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Translational stability of native and deadenylylated rabbit globin mRNA injected into HeLa cells

[α and β globin messenger RNA/poly(A)/cell injection/two-dimensional gel electrophoresis]

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ABSTRACT **HeLa human cells were injected with a natural mixture of rabbit α and β globin mRNA. They were incubated for 6 hr with [^{35}S]methionine either immediately after injection or 20 hr later. The labeled proteins in the injected cells were analyzed by fluorography of two-dimensional electrophoresis gels. By using this procedure, it was possible to show that, during the first few hours after injection, both α and β globin molecules are synthesized with an α to β ratio approximately equal to 0.6. The rate of synthesis of α globin decreased significantly faster than that of β globin over a 26-hr period after injection of the two mRNAs. It thus seems that two messenger RNAs coding for closely related polypeptides possess a markedly different translational stability. When deadenylylated rabbit globin mRNAs were injected into HeLa cells, no globin synthesis could be detected by the techniques used. We conclude that the translational half-life of mRNAs lacking poly(A) is very short in these cells. It is thus clear that the poly(A) segment is required to ensure stability to globin mRNA in somatic cells as in *Xenopus* oocytes.**

In eukaryotic cells, about 60% of the population of messenger RNA molecules contains a poly(A) segment at the 3′ end (1). The role of this segment has been the subject of a wealth of research for a long time. A few years ago, we compared the half-lives of native and enzymatically deadenylylated rabbit globin mRNAs in a stable translation system, the *Xenopus* oocyte (2–4). We concluded from these experiments that the presence of the poly(A) segment is a prerequisite for ensuring stability to these messengers in oocytes.

This conclusion was not restricted to globin mRNA, because similar results were obtained with histone mRNAs either lacking poly(A) or having artificially attached poly(A) (5). One could, however, ask the question whether these findings were peculiar to the translation system utilized. It thus appeared to be of interest to compare the translational stabilities of a native and a deadenylylated mRNA after their introduction into somatic cells in culture.

In the present work, we injected a mixture of rabbit α and β globin mRNA molecules into HeLa human cells. These molecules were either native or deadenylylated. We then followed the synthesis of both α and β globin chains over a 26-hr period. HeLa cells were chosen for these experiments because it has been shown (6, 7) that they are able to efficiently support globin mRNA translation.

MATERIALS AND METHODS

Globin mRNA. Rabbit globin mRNA was prepared as described in ref. 8.

Deadenylylation of Globin mRNAs. Deadenylylation was performed by a modification of the method of Sippel *et al.* (9) using nuclease H from *Escherichia coli* (Enzo Biochemicals, New York). In a typical experiment, 20 μg of RNA was incubated for 15 min at 20°C in 0.05 M KCl with 2 μg of $(dT)_{12-18}$ (P-L Biochemicals). Nuclease H (4 units) were then added and the mixture was subsequently incubated for 20 min at 37°C. Twenty volumes of buffer containing 0.3 M NaCl, 0.01 M Tris·HCl at pH 7.4, 0.5% $NaDodSO_4$, and 200 μg of proteinase K per ml (Merck) was added to stop the reaction. The mixture was incubated at 0°C for 10 min and at 20°C for 20 min to allow the digestion of nuclease H by proteinase K. The RNA solution was then immediately submitted to oligo(dT)-cellulose chromatography at 2°C. Fractions containing the RNA excluded from the column were collected, pooled, and submitted to phenol extraction. The deadenylylated RNA (about 60% of the input) was recovered by ethanol precipitation.

Readenylylation of the Deadenylylated RNA. Readenylylation of the previously deadenylylated RNA was performed with *E. coli* RNA-ATP transferase as described (4). The enzyme preparation was kindly supplied by R. Devos (University of Ghent, Belgium).

Oocyte Injection. *Xenopus* oocytes were injected with a mRNA solution at a concentration of 0.1 mg/ml and incubated with [^{3}H]histidine in Barth's medium at 1 mCi/ml (1 Ci = 3.7×10^{10} becquerels). The injection was 50 nl per oocyte. Analysis of the proteins was performed as described in ref. 5.

