TRANSLATION OF MESSENGER RNA SPECIFIC FOR TYROSINE AMINOTRANSFERASE IN OOCYTES OF XENOPUS LAEVIS*

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SUMMARY.— Injection of rat liver polysomal poly(A)-containing RNA into oocytes of Xenopus laevis results in synthesis of a protein whose immunochemical and electrophoretic characteristics are identical to liver tyrosine aminotransferase. Poly(A)-RNA from hydrocortisone-treated rat liver stimulates about five times more aminotransferase synthesis than does poly(A)-RNA from controls, establishing that the steroid hormone increases the level of functional hepatic tyrosine aminotransferase mRNA.

The induction of rat liver tyrosine-α-ketoglutarate aminotransferase [L-tyrosine:2-oxoglutarate aminotransferase (E.C.2.6.1.5)] has been extensively investigated since Lin and Knox first reported an increase in its activity following injection of rats with hydrocortisone (1). Increased aminotransferase activity in the liver is also stimulated by the polypeptide hormones insulin and glucagon (2). These changes in aminotransferase activity following hormone administration are due to enhanced enzyme synthesis (2). Indirect evidence (induction kinetics of each hormone, effects of different inhibitors on induction, and the complementarity of different inducers) has indicated that the steroid induces aminotransferase synthesis by increasing the functional mRNA level, whereas the peptide hormones act at some translational level (3). To verify conclusively these proposed hormonal mecha-

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nisms of action, an effective assay system to quantitate tyrosine aminotransferase mRNA is needed. Here, we report the translation of hepatic aminotransferase mRNA in oocytes of Xenopus laevis (4), and the use of this assay to show that during hydrocortisone induction there is an increase in the amount of active polysomal mRNA specific for tyrosine aminotransferase.

METHODS AND MATERIALS:

Hormone administration. Hydrocortisone (2.5 mg/100 g body weight) was injected intraperitoneally into fasted male rats three hr before killing.

Isolation of RNA. Total rat liver polysomes were isolated by detergent treatment of postmitochondrial supernatants, and centrifugation through a sucrose cushion (5). Poly(A)-containing RNA was isolated from total polysomes by two passages through oligo dT cellulose in high salt-SDS[1] (6).

Heterologous translation system. Twenty-three nl of poly(A)-RNA was microinjected into Xenopus laevis oocytes manually dissected free of ovarian tissue. The injected oocytes were cultured in salt medium (7) containing 1 mCi/ml [^{3}H]leucine for 18 hr at 19°C.

Characterization of the translation products. At the end of incubation the oocytes were washed with unlabeled incubation media, homogenized with a Teflon homogenizer in phosphate-buffered saline containing 5 mM α-ketoglutarate and 3 μg/ml pyridoxal-5'-phosphate to stabilize the newly synthesized tyrosine aminotransferase. Postribosomal supernatants were prepared, and the labeled aminotransferase was selectively precipitated using specific anti-aminotransferase antibody and carrier aminotransferase. These immunoprecipitated products were washed three times with phosphate-buffered saline containing 1% triton X-100 and sodium deoxycholate, then centrifuged over a 1 M sucrose cushion containing the detergents. The immunoprecipitates were then characterized by SDS-polyacrylamide gel electrophoresis (8). The amount of aminotransferase synthesized by the oocytes was determined by quantitation of the ^{3}H radioactivity peak migrating with the mobility of tyrosine aminotransferase subunit.

RESULTS AND DISCUSSION:

Incorporation of [^{3}H]leucine into total protein was linear during the incubation period (18 hr), and was stimulated only slightly in oocytes microinjected with hepatic poly(A)-RNA up to 30 ng/oocyte. However, the RNA-injected oocytes synthesized more radioactive proteins specifically immunoprecipitable by anti-aminotransferase and carrier enzyme, compared to the buffer-injected control oocytes. For further characterization, these immunoprecipitates were analyzed by SDS-polyacrylamide gel electro-

[1] The abbreviation used is: SDS, sodium dodecyl sulfate.

