TRANSLATION OF GLOBIN mRNA AMONG EUKARYOTES

C. VAQUERO, L. REIBEL, J. DELAUNAY and G. SCHAPIRA
Institut de Pathologie Moléculaire*
24, rue du Faubourg Saint-Jacques
75014 PARIS

Received July 20, 1973

SUMMARY : Rabbit globin mRNA can be translated onto ribosomes from trout liver and kidney bean root tips. The demonstration is performed by incorporating labelled tyrosine into the six tyrosine containing peptides specific for rabbit globin. These results strongly support the view that there is no translational barrier among eukaryotes.

It has been previously demonstrated that a mRNA from one species can be translated onto ribosomes from another species, but the question as to whether any ribosome can translate any messenger RNA is still debated.

A mRNA of a variant of rabbit globin was translated onto ribosomes from rabbit having another globin variant and from guinea-pig (1, 2). mRNAs of mouse hemoglobin (3), of calf lens crystallin (4), of ovalbumin (5) and other mammalian proteins were translated in different cell-free systems. Duck mRNA could be translated onto mouse liver ribosomes (6). LANE et al. (7) succeeded the translation of globin mRNA in frog oocytes, i.e. in living cells. Thus, no barrier appears to exist within eukaryotes investigated so far.

However, ribosomal proteins of almost all the eukaryotic species which have been crossed until now display identical fingerprints when they are electrophoresed two-dimensionally on polyacrylamide gel (8). Only ribosomal proteins from frogs start to deviate slightly. It seemed interesting to look for the translation of purified rabbit globin mRNA onto ribosomes of phylogenetically more distant species from the rabbit that those already investigated, and having a set of ribosomal proteins with a more different pattern.

In the present work, we used ribosomes from trout liver and kidney bean root tips. Fish and papilionaceous plant ribosomes

*Université de Paris, Groupe U 15 de l'Institut National de la Santé et de la Recherche Médicale , Laboratoire Associé au Centre National de la Recherche Scientifique.

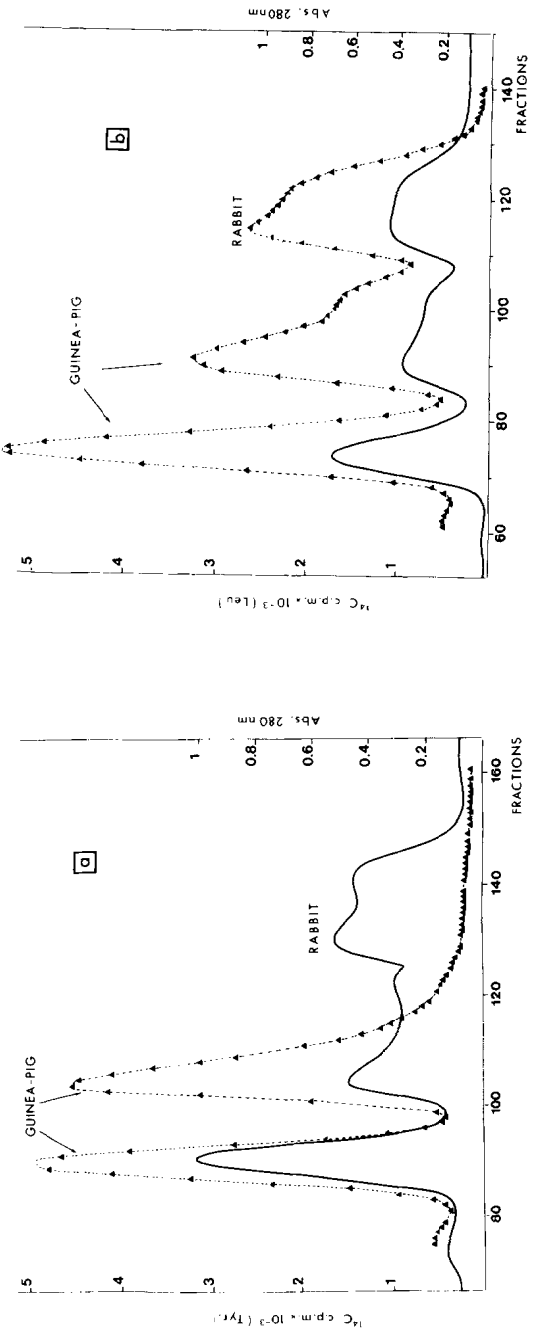

Figure 1 : Trout ribosomes were incubated with "pH 5 enzyme" and 0.5 M KCl ribosome wash from guinea-pig reticulocytes. Chains were separated by chromatography and fractions counted in a liquid scintillation counter as described in "Material and Methods".