Cell Injection. Injection of mRNA was performed with glass capillaries according to the method of Graessmann (10). Cells to be injected were grown on fragments of microscope slides (2 mm^2) dispersed in 6-cm petri dishes in minimal essential medium plus 10% fetal calf serum. In a typical experiment, 100–150 individual cells were injected. mRNA was dissolved in water at a concentration of 1 mg/ml. The volume injected into each cell was $5 \cdot 10^{-5}$ nl (average value).

Cell Labeling. Cell labeling was performed as described in ref. 7. Immediately after injection or 20 hr later, the small pieces of glass carrying the cells were transferred under sterile conditions into the wells of a microtest plate (96 wells). They were then covered with 50 μl of minimal essential medium lacking methionine and supplemented with 10% dialyzed fetal calf serum, kanamycin at 100 μg/ml, and [^{35}S]methionine (700 Ci/mmol) at 3 mCi/ml. About 250,000 cpm was recovered in the proteins from 150 cells.

Gel Electrophoresis and Autoradiography. At the end of the incubation period, the cells were washed twice with phosphate buffer (0.015 M NaH_2PO_4/0.065 M K_2HPO_4, pH 7.2) and lysed by the addition of 40 μl of the sample buffer of the isoelectric focusing gel according to O'Farrell *et al.* (pH range 6–9.5, Ampholines from LKB) (11). Electrophoresis in the isoelectric focusing gel, as well as in the second-dimension gel, was performed according to ref. 11; autoradiographies were according to ref. 12. Exposure time was 7 days (unless otherwise specified).

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Purification of α and β Globins. ^{14}C-labeled α and β globin molecules were obtained by incubation of a reticulocyte lysate with [^{14}C]histidine. α and β globin were separated by carboxymethyl-cellulose chromatography (5).

RESULTS

Fig. 1*A* displays the protein pattern obtained when HeLa cells were injected with water (controls). In Fig. 1*B*, the cells were injected with native rabbit globin mRNAs and subsequently incubated for 6 hr in a medium containing labeled methionine. As expected, the pattern is very complex, but few species migrate in the region of the gels corresponding to low molecular weight basic proteins (bottom right of the figures). Comparison between Fig. 1 *A* and *B* shows the presence of two large additional spots (arrows) when the cells have been injected with globin mRNA. In the area of the gel where these two additional spots are located, one cannot detect any spot corresponding to endogenous proteins in control cells injected with water. This is true even if the exposure time of the autoradiography is prolonged up to 5 weeks.

In order to characterize the polypeptides corresponding to the large additional spots in the mRNA-injected cells, we submitted to the same electrophoresis separation procedure a mixture of labeled proteins from uninjected cells together with a small amount of purified labeled rabbit β globin (Fig. 2*A*) or α globin (Fig. 2*B*). A comparison between these two patterns shows that the positions of the spots corresponding to α and β globin are different from each other in this kind of gel. The "β spot" is more or less in line with four spots (called 1, 2, 3, and 4 in Fig. 2*A*) corresponding to polypeptides with higher molecular weights. The "α spot" is on the right of this line. We thus conclude that the two strong additional spots shown in Fig. 1*B* correspond to β and α rabbit globin, respectively (from left to right).

In Fig. 1*B* it seems that the intensity of the β spot is stronger than that of the α spot. We measured the radioactivity of each globin spot by cutting out the appropriate areas of the dried gel and dissolving them in NCS tissue solubilizer (Amersham). This showed that the amount of α globin synthesized represented about 60% of that of β globin (average of three experiments). The radioactivity present in the two spots corresponded to 0.02% of the total radioactivity incorporated into the proteins submitted to electrophoresis.

Fig. 3 shows the result of an experiment in which the cells were injected with native rabbit globin mRNA and labeled 20 hr after injection. Again, two spots of radioactivity corresponding to α and β globins are detectable; but in this case the α spot is considerably less intense than the β one. When one compares, in Figs. 1*B* and 3, the intensity of the β spot with that of the endogenous proteins in the vicinity, one can conclude that, 20 hr after globin mRNA injection, the rate of β globin synthesis is significantly lower than during the first few hours.

