CRYPTIC FORM OF mRNA IN DORMANT ARTEMIA SALINA CYSTS

H. Grosfeld and U.Z. Littauer

Weizmann Institute of Science, Neurobiology Unit
Rehovot, Israel

Received September 2, 1975

SUMMARY: Dormant Artemia salina cysts are almost devoid of polysomal structures but contain appreciable quantities of mRNA that sediments mainly as a 40S complex in sucrose gradients. The mRNA can be isolated from this complex and efficiently translated in a wheat germ cell-free system, although the 40S complex itself is inactive. During rehydration of the cysts, mRNA becomes increasingly involved in polysomal complexes which can be actively translated in the cell-free system.

Cysts of the brine shrimp Artemia salina represent a convenient model system for the study of mRNA function during development. We have previously shown that the postmitochondrial supernatant of dormant and incubated developing cysts of Artemia salina contain approximately the same quantities of poly(A)-rich mRNA (1). However, as unicubated cysts appear to be devoid of polysomes (2-4) and mRNA activity could not be detected in isolated ribosomes from dormant cysts (5), it was of considerable interest to elucidate the form in which mRNA occurs within such cells. We show here that the mRNA isolated from the postmitochondrial fraction of 0 hr cysts, sediments mainly as a 40S complex in sucrose density gradients. During rehydration of the cysts, mRNA becomes increasingly involved in polysomal complexes, with a consequent diminution of the mRNA found in the 40S pool.

MATERIALS AND METHODS: Sucrose gradient analysis of cyst extracts.

5g of either dormant (0 hr cysts) or cysts incubated in artificial sea water for 22 hrs at 30° (22 hr cysts) were extensively washed with cold deionized water followed by cold TMK buffer (0.2 M KCl, 5mM $MgCl_2$, 20 mM Tris buffer pH 7.6) on a number 1 sintered glass funnel. Cysts were then broken in 15 ml of the same buffer with 10 strokes of a loose fitting motor-driven Potter-Elvehjhem homogenizer, the extract being passed through Miracloth and then centrifuged at 12,000 g for 10 min. Five ml of the postmitochondrial supernatant, freed of the lipid layer by aspiration, was layered onto 30 ml of a 10-40% sucrose gradient in TMK buffer, in Beckman SW 27 rotor nitrocellulose tubes. The gradients were run for 3.5 hr at 25,000 rpm, and the distribution of the 260 nm absorbing material was followed by scanning in a Gilford 2400-S spectrophotometer equipped with a flow-through cell, prior to collection of 3 ml fractions using a Gilson fraction collector. 200µl of

each fraction was kept on ice for immediate translation in the wheat germ cell-free system. To the remaining fractions, 2 volumes of cold ethanol were added, and after vigorous mixing, stored overnight at -20°. The ethanol-precipitated material was collected at 12,000g for 10 min, lyophilized and resuspended in 1 ml of mRNA extraction buffer (0.1 M Tris-HCl, pH 9.0; 0.1M NaCl; 1mM EDTA). SDS was added to a final concentration of 1% and RNA from each fraction was twice extracted with phenol-chloroform-isoamylalcohol (50:50:1) (6). Reprecipitation of RNA was performed from the aqueous phase with 2% CH_3COOK pH 5.5 and 2 volumes of ethanol, at -20°C for 6 hr, and after collection by centrifugation the precipitate was washed twice in ethanol -0.2 M NaCl (2:1 V/V), before lyophilization.

To obtain a better resolution of the 40S region, a similar procedure to that just described, but with the substitution of a 20-40% sucrose gradient, was employed.

Translation in a wheat germ S-30 cell-free system: The system described by Roberts and Paterson (7) was used with the following modifications: (a) spermine was added to a final concentration of 95 µM, (b) HEPES buffer was used at pH 7.9 instead of pH 7.6, (c) the final GTP concentration was 0.22 mM, (d) K^+ concentration was 94 mM. 7.5 microcuries of (^{35}S)-methionine (220 Ci/mmole, The Radiochemical Center, Amersham, England) were added per 50 µl of reaction volume. In all figures, the counts per minute values quoted are for 5 µl samples of the reaction mixtures.

Product analysis: ^{35}S-Methionine labeled proteins synthesized in vitro were subjected to electrophoresis on SDS-polyacrylamide gel slabs, using a 10-18% gradient (8). Identical volumes of the reaction mixture were applied to the gel regardless of differences in radioactive content. After staining with Coomassie-Brilliant Blue, the gel was prepared for fluorography with DMSO-PPO (9). Various exposure times of between 12 hr to 6 days were employed in order to effectively compare bands of high and low radioactive content respectively.

RESULTS

The distribution of RNA obtained on fractionation of sucrose gradients from postmitochondrial supernatants from 0 hr and 22 hr incubated cysts was found to be markedly different. Whereas 22 hr cysts showed a typical polysomal region containing about 54% of the total A_{260}-absorbing material, 0 hr extracts appeared to be practically devoid of polysomes, in agreement with previous data (4).

