TRANSLATION OF PLANT-SPECIFIC MESSENGER RNAs IN LIVING ANIMAL CELLS

J. SCHRÖDER, F. KREUZALER and J. SCHMOCK
Biologisches Institut II der Universität, Schänzlestr. 1, D-7800 Freiburg, FRG

Received 11 July 1977

1. Introduction

Many reports in the past few years have demonstrated that mRNAs from animal cells were translated in the wheat germ extract, a cell-free system for protein synthesis prepared from a plant tissue. We have shown recently with RNA fractions from parsley cell suspension cultures (*Petroselinum hortense* Hoffm.) that a rabbit reticulocyte lysate synthesized complete subunits of phenylalanine ammonia-lyase (PAL), a plant-specific enzyme [1–3]. This seemed to suggest a rather close similarity between plant and animal mRNAs. It could be argued, though, that the cell-free systems lack some properties of the intact cells, for example the capacity to distinguish between mRNAs from different sources. We tested this possibility by injecting mRNA from the parsley cell cultures into living oocytes from *Xenopus laevis*. A subsequent analysis with specific antisera against two plant-specific enzymes showed that the oocytes synthesized radioactive proteins which could not be distinguished from the enzyme subunits made by the plant cells.

2. Experimental

The isolation of polyribosomal RNA and poly(A)-containing RNA from parsley cell suspension cultures (*Petroselinum hortense* Hoffm.) has been described [1]. The oocytes from *Xenopus laevis* were prepared according to a published procedure [4]. They were injected with 50 nl polyribosomal RNA (120–150 ng) or poly(A)-containing RNA (20–22 ng) from the parsley cells. Groups of five oocytes were incubated at 19°C for 15–20 h in 50 μl of Barth's modified medium [5] containing 50 μCi [^{35}S]methionine (400–450 Ci/mmol, Amersham Buchler, Braunschweig, FRG). The oocytes were washed briefly with distilled water after the incubation, frozen in liquid nitrogen and stored at −70°C.

The labelled oocytes were homogenized with a glass rod in a buffer containing 20 mM sodium phosphate (pH 7.5), 2% Triton X-100, 2% sodium deoxycholate, 0.3 M NaCl and 8 mM unlabelled methionine. One millilitre of this buffer was used for 5 oocytes. Insoluble material was removed by centrifuging the homogenate for 8 min at 18 000 × *g*. The supernatant fluids were mixed with an unlabelled, partially purified preparation of flavanone synthase (FS) or PAL from the parsley cultures. After addition of a 1.5–2-fold excess of the specific antiserum against the respective enzyme, the extracts were left for 30 min at room temperature and for 5 h at 4°C. The immunoprecipitates were isolated by the sucrose-cushion technique [1,6], washed twice and analyzed by polyacrylamide gel electrophoresis in the presence of SDS for radioactivity at the position of the enzyme subunits [1].

Radioactive FS and PAL from the parsley cells were isolated after labelling 40 ml of a light-induced culture (continuous light for 8 h [2]) with 140 μCi of a [^{3}H]amino acid mixture (New England Nuclear, Dreieichenhain, FRG) for an additional 2.5 h. The immunoprecipitation of the labelled enzymes was performed as outlined above. The monospecific antisera against PAL [7] and against FS (Kreuzaler, F. unpublished) were raised in rabbits.

Abbreviations: PAL, phenylalanine ammonia-lyase; FS, flavanone synthase; poly(A), polyadenylic acid; SDS, sodium dodecyl sulfate

North-Holland Publishing Company – Amsterdam

3. Results and discussion

The question whether the normal living oocytes translated plant mRNA could be answered most convincingly by using mRNAs for proteins which are not existent in animal cells. FS and PAL belong to a pathway of secondary metabolism in plants which leads to flavonoid glycosides [8] and neither one of these enzymes has been found in animals. The parsley cell cultures offer furthermore the advantage that the concentration of the mRNAs for these enzymes are very low in dark-grown cultures and large increases were observed after irradiation of the cultures ([2], Schröder, J., Kruezaler, F. and Hahlbrock, K. unpublished). It was therefore possible to use RNA from dark-grown cells as control for the results obtained with mRNA from light-induced cell cultures.

Figure 1 describes the results of an experiment in which the oocytes were injected with poly(A)-containing RNA from light-induced parsley cell cultures. The gel-electrophoretic analysis of the immunoprecipitates showed that the oocytes synthesized two radioactive proteins which were precipitated by the specific antisera against FS (fig.1A) or against PAL (fig.1B). In this experiment the ^{35}S-labelled, immunoprecipitated proteins from the oocytes were co-electrophoresed with ^{3}H-labelled enzyme subunits which had been synthesized by the parsley cells. Figure 1 shows that the radioactive proteins from the oocytes and from the parsley cells comigrated in the polyacrylamide gels. Similar results were obtained with total polyribosomal RNA from light-induced parsley cells. After injection of the oocytes with RNA fractions from dark-grown cultures or with distilled water, the immunoprecipitates contained no significant amounts of radioactivity at the positions of either FS or PAL subunits.

