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Translation of phenylalanine hydroxylase-specific mRNA *in vitro*: Evidence for pretranslational control by glucocorticoids

(*in vitro* protein synthesis/wheat-germ lysate/gene expression/liver enzyme/rat hepatoma cells)

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ABSTRACT **We have found that the induction of phenylalanine hydroxylase by hydrocortisone and serum in confluent cultures of H4-II-E-C3 rat hepatoma cells is accompanied by an increase in polysomal mRNA specific for phenylalanine hydroxylase, as measured by translation in a cell-free protein-synthesizing system. Thus, the induction is mediated largely, if not entirely, by a pretranslational mechanism, possibly by stimulation of the transcription of the phenylalanine hydroxylase gene.**

It has been shown that two cultured rat hepatoma cell lines, H4-II-E-C3 (H_4) and MH_1C_1, contain phenylalanine hydroxylase but at a very low level (1). Addition of hydrocortisone or dexamethasone at submicromolar concentrations and of steroid-free blood serum, from diverse mammalian species, to confluent cultures of the H4 cells caused an increase in the hydroxylase content of the cells to levels comparable with those found in normal rat liver (1, 2). This increase resulted from an increase in the amount of hydroxylase protein and not from activation of an inactive (pro)enzyme (3). The stimulation by glucocorticoids was shown to be mediated by an increase in the rate of the synthesis of the enzyme (4) without any change in the rate of its degradation (5).

By the use of a cell-free protein-synthesizing system of wheat germ and specific antiserum against rat liver phenylalanine hydroxylase, we now show that the induction of the hydroxylase by hydrocortisone and serum in H4 cells is associated with an increase of polysomal mRNA specific for phenylalanine hydroxylase ($mRNA_{phx}$).

METHODS AND MATERIALS

Cell Cultures and Assay for Phenylalanine Hydroxylase. H4 cells were grown to confluency in 75-cm^2 flasks (Lux Scientific, Thousand Oaks, CA) in modified Swim's medium S-77 supplemented with 5% (vol/vol) fetal bovine and 10% horse sera (1, 2). The harvesting and extraction of postconfluent cultures for assay of phenylalanine hydroxylase by the method of Ayling *et al.* (6) have been described (1–3). One enzyme unit is defined as the phenylalanine-dependent oxidation of 1 nmol of 6,7-dimethyltetrahydropterin to 6,7-dimethyldihydropterin or the formation of 1 nmol of tyrosine per min at 27°C. Protein concentrations were measured by dye-binding (7).

To prepare the basal and fully induced cells used for the preparation of poly(A)$^+$RNA (see Table 1 and Fig. 2), postconfluent cultures of H4 cells were first washed with modified S-77 medium and then given either the same medium without serum and hydrocortisone or serum-containing medium supplemented with 1 μM hydrocortisone. The cells were harvested 68–160 hr later. Such long exposure of the H4 cells to serum-free medium, or to supplemented medium results in the decay of phenylalanine hydroxylase to basal levels under the former conditions and in full induction of the enzyme under the latter (1–3).

For labeling of proteins in intact cells and subsequent immunoprecipitation of [^{14}C]phenylalanine hydroxylase from extracts of the cells, ten 75-cm^2 flasks were inoculated with 1.6×10^7 cells per flask into 20 ml of growth medium supplemented with 2 μCi (1 Ci = 3.7×10^{10} becquerels) of L-[U-^{14}C]leucine and 1 μM hydrocortisone. This labeling medium was renewed after 68 hr. The cells were harvested and extracted after 113 hr. The incorporation of ^{14}C into total soluble cellular protein was $\approx$0.35 μCi per flask; of this, $\approx$3% could be precipitated by antiserum against rat liver phenylalanine hydroxylase.