-In experiment a, no rabbit hemoglobin mRNA was added.
-In experiment b, rabbit hemoglobin mRNA was added.

The continuous line (———) refers to optical density at 280 nm and the closed triangles (▲—▲) to cts/min.

share in common with rabbit ribosomes 90 p.100 and 25-30 p.100 respectively of their proteins in terms of electrophoretic mobility.

MATERIAL AND METHODS

The cell-free system contained trout liver or kidney bean root tips polysomes prepared essentially according to FALVEY and STAEHELIN (9), 0.5 M KCl wash from guinea-pig reticulocyte polysomes (10), pH 5 enzyme from guinea-pig reticulocytes (9), and purified mRNA prepared from rabbit reticulocytes (11) with some modifications which have been reported previously (2). The incubation medium used has been described elsewhere (2) and either L-(^{14}C) leucine (S.A. 150 mC/mM) for the characterization of globins or L-(^{3}H) tyrosine (S.A. 21 C/mM) for the characterization of peptides were added. The incubations were carried out for 2 hrs at 37° C, in the absence or in the presence of rabbit globin mRNA (10 µg/ml of the incubation medium) and both guinea-pig and rabbit hemoglobins were added as carriers.

Globins were separated on a CM52 column (12). The guinea-pig α and β chains were well-resolved from the rabbit chains : the latter were eluted together. The fractions were measured for radioactivity in a Nuclear Chicago Mark I scintillation counter. The tyrosine containing peptides were analyzed according to SCHAPIRA et al. (13). The incubation was carried out with L-(^{3}H) tyrosine for 2 hrs ; hemoglobin, labelled with L-(^{14}C) tyrosine in whole cells, was added as carrier.

RESULTS

Figures 1 and 2 show the CM52 elution profile of guinea-pig and rabbit globin chains.

In the absence of rabbit globin mRNA, either with trout or kidney bean polysomes, the incubation results in the synthesis of radioactive material which elutes with guinea-pig α and β chains. This is due to the free mRNA present in guinea-pig pH 5 enzyme (13). The presence of rabbit mRNA results in the appearance of a peak of radioactive rabbit globin chains. Further evidence that rabbit globin has been synthesized is brought by the study of the specific tyrosine containing peptides.

Figure 3 (a and b) shows the high voltage electrophoretic pattern of the six tyrosine containing peptides specific for rabbit globin. The precise coincidence between the ^{14}C rabbit globin