Fractions from both gradients were directly checked for in vitro translation capacity by their ability to stimulate protein synthesis in a wheat germ cell-free system. The activity pattern found in the 22 hr cysts corresponded to the polysomal region but almost no activity could be detected in gradients from 0 hr cysts (Fig. 1).

A very different pattern resulted when RNA was extracted from each gradient fraction and then assayed in the same cell-free system. Both gradients were found to possess substantial template activity in most fractions, indicating that 0 hr mRNA participates in polysomal complexes, although these can hardly be

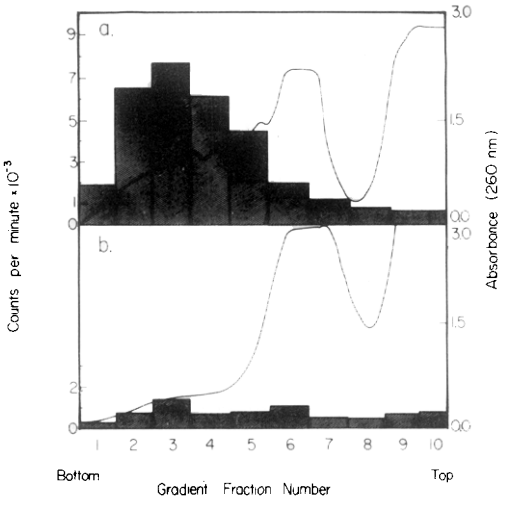

Fig. 1: A_{260} tracing (solid lines) and protein synthetic activity in fractions from a 10-40% sucrose gradient of postmitochondrial supernatants from 0 hr (a) and 22 hr (b) cysts (see Materials and Methods). Bars in the histogram represent counts incorporated by 5 µl from a total reaction volume of 50 µl in the cell free system.

detected by UV absorbance (Fig. 2). In both extracts, a second region of activity occurred in the upper part of the gradient, consisting of mRNA apparently not bound to polysomes at the time of extraction. This fraction was more pronounced in the 0 hr than in the 22 hr extracts. In order to obtain better resolution of this area, 0 hr cysts extracts were subjected to centrifugation in a 20-40% sucrose gradient (Fig. 3a). Subsequent extraction of RNA and translation in vitro show that a major part of the messenger activity of unincubated cysts occurs as a peak in the 40S region of such a gradient (Fig. 3b). In contrast, 22 hr incubated cysts exhibited only poor messenger activity in the same region (Fig. 2).

The pattern of proteins synthesized by mRNA extracted from the 40S pool differed markedly from that obtained with polysomal mRNA from either 0 hr or 22 hr cysts and appeared to consist mainly of a restricted number of low molecular weight polypeptides (Fig. 2). Polysomal mRNA products from either gradient were generally similar and only minimal variations between different fractions within each gradient was noted.

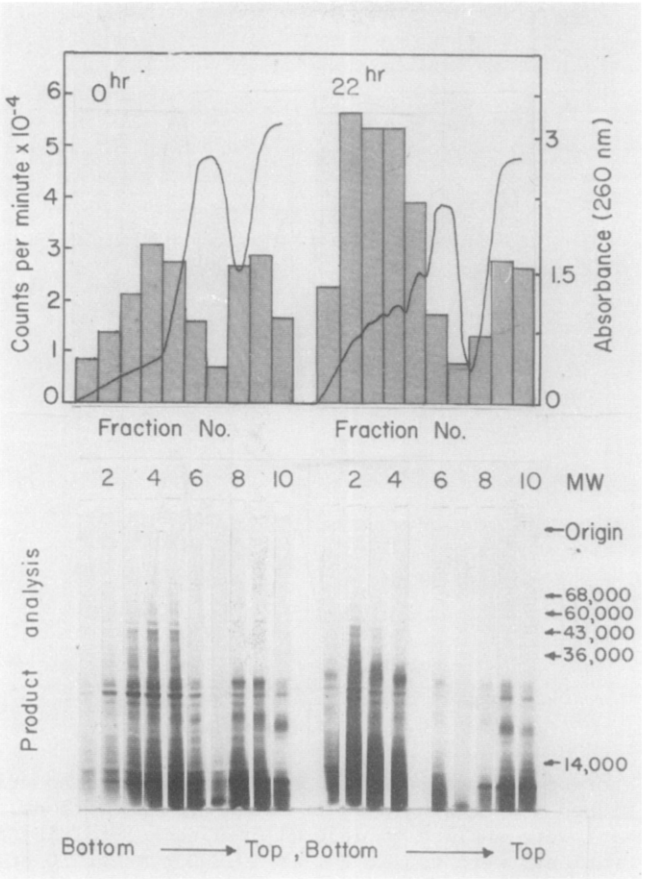

Fig. 2: A_{260} tracing of the gradient (solid lines) and histogram of template activity of total RNA extracted from 10-40% sucrose gradient fractions shown in Fig. 1. 5 µl of the reaction mixture were counted as in Fig. 1. The lower radioautograph represent translation products of the respective RNA fractions, as analyzed by SDS-polyacrylamide gradient gel electrophoresis.