In Fig. 4, we show the protein pattern obtained when HeLa cells are injected with deadenylylated globin mRNA and are labeled for 6 hr with [^{35}S]methionine immediately after injection. In contrast with the results shown in Fig. 1*B*, no spot cor-

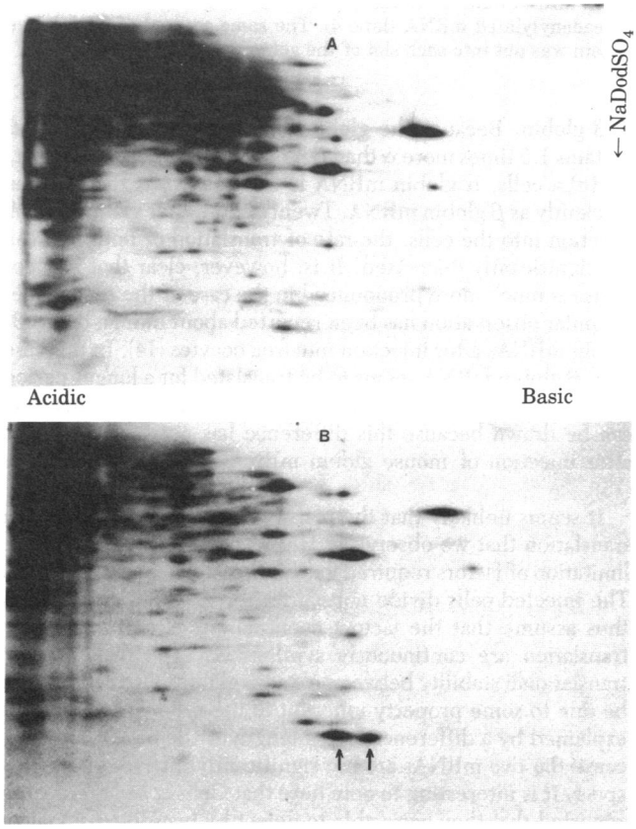

FIG. 1. Fluorogram of two-dimensional polyacrylamide gel electrophoresis of proteins extracted from HeLa cells after injection with water (*A*) or native rabbit globin mRNA (*B*). The cells were incubated for 6 hr with labeled methionine after injection.

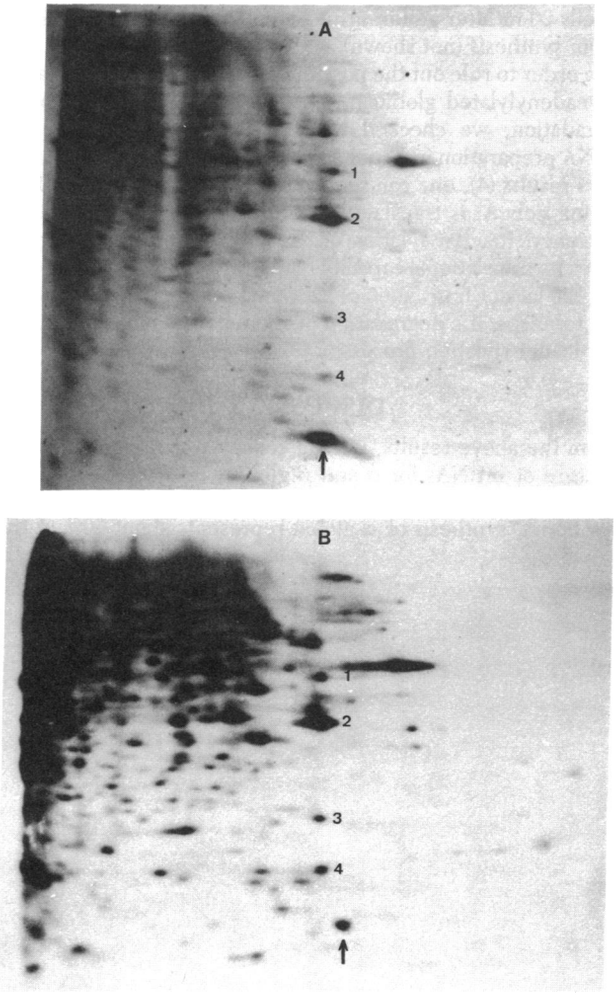

FIG. 2. Fluorogram of two-dimensional polyacrylamide gel electrophoresis of a mixture of ^{14}C-labeled purified rabbit β globin (*A*) or α globin (*B*) and proteins from uninjected HeLa cells labeled for 6 hr with [^{35}S]methionine.