The results suggest that the mRNAs for these two plant-specific enzymes were translated in the normal living animal cell. It seems therefore reasonable to conclude that mRNAs from animals and higher plants share at least some of the properties which are essential for translation. The finding that mRNAs from mammalian cells [9–13], insects [14], yeast [15] and from plant [16] and animal [17] viruses were successfully translated in oocytes seems to indicate that this conclusion might apply to all mRNAs which are normally translated in the cytoplasm of eucaryotic cells.

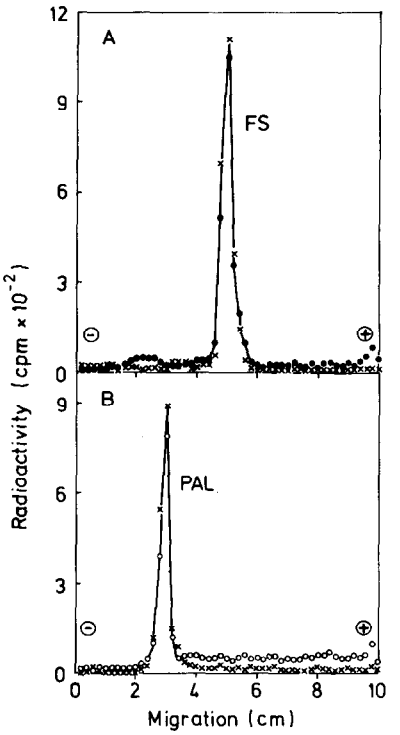

Fig.1. Synthesis of FS (A) and PAL (B) in frog oocytes: Analysis of the specific immunoprecipitates on 10% polyacrylamide gels in presence of 0.1% SDS. The oocytes had been injected with poly(A)-containing RNA from light-induced parsley cell cultures (continuous light for 7 h) and the immunoprecipitates were isolated as described in the Experimental section. The ^{35}S-labelled immunoprecipitates from the oocytes (FS (●–●) PAL (○–○)) were co-electrophoresed with the respective ^{3}H-labelled immunoprecipitates (X–X) from light-induced parsley cells to compare the size of the proteins synthesized in the animal and plant cells.

Acknowledgements

The skillful technical assistence of Ms G. Braun is gratefully acknowledged. This research was supported by the Deutsche Forschungsgemeinschaft (SFB 46).

References

[1] Ragg, H., Schröder, J. and Hahlbrock, K. (1977) Biochim. Biophys. Acta 474, 226–233.
[2] Schröder, J. (1977) Arch. Biochem. Biophys. in press.
[3] Schröder, J., Betz, B. and Hahlbrock, K. (1977) Plant Physiol. in press.

[4] Scheer, U. (1973) Develop. Biol. 30, 13–28.
[5] Gurdon, J. B. (1968) J. Embryol. Exp. Morph. 20, 401–414.
[6] Palmiter, R. D. (1973) J. Biol. Chem. 248, 2095–2106.
[7] Schröder, J., Betz, B. and Hahlbrock, K. (1976) Eur. J. Biochem. 67, 527–541.
[8] Hahlbrock, K. and Grisebach, H. (1975) in: The Flavonoids (Harborne, J., Mabry, T. and Mabry, H. eds) pp. 866–915, Chapman and Hall, London.
[9] Berns, A. J. M., Van Kraaikamp, M., Bloemendal, H. and Lane, C. D. (1972) Proc. Natl. Acad. Sci. USA 69, 1606–1609.
[10] Mach, B., Faust, C. and Vassalli, P. (1973) Proc. Natl. Acad. Sci. USA 70, 451–455.
[11] Vassart, G., Brocas, H., LeCocq, R. and Dumont, J. (1975) Eur. J. Biochem. 55, 15–22.
[12] Gurdon, J. B., Lane, C. D., Woodland, H. R. and Marbaix, G. (1971) Nature 233, 177–182.
[13] Beato, M. and Rungger, D. (1975) FEBS Lett. 59, 305–309.
[14] Kindas-Mügge, I., Lane, C. D. and Kreil, G. (1974) J. Mol. Biol. 87, 451–462.
[15] Eggitt, M. J. and Scragg, A. H. (1975) Biochem. J. 149, 507–512.
[16] Van Vloten-Doting, L., Bol, J., Neeleman, L., Rutgers, T., Van Dalen, D., Castel, A., Bosch, L., Marbaix, G., Huez, J., Hubert, E. and Cleuter, Y. (1977) in: Nucleic Acids and Protein Synthesis in Plants (Bogorad, L. and Weil, J. H. eds) pp. 387–411, Plenum Press, New York and London.
[17] Laskey, R. A., Gurdon, J. B. and Crawford, L. V. (1972) Proc. Natl. Acad. Sci. USA 69, 3665–3669.