TRANSLATION OF RABBIT GLOBIN mRNA UPON INJECTION INTO FUSED HeLa CELLS

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1. Introduction

Introduction into a living cell of an isolated messenger RNA allows to answer many interesting questions concerning the translation as well as the stability of this mRNA. Injection of mRNA into frog oocytes is one of the most successful examples of such an approach [1,2]. However, due to the very nature of the frog oocyte, it may be interesting in some circumstances to be able to introduce a mRNA into somatic cells. This is especially true if one wants to study some of the regulatory mechanisms of the somatic cell. So far, the possibility of injection and translation of mRNA in these cells has been demonstrated using different methods.

One method consists in injecting directly the mRNA into the cells using a glass capillary [3,4]. Another method consists in incubating the cells with either an aqueous RNA solution [5] or an RNA solution encapsulated into liposomes [6,7] or red blood cell 'ghosts' [8]. Injection with glass capillaries offers some advantages over the other methods. Indeed, one needs but a very small volume of mRNA solution (<1 μl) for a typical injection experiment. Furthermore, the amounts of mRNA injected can be accurately determined.

So far, the detection of the translation products of an mRNA injected by this technique has been performed by immunofluorescence or autoradiography of the recipient cell [3,4]. As these detection methods present some limitations, it would be useful in some cases to use classical biochemical techniques like gel electrophoresis followed by autoradiography. This implies that a relatively large amount of labelled proteins has to be extracted from the injected cells. This can of course be obtained by injecting a large number of cells (>10^3) but is very tedious. A better approach is to fuse the cells before injection as proposed in [9]. By this way, giant cells are formed which can be injected very easily. The equivalent of 10^3 individual cells can thereby be injected in a matter of minutes.

Here, we demonstrate that rabbit globin mRNA can be translated in such fused cells and furthermore, we show that the synthesis of globin can be detected using polyacrylamide gel electrophoresis.

2. Material and methods

2.1. *Cell fusion*

Cell fusion was performed essentially according to [9]. HeLa cells are grown to ~80% confluence in MEM supplemented by 10% foetal calf serum (FCS) on fragments of microscopic glass slides (~1 mm^2) sitting in 6 cm Petri dishes. For fusion to occur, the medium was removed and the cells were briefly washed (10 s) with 1.5 ml PEG (49% polyethylene glycol (PEG) 1000 Sigma in MEM w/v). The fluid was removed and 2 ml PEG solution were added again for 40 s. The cells were then washed twice with MEM containing 15% dimethylsulfoxide v/v and 3 times with MEM; they were then incubated in standard conditions and washed again with MEM + 10% FCS 4 h later.

2.2. *Globin mRNA*

Rabbit globin mRNA was prepared according to [10] and dissolved in water at 1 mg/ml.

2.3. *Microinjections*

Microinjections were performed as in [3] using

glass capillaries, under a phase contrast microscope (total magnification 320X) and with the help of a Leitz micromanipulator. The glass needles were obtained by pulling glass tubes (1.5 mm external diameter from SGS Scientific) with an home-made puller. The external diameter of the glass needles was 0.5–1 μm at the tip. The solution to be injected was sucked into the needle from the tip by an air depression created with a 50 ml syringe. For the injection, the needle was gently lowered into the cell until a change in the darkness of the cell surface was observed around the tip of the needle (indicating that the needle is just entering the cell). The liquid is then injected into the cell by air pressure. 10^{-8}–5×10^{-8} ml are transferred per fused cell. For each assay 20 fused cells (= 500–1000 mononucleated cells) were injected. Only the cells sitting on the glass fragments were injected.

2.4. *Cells labelling*

After injection, the small pieces of glass carrying the injected cells were transferred under sterile conditions into the wells of a microtest plate (Nunck no. 167008). The slide fragments were covered with 100 μl of (His-) MEM medium, [^{3}H]histidine (50 Ci/mmol) was added to 300 μCi/ml, and the cells were incubated for 8 h. In a typical experiment, 300 000 cpm were recovered in proteins from 20 syncitia.

2.5. *Immunoprecipitation and gel electrophoresis*

At the end of the incubation period the cells were washed 3 times with unlabelled MEM medium. They were subsequently lysed by the addition of 100 μl buffer containing 20 mM Tris (pH 7.5), 50 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 1 mM phenylmethyl sulpholylfluoride and 1% histidine. Half of the lysate (= 150 000 cpm) was submitted to an immunological precipitation [11] using a rabbit globin goat antibody obtained after 3 subcutaneous injections of a solution of crystallized rabbit globin (1 mg/ml in complete Freund adjuvant). The other half of the lysate was used for direct electrophoresis after addition of SDS and mercaptoethanol (1% and 5%, respectively). Gel electrophoresis was performed according to [12] on 20% slab gels. The gels were submitted to autoradiography as in [13]; exposure time was 7 days.