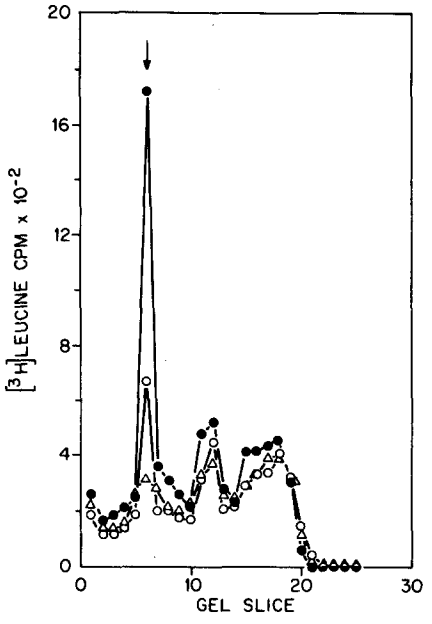

Figure 1

SDS-polyacrylamide gel electrophoretic analysis of oocyte-translation products immunoprecipitated with specific anti-tyrosine aminotransferase antibody and carrier aminotransferase. Each oocyte was injected with 7 ng poly(A)-containing RNA isolated from polysomes of uninduced (o—o) or hydrocortisone-induced (•—•) rat liver. A second set of control oocytes was injected with an equal volume (23 nl) of injection buffer alone (Δ—Δ). Direction of migration is from left to right. Bromophenol-blue tracking dye migrated to slice 17; the arrow indicates the position of the 50,000 molecular weight subunits of tyrosine aminotransferase (TAT) under these conditions.

phoresis (Fig. 1). The ^{3}H radioactivity gel profile of the immunoprecipitates from oocyte samples injected with liver mRNA shows a major peak with mobility identical to that of authentic tyrosine aminotransferase subunits, whereas this peak is absent from control oocytes injected with buffer alone.

In addition, the relative size of this major peak is greatly increased in gel profiles of immunoprecipitates from oocytes injected with an equivalent amount of poly(A)-RNA from hydrocortisone-treated livers. There are two additional peaks, whose presence in equal amounts in all three profiles suggests that they represent contamination coprecipitating with the immune

TABLE I. SPECIFICITY OF IMMUNOPRECIPITATION REACTION

RNA INJECTED	CPM PRECIPITATED[1] BY	
	Anti-Ovalbumin γ- Globulin	Anti-Tyrosine Aminotransferase γ-Globulin
Rat Liver	100	8480
Hen Oviduct	15,398	60

[1]Calculated from the ^{3}H radioactivity migrating on gels in the position of authentic aminotransferase subunits and of ovalbumin. Background radioactivities of 150-200 CPM were subtracted.

complex. These major contaminations were expected since immunoprecipitations were carried out on total crude translated proteins (cf. ref. 9). Further control immunoprecipitations were done to insure that the oocyte translated protein which electrophoretically migrates as authentic aminotransferase subunit and which is precipitable by specific anti-TAT antibody, is indeed tyrosine aminotransferase. Proteins that were synthesized by oocytes programmed by injection of polysomal RNA from hen oviduct or rat liver were precipitated by either anti-tyrosine aminotransferase or anti-ovalbumin with their respective carrier antigens. The results (Table I) clearly demonstrate that aminotransferase or ovalbumin was detectable only by immunoprecipitation with their corresponding antibodies, and only from oocyte samples injected with RNA of the proper source.

RNA titrations were run on control and induced samples of rat liver mRNA to assess more clearly their relative abilities to stimulate the synthesis of aminotransferase (Fig. 2). Serial dilutions of RNA were injected into oocytes, and the radioactivity incorporated into aminotransferase was quantitated as already described. For an equivalent amount of RNA injected, the induced sample stimulated the synthesis of approximately fivefold more tyrosine aminotransferase than the control.

amount of functional tyrosine aminotransferase mRNA on polysomes.

Steroid hormone regulation of cellular RNA has been reported for several other steroid-dependent proteins (5,11,12). We present evidence that tyrosine aminotransferase mRNA in rat liver is also regulated by the steroid hormone hydrocortisone. The increased rates of aminotransferase synthesis during hydrocortisone induction can be attributed to the hormone's increasing the level of functional mRNA encoding the enzyme.

Success in the translation of various mammalian mRNA's in different heterologous cell-free systems has been reported (13). However, the in vivo oocyte system was the only heterologous system that in our hands faithfully translated tyrosine aminotransferase mRNA. Using the immunochemical approach described here, we were unable to identify the presence of aminotransferase in translation products of wheat germ or reticulocyte lysate systems. These systems are currently being investigated to see if they are simply not able to translate tyrosine aminotransferase mRNA, or if the aminotransferase translation product in these systems cannot be identified by techniques currently used.