DISCUSSION

The results presented suggest that the total mRNA pool from the postmitochondrial fraction of both dormant and developing A. salina cysts can be separated into two major groups; firstly, mRNA involved in polysomal complexes, which is more pronounced in the developed cysts, and secondly an mRNA sedimenting at 40S which may be in the form of a messenger ribonucleoprotein complex. This latter fraction comprises the majority of the mRNA molecules in undeveloped cysts. By analogy with the situation in unfertilized sea urchine eggs, where a putative

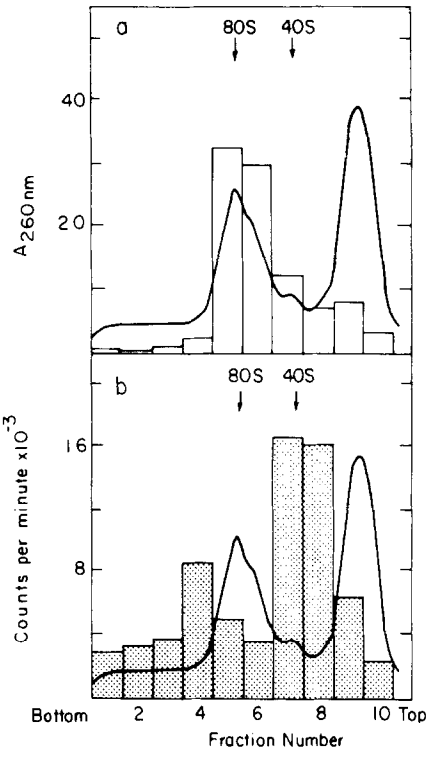

Fig. 3: 20-40% sucrose TMK gradient of 0 hr cysts postmitochondrial supernatant. 0 hr cysts were washed and extracted as in Materials and Methods, and 2 ml of the 12,000 g supernatant were applied to a 30 ml gradient containing a cushion of 100% glycerol. Gradients were run at 4°, at 20,000 rpm for 10 hr in the Spinco SW25 rotor. (a) Absorbance tracing of the gradient (solid line) and A_{260} of RNA extracted from the corresponding fractions, and suspended in 100µl H_2O. (b) mRNA activity of the corresponding RNA fractions, as assayed in the wheat germ system. 25 µl of each RNA sample was assayed in final volume of 50 µl of which 5 µl was counted.

40-60S mRNP complex (maternal mRNA) becomes translated only after fertilization (10), the 40S complex in Artemia may represent a stored form of biologically active mRNA.

Our results suggest that the lack of protein synthesis characteristic of the dormant stage of A. salina, is partially due to the occurence of a major fraction of the mRNA in a cryptic nonpolysomal state. In addition, the poor template activity exhibited by the minor polysomal region of undeveloped cysts (Fig. 1b) may be explained by the presence of an endogenous inhibitor, the existence of which has been shown by Huang and Warner (11).

The fate of the mRNA in the presumptive mRNP particles is not yet clear. It is probable that during cyst development there is a flow of mRNA from this fraction into polysomal complexes, together with de novo synthesized mRNA. Such a movement of mRNA from the pool of 40S complexes into the polysomal fraction, coupled with the gradual disappearance of the inhibitor could then trigger the resumption of protein synthesis characteristic of rehydrated cysts.

REFERENCES

1. Grosfeld, H. and Littauer, U.Z. The Israel Journal of Medical Sciences, in press.
2. Golub, A., and Clegg, J.S. (1968) Dev. Biol. 17, 644-656.
3. Clegg, J.S. and Golub, A.L. (1969) Dev. Biol. 19, 178-200.
4. Finamore, F.J. and Clegg, J.S. (1969) in "The Cell Cycle", G.M. Padillor, Editor, Academic Press, Inc. New York, 249-278.
5. Sierra, J.M., Meier, D. and Ochoa, S., (1974). Proc. Nat. Acad. Sci. U.S.A., 71, 2693-2697.
6. Aviv, H., and Leder, P. (1972). Proc. Nat. Acad. Sci., U.S.A. 69, 1408-1412.
7. Roberts, B.E. and Patterson, B.M. (1973). Proc. Nat. Acad. Sci. U.S.A. 70, 2330-2334.
8. Maizel, J.V. (1971). In Methods in Virology, eds. Maramorosch, K., and Koprowski, H. (Academic Press, New York) 5, 180-247.
9. Bonner, W.M. and Laskey, R.A. (1974). Eur. J. Biochem. 46, 83-88.
10. Gross, K.W., Jacobs-Lorena, M., Baglioni, C., and Gross, P.R. (1973). Proc. Nat. Acad. Sci. U.S.A. 70, pp. 2614-2618.
11. Huang, F.L., and Warner, A.H. (1974). Arch of Biochem. and Biophys. 163, 716-727.