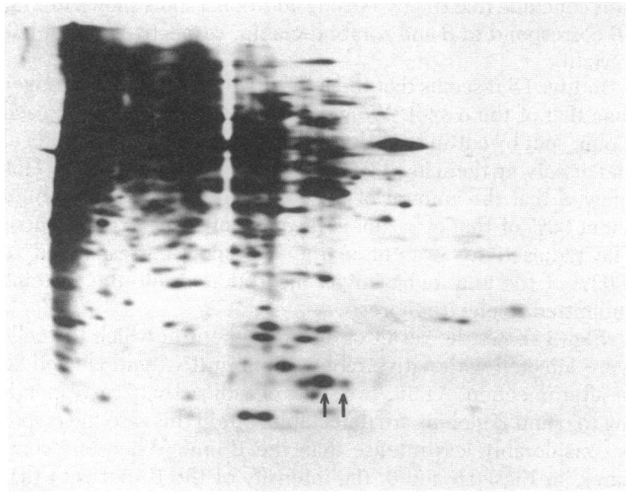

FIG. 3. Fluorogram as in Fig. 1*B*, except that the cells were incubated for 20 hr in an unlabeled medium and then incubated for 6 hr with [^{35}S]methionine. The exposure time of the autoradiogram was increased to 5 weeks in order to allow the visualization of the weak spot corresponding to α globin (right arrow).

responding to α or β globin can be detected. Similarly, labeling of cells 20 hr after globin mRNA injection does not reveal any globin synthesis (not shown).

In order to rule out the possibility that the lack of translation of deadenylylated globin mRNA could be due to nucleolytic degradation, we checked the translational capacity of this mRNA preparation in *Xenopus* oocytes. In agreement with previous results (4), one can see in Fig. 5 (lane 3) that the mRNA lacking poly(A) is translated in the oocytes, but with a lower efficiency. Readenylylation of this preparation completely restores its translational capacity (lane 4 of Fig. 5). It is thus clear that the lack of translation of deadenylylated mRNA cells is not due to unspecific degradation of the message molecule during the deadenylylation process.

DISCUSSION

From the above results, we can conclude that when a natural mixture of mRNAs for α and β globins is injected into HeLa cells, both types of molecules are translated. During the first few hours, synthesis of α globin represents about 60% of that

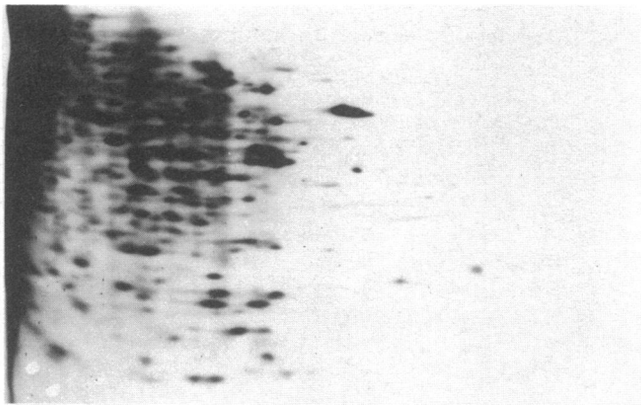

FIG. 4. Fluorogram as in Fig. 1*B*, except that the cells were injected with deadenylylated rabbit globin mRNA. Although the exposure time was extended to 5 weeks, no significant spot can be detected at the position of α or β globin.