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REFERENCES:

1. Lin, E. C. C. and W. E. Knox, Biochim. Biophys. Acta, 26: 85-88 (1957).
2. Kenney, F. T., J. R. Reel, C. B. Hager and J. L. Wittliff, In: Regulatory Mechanisms for Protein Synthesis in Mammalian Cells (San Pietro, A., Lamborg, M. R., and Kenney, F. T., eds.) pp. 119-142, Academic Press, N. Y. (1968).
3. Lee, K.-L., J. R. Reel and F. T. Kenney, J. Biol. Chem., 245: 5806-5812 (1970).
4. Gurdon, J. B., C. D. Lane, H. R. Woodland and G. Marbaix, Nature, 233: 177-182 (1971).

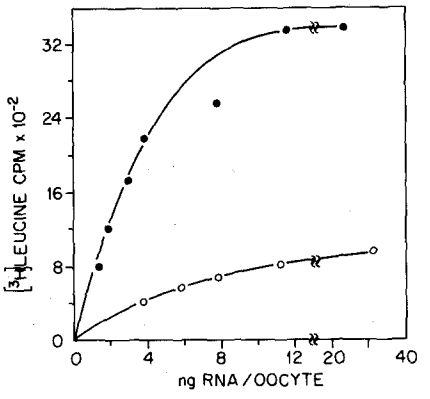

Figure 2

Titration of poly(A)-containing RNA isolated from polysomes of control and hydrocortisone-induced rat liver. Serial dilutions of RNA were injected into oocytes, and translation products were immunoprecipitated by specific and anti-aminotransferase antibody. The amount of aminotransferase synthesized was calculated from the amount of ^{3}H radioactivity migrating to the position of authentic aminotransferase subunit upon SDS-polyacrylamide gel electrophoresis of the immunoprecipitates. Control RNA o—o; hydrocortisone induced RNA •—•.

It is impossible to measure the total translational ability of RNA preparations by injecting them into Xenopus oocytes because in these cells there is an insignificant change in total protein incorporation in response to injected RNA. Therefore, the ability of rat liver polysomal poly(A)-RNA to stimulate total protein synthesis was analyzed in a wheat germ heterologous translation system (10). The two mRNA preparations used in the experiments reported in Fig. 2 were found to have essentially identical total messenger activity when assayed in the wheat germ system. The specific activity of control RNA was 8.8×10^{5} CPM [^{3}H]leucine incorporated/µg RNA and that of the hydrocortisone-treated preparation was 8.2×10^{5} CPM/µg RNA. Since the yield of polysomal poly(A)-RNA from control and induced rat liver is identical, we conclude that the differential abilities of the two RNA preparations to direct aminotransferase synthesis in the oocyte is not because hydrocortisone increased total mRNA, but because the hormone selectively increased the

5. Schimke, R. T., R. Palacios, D. Sullivan, M. L. Kiely, C. Gonzales and J. M. Taylor, In: Methods in Enzymology, Vol. XXX (Moldove, K., and Grossman, L., eds.) pp. 631-648, Academic Press, N. Y. (1974).
6. Kabat, D., J. Biol. Chem., 250: 6805-6809 (1975).
7. Wallace, R. A., D. W. Jared, J. N. Dumont and M. W. Sega, J. Exp. Zool., 184: 321-333 (1973).
8. Weber, K. and M. Osborn, J. Biol. Chem., 244: 4406-4412 (1969).
9. Lee, K.-L. and F. T. Kenney, Acta Endocrin., (Kbh), Supp. 153: 109-125 (1971).
10. Marcu, K. and B. Dudock, Nucl. Acids Res., 1: 1385-1397 (1974).
11. Schutz, G., L. Killewich, G. Chen and P. Feigelson, Proc. Nat. Acad. Sci. U.S.A., 72: 1017-1020 (1975).
12. Sarkar, P. K. and B. Griffith, Biochem. Biophys. Res. Commun., 68: 675-681 (1976).
13. Brawerman, G., In: Annual Rev. Biochem., 43: 621-642 (1974).