INFIDELITY OF TRANSLATION OF ENCEPHALOMYOCARDITIS VIRAL RNA WITH tRNA FROM HUMAN MALIGNANT TROPHOBLASTIC CELLS

Opendra K. Sharma* and Yoshiyuki Kuchino**

Department of Microbiology
University of Colorado Medical Center
Denver, Colorado 80262

Received July 29,1977

SUMMARY: We have investigated tRNA from the human malignant trophoblastic cells (BeWo cell) and human chorionic tissue for the translation of specific mRNAs, in a tRNA-dependent protein synthesizing system from Ehrlich ascites cells. BeWo cell tRNA and chorionic tRNA supported oviduct mRNA or encephalomyocarditis (EMC) viral RNA directed amino acid incorporation into polypeptides equally effectively. Polypeptides synthesized with oviduct mRNA and tRNA from both sources were identical upon sodium dodecylsulfate polyacrylamide gel electrophoresis. But the EMC RNA directed polypeptides synthesized with BeWo cell tRNA were different from those synthesized with chorionic tRNA. A polypeptide (molecular weight 58,000) was apparently not synthesized and the synthesis of a faster moving component (molecular weight, 14,000) was enhanced when BeWo cell tRNA was used. These results imply a functional difference in tRNA from human malignant cells compared to their normal counterpart.

Alterations in the populations of tRNAs have been observed in every malignant neoplasm tested (See Ref. 1). But the functional attributes of the altered tRNAs in the process of neoplastic transformation have remained obscure. Bridges and Jones (2) have investigated cell-free protein synthesis in vitro with tRNA prepared from mouse plasmacytomas MOPC-41 and RPC-20. These plasmacytomas produce different immunoglobulin chains. In the protein synthesizing system from RPC-20 plasmacytoma, quantitative differences in serine-containing peptides were observed with tRNA from two plasmacytomas. Novikoff hepatoma tRNA inhibited ovalbumin synthesis when added to fragmented immature chick oviduct explants in culture (3). Whereas, tRNA from rat liver did not affect ovalbumin synthesis.

---

*Present mailing address: AMC Cancer Research Center and Hospital, 6401 West Colfax Avenue, Lakewood, Colorado 80214
**Present address: Biology Division, National Cancer Center Research Institute, Tsukiji 5-1-1 Chuo-Ku, Tokyo, Japan.

ISSN 0006-291X

We recently reported changes in tRNAs from human malignant trophoblastic cells (BeWo cell) compared to their normal counterpart, human chorionic tissue (1). The BeWo cell retains the property of normal trophoblasts, in secreting chorionic gonadotropin (4). We have examined tRNAs from the BeWo cell and human chorionic tissue for the translation of specific mRNAs, in an in vitro tRNA-dependent protein synthesizing system from Ehrlich ascites cells (5). Such a system is essential for studying the efficiency and fidelity of translation of a specific mRNA, otherwise the residual indigenous tRNA can mask any specific effect (6,7). We describe here that EMC RNA is not translated with fidelity with BeWo cell tRNA compared to tRNA from chorionic tissue.

MATERIALS AND METHODS

Isolation of tRNA. tRNA was isolated from human malignant trophoblastic cells and normal human chorionic tissue as described earlier (1). The tRNA preparations were deacylated and purified by gel filtration on Sephadex G-100 (3).

Assay of Protein Synthesis in a tRNA-Dependent Protein Synthesizing System From Ehrlich Ascites Cells. Ribosomes and ammoniom sulfate fractions were freed from tRNA by passing through DEAE cellulose immediately before addition to the protein synthesizing system (5). All incubations were carried out in duplicate for 90 min, a time period for which amino acid incorporation was linear. 0.2 ml of 0.2 M KOH was added and the tubes were further incubated for 20 min when 1 ml of 5% trichloroacetic acid was added. The samples were filtered through Whatman GF/C filters, washed 4-5 times with 5% trichloroacetic acid and once with ethanol. The filters were dried and counted in a liquid scintillation counter: EMC viral RNA, oviduct mRNA and Ehrlich ascites cell tRNA were isolated as described earlier (5).

RESULTS AND DISCUSSION

In the reconstituted tRNA-dependent protein synthesizing system from Ehrlich ascites cells, addition of tRNA from BeWo cell and chorionic tissue supported oviduct mRNA or EMC RNA directed amino acid incorporation into polypeptides equally effectively (data not shown). Protein synthesis plateaued with 5 µg of tRNA in the assay mixture. The products of cell-free protein synthesis were analyzed by SDS-polyacrylamide slab gel electro-

---

Abbreviation: SDS, Sodium dodecylsulfate.