Isolation of Total Polysomal Poly(A)$^+$RNA. Poly(A)$^+$RNA was isolated from disrupted H4 cells by a combination of previously described methods (8–10) with minor modifications, such as the use of Hepes for all buffers. A total of 12 preparations was made; each was derived from cells pooled from sets of 10, 25, 30, or 32 similarly grown cultures. Nine preprations were made from induced cells and three were made from basal cells. The cells were harvested with trypsin (1), washed with ice-cold buffer A [25 mM Hepes, pH 7.6/100 mM KCl/40 mM NaCl/7.5 mM $MgCl_2$/50 mM NH_4Cl/6 mM 2-mercaptoethanol/heparin (0.5 mg/ml)/yeast tRNA (1.5 mg/ml)] and pelleted by centrifugation. They were lysed at 0°C for 20 min with 0.5% Triton X-100 in buffer A (5 ml for cells from 10 cultures); the lysates were centrifuged at 30,000 $\times$ *g* for 10 min. The supernatants were fortified with heparin to 3.0 mg/ml and with Triton X-100 and sodium deoxycholate to 1% each. Four milliliters of the mixtures were layered over 2.5 ml of 2.0 M sucrose + 3.5 ml of 0.5 M sucrose, each in 50 mM Hepes, pH 7.6/5 mM $MgCl_2$/2.5 mM NaCl/heparin, (50 μg per ml), and these systems were centrifuged at 120,000 $\times$ *g* at 4°C for 17–20 hr. The sucrose layers were removed, and the sides of the centrifuge tube(s) and the top(s) of the pellet(s) were gently rinsed with 2 ml of buffer A. The pellet(s) of the polysomes, derived from cells of 10 cultures, were suspended in 1 ml of buffer A, and the suspensions were diluted with 8 ml of buffer B (0.1 M Hepes, pH 7.6/1 mM disodium EDTA/0.5 M NaCl/0.5% $NaDodSO_4$) and 1 ml of 1% $NaDodSO_4$. The yields of polysomes from induced and basal cells were similar: 94 $\pm$ 9 A_{260} units/g of fresh cells. (Ten 75-cm^2 cultures give $\approx$0.7 g of cells.) The suspensions of polysomes (10 ml) were warmed to 37°C for 5 min, and the warm mixtures were applied to 50-mg oligo(dT)-cellulose columns

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Abbreviations: H4, H4-II-E-C3; hydrocortisone, hydrocortisone 21-sodium succinate; $mRNA_{phx}$, phenylalanine hydroxylase-specific mRNA.

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presaturated with yeast tRNA (10). Columns were first eluted with buffer B until all material absorbing at 260 nm disappeared from the eluate (15–20 ml). This step was followed by elution of the poly(A)$^+$RNA with buffer C (10 mM Hepes, pH 7.6/1 mM disodium EDTA/0.5% $NaDodSO_4$). The fractions absorbing at 260 nm were pooled, and the RNA was precipitated in the presence of 0.2 M NaCl with 2 vol of EtOH at −20°C overnight. This still-impure poly(A)$^+$RNA represented 2.4 ± 0.6% of the total polysomal RNA (87 ± 14 μg/g of fresh cells). The precipitated RNA was dissolved in 50 μl of buffer C without $NaDodSO_4$, and 50 μl of 10 mM Hepes, pH 6.5/1 M LiCl/50 mM disodium EDTA/2% $NaDodSO_4$ and 450 μl of Me_2SO were added (11). The mixture was heated at 55°C for 5 min; 550 μl of 1 M NaCl was added and the sample was then quickly cooled to room temperature. The mixture was diluted with 4 ml of buffer B without $NaDodSO_4$, which was also the first elution buffer in the second chromatography of the RNA on the oligo(dT)-cellulose column. The fraction eluted by the low-salt buffer (buffer C without $NaDodSO_4$) represented 62 ± 6.8% of the RNA put on the column [1.4 ± 0.7% of the total polysomal RNA (54 μg/g of cells)]; this RNA was precipitated as described above with EtOH and stored as a suspension at −20°C; it maintained its template activity without loss for several months.