3. Results and discussion

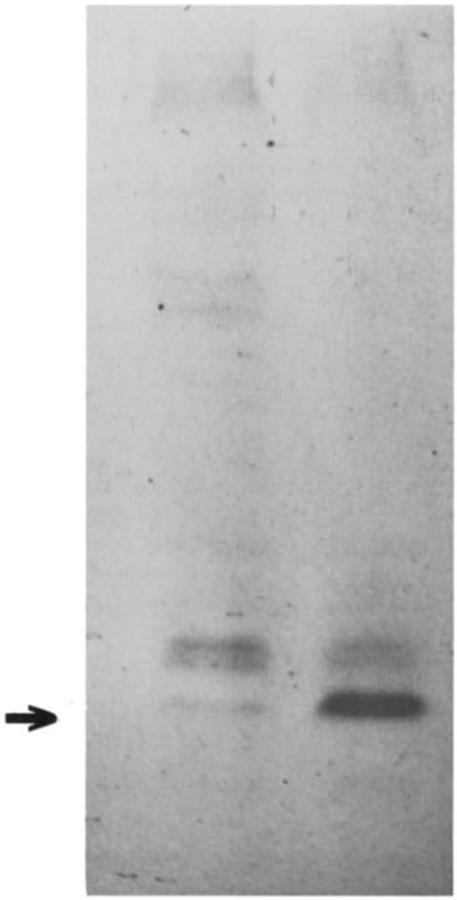
When cell fusion is performed as in section 2 syncitia containing 25–50 nuclei are formed. We have found that, in our hands, fused cells of that size do stick more strongly to glass than do giant cells containing up to 500 nuclei as used in [9]. In order to increase as much as possible the specific activity of the proteins recovered from the injected cells, such cells have to be incubated in a medium containing a large amount of labelled amino acids. To make it economically more practicable the cells to be injected were grown on small fragments of glass slides (1 × 1 mm).

After fusion and injection, these pieces of glass carrying the cells were transferred into the wells of microtest plates. In this way, it was possible to incubate the cells with a minimal volume of highly labelled medium. One can see in fig.1 that injection of fused HeLa cells with rabbit globin mRNA leads to the appearance of a labelled protein that migrates exactly at the position of marker rabbit globin by gel electrophoresis. In control cells injected with water one observes but a faint band corresponding to an unidentified material which migrates in this region of the gel.

In order to prove more strongly that the protein made by injection of globin mRNA is really rabbit globin, we submitted the cell extracts to immunoprecipitation with an antibody directed against highly purified rabbit globin. The quality of this antibody was tested by immunoprecipitation of an extract of oocytes injected with globin mRNA. In this case globin only is precipitated by the antibody (data not shown).

Figure 2 gives the result of the immunoprecipitation of the extract of fused HeLa cells injected with either water or globin mRNA. In the latter extract one observes a strong radioactive band corresponding to globin which is not present in the control cells extract. One should note here that the faint background observed is due to some unspecific adsorption of proteins on the immunoprecipitate.

One can conclude from the above results that globin mRNA can be efficiently translated upon microinjection into fused HeLa cells. The main interest of injection into fused cells is that syncitia equivalent to a large number of individual cells can easily be injected (i.e., the equivalent of several thousand cells can be injected). This, together with the labelling technique



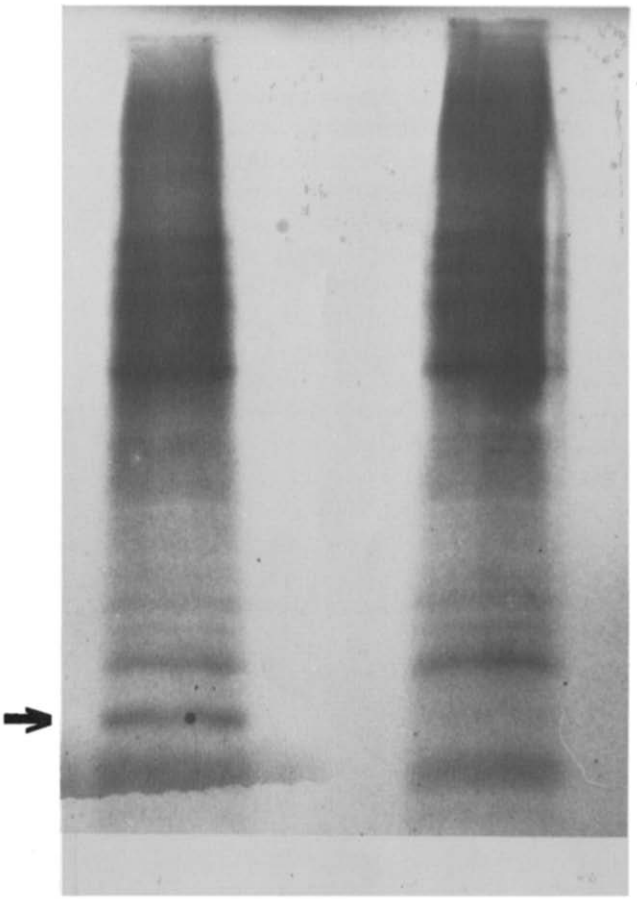


Fig.1. Fluorogram of SDS–polyacrylamide gel electrophoresis of HeLa cell extracts (20% acrylamide gel). Fused cells (20) were injected with globin mRNA (lane A) or water (lane B) and subsequently labelled for 8 h with [^{3}H]histidine. The arrow shows the position of marker rabbit globin. For details see text.

Fig.2. Fluorogram of SDS–polyacrylamide gel electrophoresis of the immunoprecipitates obtained with anti-rabbit globin antibody from the same extracts of HeLa cells as in fig.1. Lane (A), cells injected with water. Lane (B), cells injected with globin mRNA. The arrow shows the position of the globin marker. For details see text.

of the injected cells described above, allows the use of the gel electrophoresis (followed by fluorography) as a convenient means to detect the translation products of an injected mRNA. With this method of analysis, quantitative data can be obtained more easily than with the techniques used so far (immunofluorescence and autoradiography of the recipient cells). Moreover the immunological precipitation of the cell extracts, as described above, should certainly permit the detection of very low synthesis of a given protein which would probably not be detectable by other techniques.

The fact that the cells are fused does not seem to affect their translational or transcriptional capacity. Indeed fused cells are shown [9] to be capable of supporting simian virus 40 gene expression in the same way as the parental mononucleated cells. As the fused cells can be kept in culture for $\geqslant$24 h time course experiments could be performed over a reasonable time. The present method is thus most suitable if one wants to study the translation or the stability of an isolated mRNA after its introduction into a somatic cell.

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