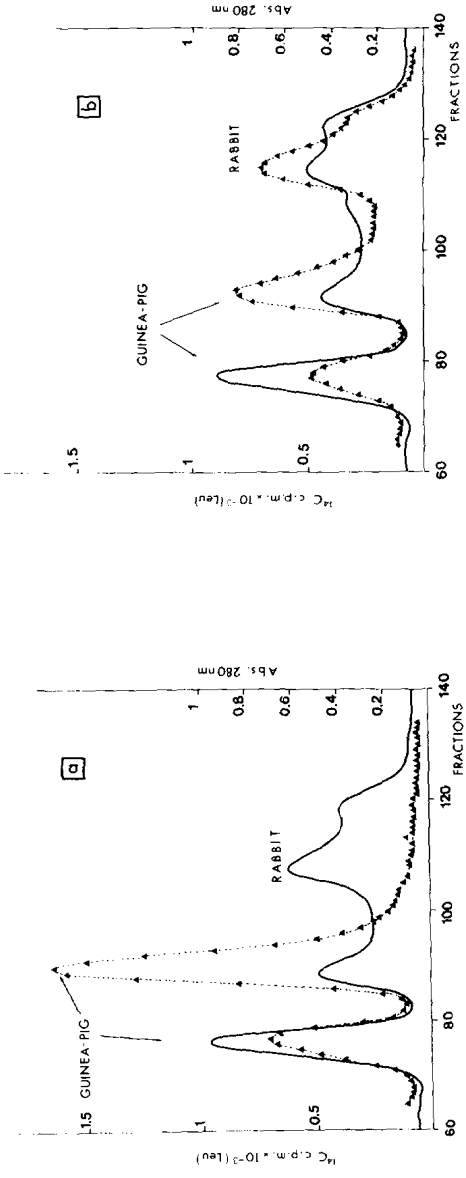

Figure 2 : Kidney bean polysomes were incubated with "pH 5 enzyme" and 0.5 M KCl wash from guinea-pig reticulocyte ribosomes. Details are given in the legend of Figure 1.

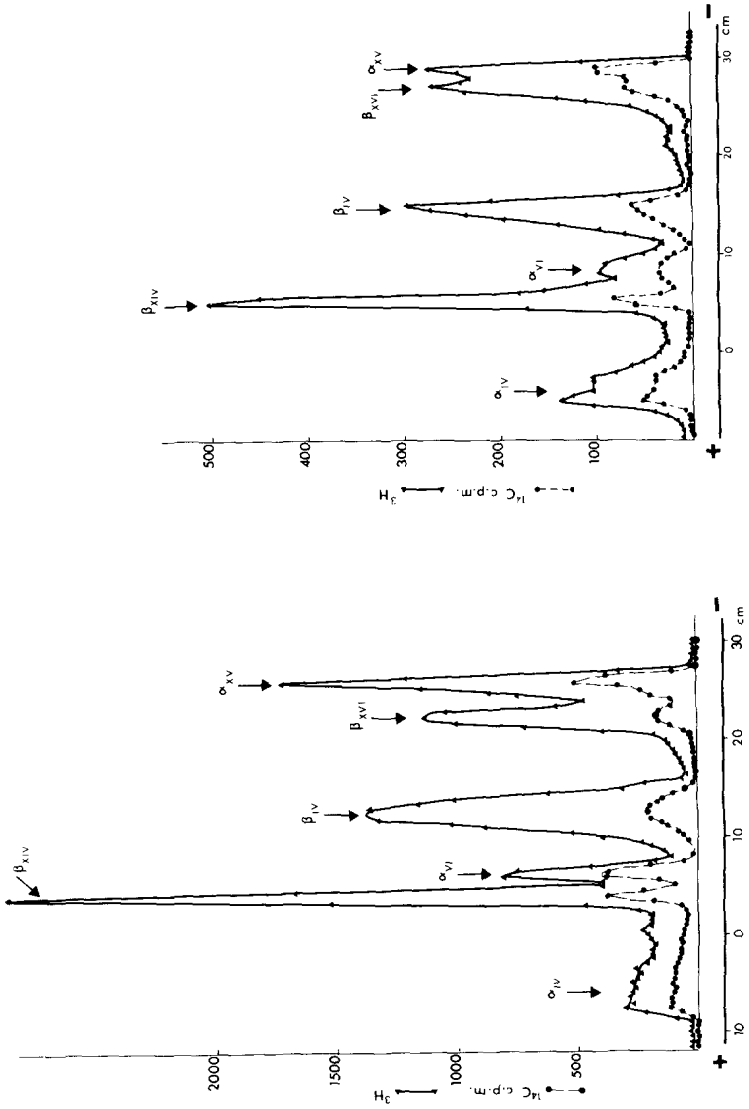

Figure 3 : Pattern of high voltage electrophoresis of rabbit globin tyrosine containing peptides synthesized in a cell-free system with either trout liver or kidney bean root tips polysomes and rabbit mRNA. These incubations have been done in presence of L-(^{3}H) Tyr and L-(^{14}C) Tyr rabbit hemoglobin, added as carrier. The ^{3}H product comigrating with rabbit hemoglobin on starch-block electrophoresis was digested with trypsin.

a) Incubation with trout polysomes,
b) Incubation with kidney bean polysomes.

(●— —●) refers to ^{14}C cts/min. and (▲— —▲) to ^{3}H cts/min.

carrier and ^{3}H cell-free product indicates clearly that rabbit hemoglobin has been synthesized.