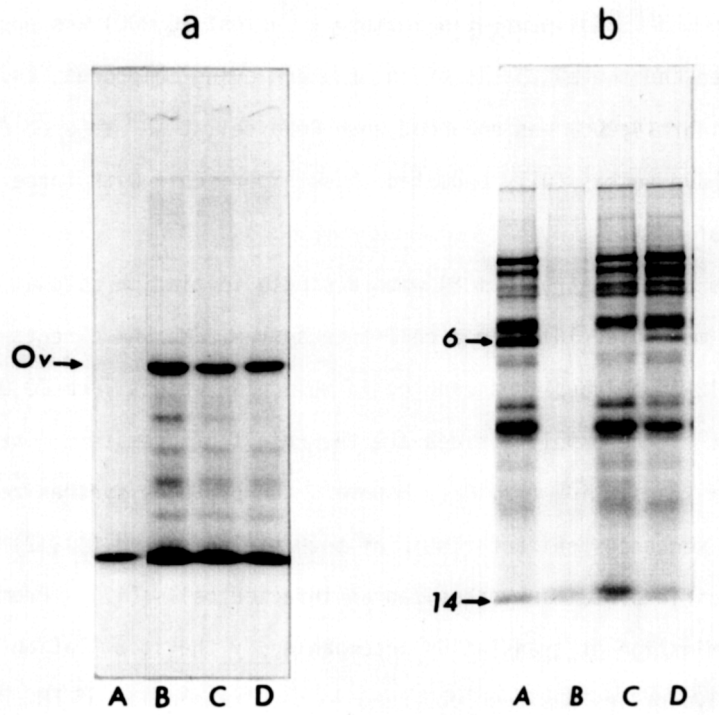

Fig. 1 Autoradiography of ^{14}C-labeled products of cell-free translation. Aliquots (25 µl) of protein synthesis reaction mixtures were precipitated with 5% trichloroacetic acid, the precipitates were washed with trichloroacetic acid, solubilized, electrophoresed on SDS-10% polyacrylamide slab gels (8,9) and subsequently, stained with Coomassie brilliant blue for 1 hour and destained with 5% methanol - 7% acetic acid. The slab was dried under vacuum and autoradiographed using Kodak blue x-ray film. Figure a, cell-free translation of oviduct mRNA (5 µg). Slot A, oviduct mRNA (without added tRNA); Slot B, oviduct mRNA and 5 µg Ehrlich ascites cell tRNA; Slot C, oviduct mRNA and 5 µg BeWo cell tRNA; Slot D, oviduct mRNA and 5 µg chorionic tRNA. OV, indicates mobility of ovalbumin. Figure b, translation of EMC RNA (3 µg). Slot A, EMC RNA and 5 µg Ehrlich ascites cell tRNA; Slot B, EMC RNA (without added tRNA); Slot C, EMC RNA and 5 µg BeWo cell tRNA; Slot D, EMC RNA and 5 µg chorionic tRNA.

phoresis. The electrophoretic mobility of proteins synthesized with oviduct mRNA, showed a prominent peak of radioactivity with the mobility of ovalbumin (Fig. 1 a). The polypeptides synthesized with BeWo cell and chorionic tRNAs were identical.

With EMC RNA, the tRNAs from both sources were equally effective in supporting protein synthesis. But the EMC RNA directed polypeptides synthesized with BeWo cell tRNA were different from those synthesized

with chorionic tRNA. Polypeptide 6 (molecular weight 58,000) was apparently not synthesized and the synthesis of the faster moving component, 14 (molecular weight 14,000) was enhanced when BeWo cell tRNA was used (Fig.1b, slot C). We have successfully repeated these experiments with three different preparations of tRNAs.

EMC RNA is a polycistronic mRNA with a single initiation site at 5' end of the molecule (10). In a cell-free system, EMC RNA directs the synthesis of 12-14 polypeptides ranging in molecular weight from 20,000 to 140,000. Most of these polypeptides are the result of premature nonrandom termination of translation (11,12). However, the proteins synthesized in vitro contain sequences characteristic of both virion capsid (11,12) and EMC virus specific proteins synthesized in infected cells (12). Premature nonrandom termination of translation accompanied by the accumulation of incomplete polypeptides has been observed in the translation of EMC RNA with rabbit reticulocyte tRNA (6), and the translation of globin mRNA and mengovirus RNA by extracts of interferon treated L cells (13).

The aberrant translation of EMC RNA with tRNA from human malignant cells does not appear to stem from some artifact of tRNA isolation, because oviduct mRNA was translated with fidelity in the presence of BeWo cell tRNA. However, the mechanism for the lack of fidelity of translation of EMC RNA with BeWo cell tRNA remains unclear.

ACKNOWLEDGEMENTS: We are grateful to Dr. Ernest Borek for his encouragement. This work was supported by a Contract from the U. S. Energy Research Development Agency.

REFERENCES

1. Kuchino, Y. and Borek, E. (1976) Cancer Res. 36:2932-2936.
2. Bridges, K. R. and Jones, G. H. (1973) Biochemistry 12:1208-1212.
3. Sharma, O. K., Mays, L. L. and Borek, E. (1975) Biochemistry 14:509-514.
4. Patillo, R. A. and Gey, G. O. (1968) Cancer Res. 28:1231-1236.
5. Sharma, O. K., Roberts, W. K., Beezley, D. N. and Borek, E. (1975) Biochim. Biophys. Acta 390:327-331.

6. Sharma, O. K., Beezley, D. N. and Roberts, W. K. (1976) Biochemistry 15:4313-4318.
7. Sharma, O. K., Beezley, D. N. and Borek, E. (1976) Nature 262:62-63.
8. Studier, F. W. (1973) J. Mol. Biol. 79:237-248.
9. Palmiter, R. D., Oka, T. and Schimke, R. T. (1971) J. Biol. Chem. 246:724-737.
10. Smith, A. E. (1973) Eur. J. Biochem. 33:301-313.
11. Boime, I. and Leder, P. (1972) Arch. Biochem. Biophys. 153:706-713.
12. Kerr, I. M., Brown, R. E. and Tovell, D. R. (1972) J. Virol. 10:73-81.
13. Content, J., Lebleu, B., Nudel, U., Zilberstein, A., Berissi, H. and Revel, M. (1975) Eur. J. Biochem. 54:1-9.