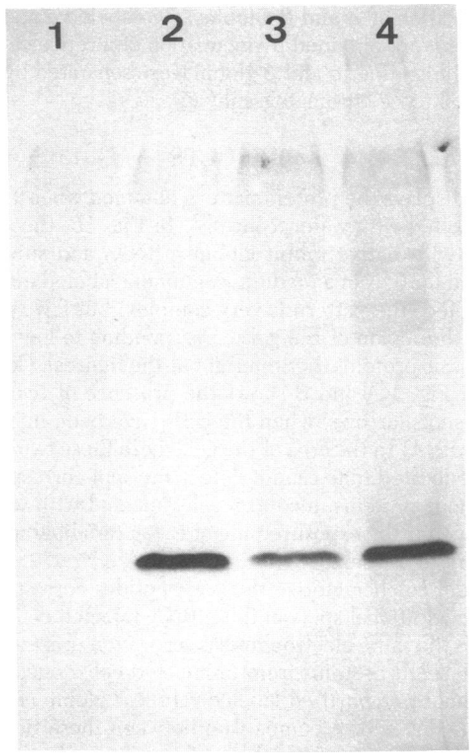

FIG. 5. Fluorogram of a one-dimensional NaDodSO$_4$ gel electrophoresis of the proteins from oocytes incubated immediately after injection for 3 hr in Barth's medium containing [^{3}H]histidine at 1 mCi/ml. Oocytes were injected with: 50 nl of water (lane 1), native rabbit globin mRNA (lane 2), deadenylylated rabbit globin mRNA (lane 3), or readenylylated mRNA (lane 4). The same amount of radioactive protein was put into each slot of the gel.

of β globin. Because the globin mRNA preparation we used contains 1.5 times more α than β messenger (13), it follows that, in HeLa cells, α globin mRNA is translated less than half as efficiently as β globin mRNA. Twenty hours after globin mRNA injection into the cells, the rate of translation of both mRNAs has significantly decreased. It is, however, clear that this decrease is much more pronounced in the case of the α message. A similar observation has been reported about human α and β^S globin mRNAs after injection into frog oocytes (14). In this case also, β globin mRNA seems to be translated for a longer period of time than α globin mRNA. However, no general conclusion can be drawn because this difference has not been observed after injection of mouse globin mRNA into *Xenopus* oocytes (15).

It seems unlikely that the marked decrease in α messenger translation that we observe in HeLa cells could be due to the limitation of factors required for the translation of this message. The injected cells divide normally after injection and we may thus assume that the factors eventually needed for α globin translation are continuously synthesized. The difference in translational stability between α and β globin mRNAs may thus be due to some property inherent to the mRNA. It cannot be explained by a difference in the length of the poly(A) tract because the two mRNAs are not significantly different in this respect. It is interesting to note here that Celis *et al.* (16) recently reported that they were able to detect by two-dimensional gel electrophoresis one additional M_r 16,000 basic protein in extracts of 3T3 cells injected with rabbit globin mRNA. The fact that they detected only one protein may be due to differences in the resolution of the electrophoretic system utilized. But,

because they analyzed the proteins of the injected cells 12–16 hr after injection of the mRNA, it might be that they could detect only the β globin spot (as in the present experiments with HeLa cells 20 hr after injection).

The results of the present experiments with deadenylylated globin mRNA confirm our previous observations on *Xenopus* oocytes (2–5). It is clear that, in both systems, the poly(A) segment contributes largely to the stability of globin mRNA. The fact that we cannot detect any synthesis of globin after injection of deadenylylated mRNA suggests that this mRNA has a very short half-life (maybe a matter of minutes). The relatively longer half-life of deadenylylated globin mRNA in oocytes might be at least in part explained by the fact that oocytes are incubated at a lower temperature (19°C) than HeLa cells (37°C). The short half-life of the deadenylylated globin mRNAs in HeLa cells implies that the mechanisms of cytoplasmic readenylylation that are known to exist in somatic cells (17) are not able to restore a poly(A) tract of sufficient length to preserve the deadenylylated mRNA from inactivation or degradation.

It should be pointed out here that the translational half-life of some mRNAs does not seem to be linked to the presence or the absence of a poly(A) segment at the 3′ end of the molecule. This is the case for a complementary RNA lacking poly(A), synthesized *in vitro* on simian virus 40 DNA, which is translated for several hours after its injection into mouse kidney cells (18). The alfalfa mosaic virus coat protein mRNA 4, which also lacks poly(A), is much more stable, when injected into oocytes, than histones or deadenylylated globin mRNAs (unpublished observations made in collaboration with L. Van Vloten-Doting and T. Rudgers).

On the other hand, human fibroblast interferon mRNA, which contains a terminal poly(A), seems to have a short life in oocytes. The removal of the poly(A) segment has no influence on the translational half-life of this mRNA (19).

One can thus conclude that several mechanisms probably regulate the stability of the different mRNAs. For rabbit globin mRNAs, the results presented above suggest that, while the presence of a poly(A) segment at the 3′ end of the molecules is a prerequisite for mRNA stability, this is not the only factor that regulates the half-life of these messengers.

The present work also emphasizes the fact that injection into somatic cells may be an excellent means for a direct evaluation of the translational half-life of a given mRNA.

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