DISCUSSION

These data demonstrate conclusively that globin mRNA is translated onto trout and kidney bean polysomes : we have employed two methods to identify as globin the synthesized product : chain chromatography on carboxymethylcellulose and high voltage electrophoresis of specific peptides.

The fact that it is possible to translate a rabbit mRNA onto ribosomes of eukaryotic species as remote from rabbit as trout and kidney bean, with different ribosomal proteins, suggests that no barrier exists within eukaryotes with respect to their translational machinery. But a question still remains to be answered : is there really a barrier between eukaryotes and prokaryotes ? In 1969, LAYCOCK and HUNT (14) claimed that there was none because they succeeded in translating rabbit hemoglobin in an E.Coli cell-free system. But many authors could not reproduce this experiment (15, 16). In this laboratory, we were unable to achieve the translation of rabbit hemoglobin mRNA in plasmolyzed E.Coli cells (17, results to be published).

As a conclusion, it appears that no barrier exists within eukaryotes between as distant species from rabbit than trout, and even more distant, as kidney bean. On the other hand, the possibility of a gap between eukaryotes and prokaryotes remains*.

ACKNOWLEDGEMENTS

This work was supported by grants from the "Institut National de la Santé et de la Recherche Médicale", the "Centre National de la Recherche Scientifique", the "Délégation Générale à la Recherche Scientifique et Technique" and the "Fondation pour la Recherche Médicale Française".

*While this work was being completed, EFROM and MARCUS (FEBS Lett. 33, 23-27 (1973) reported a translation of rabbit hemoglobin mRNA into a wheat embryo cell-free system.

REFERENCES

1. SCHAPIRA, G., DREYFUS, J.C. and MALEKNIA, N., Biochem. Biophys. Res. Commun. 32, 558-561 (1968)
2. SCHAPIRA, G., VAQUERO, C. and REIBEL, L., Biochimie 55, 183-187 (1973)
3. LOCKARD, R.E. and LINGREL, J.B., Biochem. Biophys. Res. Commun. 37, 204-212 (1969)
4. MATHEWS, M.B., OSBORN, M., BERNS, A.J.M. and BLOEMENDAL, H.,

Nature New Biol. 61, 5-7 (1972)
5. RHOADS, R.E., Mc KNIGHT, G.S. and SCHIMKE, R.T., J. Biol. Chem. 246, 7407-7410 (1971)
6. SCHREIER, M.H., STAEHELIN, T., STEWART, A., GANDER, E. and SCHERRER, K., Eur. J. Biochem. 34, 213-218 (1973)
7. LANE, C.D., MARBAIX, G. and GURDON, J.B., J. Mol. Biol. 61, 73-91 (1971)
8. DELAUNAY, J., CREUSOT, F. and SCHAPIRA, G., submitted to Eur. J. Biochem.
9. FALVEY, A.K. and STAEHELIN, T., J. Mol. Biol. 53, 1-19 (1970)
10. SCHAPIRA, G., REIBEL, L. and CUAULT, F., Biochimie 54, 465-469 (1972)
11. LEBLEU, B., MARBAIX, G., HUEZ, G., TEMMERMAN, J., BURNY, A. and CHANTRENNE, H., Eur. J. Biochem. 19, 264-269 (1971)
12. LINGREL, J.B., LOCKARD, R.E., JONES, R.F., BURR, H.E. and HOLDER, J.W., Ser. Haematol. 4, 37-69 (1971)
13. SCHAPIRA, G., PADIEU, P., MALEKNIA, N., KRUH, J. and DREYFUS, J.C., J. Mol. Biol. 20, 427-446 (1966)
14. LAYCOCK, D.G. and HUNT, J.A., Nature 221, 1118-1122 (1969)
15. NOLL, M., NOLL, H. and LINGREL, J.B., Proc. Nat. Acad. Sci. US 69, 1843-1847 (1972)
16. GIELKENS, A.L.J., SALDEN, M.H.L. and BLOEMENDAL, H., FEBS Lett. 28, 348-352 (1972)
17. BEN HAMIDA, F. and GROS, F., Biochimie 53, 71-80 (1971)