (c) as has been observed in the case of RLV-RNA (f). There is a striking discrepancy between the distribution of radioactivity of polypeptides synthesized under the direction of MTV-RNA as compared with those synthesized under the direction of RLV-RNA (compare b, c with e and f).

Furthermore, in case of MTV-RNA a pronounced synthesis of polypeptides which migrated with the buffer-front could be observed (b, c). The reason for this may be that, although there is a high rate of initiation, the completion of polypeptides is inefficient.

In order to examine whether eukaryotic mRNA preparations are faithfully translated we studied the effect of addition of α-crystallin 14 S and globin 9 S mRNA to the *E. coli* cell-free system. From fig. 3 B it may be concluded that both mRNA fractions do not give rise to the synthesis of their encoded proteins (b–e). Only low molecular weight polypeptides seem to be synthesized. As suggested above this may be due to the fact that proteins are initiated but, for reasons still unknown, not completed. In this connection it should be mentioned that under conditions in which globin- and α-crystallin-mRNA are translated very efficiently in a reticulocyte cell-free system and in oocytes, until now in both systems no evidence could be obtained for the translation of RLV-RNA (experiments in collaboration with Dr. J.B. Gurdon, Unpublished). In the reticulocyte system only a strong inhibition of globin synthesis, up to 85%, could be observed. Although a 0.5 M KCl-wash derived from polysomes of leukemic spleens stimulated the endogenous globin and α-crystallin synthesis, no indication could be obtained whether this fraction has a positive effect on the translation of RLV-RNA as well.

From our results the conclusion seems to be justified that in the cell-free system of *E. coli* under the direction of the different viral RNA preparations tested at least some virus-specific polypeptides are synthesized. No specific polypeptides, however, are synthesized when this cell-free system is programmed with eukaryotic mRNA's.

Acknowledgements

We thank Miss A. Versteegen and Miss R. Lanters for excellent technical assistance. A.L.J. Gielkens is a fellow of the Koningin Wilhelmina Fonds (Royal Cancer Foundation).

References

[1] R.E. Lockard and J.B. Lingrel, Biochem. Biophys. Res. Commun. 16 (1969) 522.
[2] S.M. Heywood, Cold Spring Harbor Symp. Quant. Biol. 34 (1969) 799.
[3] J. Stavnezer and R.C.C. Huang, Nature New Biol. 230 (1971) 172.
[4] R.E. Rhoads, G.S. McKnight and R.T. Schimke, J. Biol. Chem. 246 (1971) 7407.
[5] A.J.M. Berns, G. Strous and H. Bloemendal, Nature New Biol.236 (1972) 7.
[6] D. Gallwitz and M. Breindl, Biochem. Biophys. Res. Commun. 47 (1972) 1106.
[7] G.G. Brownlee, T.M. Harrison, M.B. Mathews and C. Millstein, FEBS Letters 23 (1972) 244.
[8] R. Williamson, R. Clayton and D.E.S. Truman, Biochem. Biophys. Res. Commun. 46 (1972) 1936.
[9] M.B. Mathews, Nature 228 (1970) 661.
[10] I.M. Kerr and E.M. Martin, J. Virol. 7 (1971) 438.
[11] K.L. Eggen and A.J. Shatkin, J. Virol. 9 (1972) 636.
[12] M.J. McDowell, W.K. Joklik, L. Villa-Komaroff and H.F. Lodish, Proc. Natl. Acad. Sci. U.S. 69 (1972) 2649.
[13] D.G. Laycock and J.A. Hunt, Nature 221 (1969) 1118.
[14] D.M. Rekosh, H.F. Lodish and D. Baltimore, J. Mol. Biol. 54 (1970) 327.
[15] L.V. Crawford and R.F. Gesteland, J. Mol. Biol., in press.
[16] R.N. Bryan, D.H. Gelfand and M. Hayashi, Nature 224 (1969) 1019.
[17] I.C. van Ravenswaay-Claassen, A.B.I. van Leeuwen, G.A.H. Duijts and L. Bosch, J. Mol. Biol.23 (1967) 535.
[18] J. Říman, M. Trávníček and L. Vepřek, Biochim. Biophys. Acta 138 (1967) 204.
[19] W. Siegert, R.N.H. Konings, H. Bauer and P.H. Hofschneider, Proc. Natl. Acad. Sci. U.S. 69 (1972) 888.
[20] P.H. Duesberg and W.S. Robinson, Proc. Natl. Acad. Sci. U.S. 55 (1966) 219.
[21] R.P. Perry, J. La Torre, D.E. Kelly and J.R. Greenberg, Biochim. Biophys. Acta 262 (1972) 220.
[22] J. Schlom and S. Spiegelman, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1613.
[23] J.P. Bader and T.L. Steck, J. Virol. 4 (1969) 454.
[24] R. Ward, R.N.H. Konings and P.H. Hofschneider, European J. Biochem. 17 (1970) 106.
[25] U.K. Laemmli, Nature 227 (1970) 680.
[26] K.C. Olson, A. O'C. Deeney and G.S. Beaudreau, Biochem. Biophys. Acta 161 (1968) 532.