Preparation of Wheat-Germ Lysate. Fresh wheat germ, obtained from General Mills (Vallejo, CA), was ground and extracted as described by Marcu and Dudock (12), except that $CaCl_2$ was omitted from the extraction buffer. The 30,000 × *g* supernatant of the extract was then processed according to Andersen and Weser (9), except that $CaCl_2$ was omitted from the preincubation buffer. Our yields of active lysates were similar to those given by the latter authors: 2–3 ml from 5 g of wheat germ [a total of 110–180 A_{260} units and an A_{260}/A_{280} ratio of 1.51 ± 0.02 (n = 8)]. The lysates were kept in small batches in liquid N_2 for up to 6 months.

Translation of Poly(A)$^+$RNA. The optimum composition of the incubation mixture for the stimulation of total protein synthesis by the poly(A)$^+$RNA from H4 cells with the wheat-germ lysates was 1.5 mM ATP/0.5 mM GTP/2.5 mM Mg^{2+}/65 mM K^+/50 μM spermine/2 mM dithiothreitol/15 mM creatine phosphate/ 0.1 mg of creatine phosphokinase (2.5 units per ml)/ 1.5 μM L-[^{3}H]leucine, (210 μCi/ml)/19 other natural amino acids at 0.5 mM each/10–15 mM Hepes, pH 7.5. The components were made up in two "master" mixes; their pH was adjusted to 7.6 with 1 M KOH just before use. The reaction volumes were 25–200 μl, of which one-fifth was the wheat-germ lysate (i.e., 5–40 μl), which was added to the other reagents 1 min before initiating reaction by adding the RNA. Just before the experiments, the purified RNA suspensions were centrifuged and the pellets were washed 3 times with small volumes of EtOH, dried under N_2, and dissolved at the desired concentration in 20 mM Hepes (pH 7.5) (1.0 A_{260} unit × cm^{-1} = 40 μg of RNA/ml). Unless otherwise indicated, translations were carried out at RNA concentrations of 12 or 13 μg/ml at 28°C in sterile 1.5-ml Eppendorf tubes for 60 min. The reactions were stopped by chilling the tubes to 0°C and adding unlabeled L-leucine to 1 mM.

Analysis of Translation Products. To determine the total protein synthesis, one-tenth of the reaction mixture was added to 1 ml of 1 mM L-leucine in 0.1% bovine serum albumin. After addition of 0.15 ml of 100% (wt/vol) trichloroacetic acid to this sample, the tRNA was deacylated at 90°C for 10 min (12), and the protein was precipitated at 0°C for 30 min. The precipitate was collected on a glass-fiber filter (Whatman, GF/C), washed extensively with 10% trichloroacetic acid containing 1 mM L-leucine, and dried with EtOH. The filters were assayed in 10 ml of Scinti Verse (Fisher) in a Packard Tri-Carb scintillation spectrometer. To determine the incorporation of label into "spontaneously" released peptides, the reaction mixture was centrifuged at 165,000 × *g* for 1 hr at 15°C and the same procedure was carried out on the supernatant.

Immunoprecipitation of Phenylalanine Hydroxylase. For the immunological identification of phenylalanine hydroxylase, either from extracts of H4 cells grown in the presence of [^{14}C]leucine or from the *in vitro* experiments with wheat-germ lysates after translation of the hepatoma poly(A)$^+$RNA, we used rabbit antiserum against rat liver phenylalanine hydroxylase that had been purified by a modification (13) of the method of Cotton and Grattan (14). This antiserum gave a single precipitin line on Ouchterlony plates with extracts from either basal or induced H4 cells, in accord with our earlier observations (3). Immunotitrations involving precipitation of a constant amount of the hydroxylase from extracts of H4 cells by increasing volumes of the antiserum gave a straight line with a single slope. Ten microliters of the unfractionated antiserum precipitated 3 units (≈2 μg of pure enzyme)† of phenylalanine hydroxylase from extracts of H4 cells. In all immunoprecipitations, this titration equivalent of the antiserum was used.

To precipitate the newly synthesized [^{3}H]hydroxylase from the *in vitro* translation experiments, we added a measured amount of a standard extract of fully induced unlabeled H4 cells as carrier to the nine-tenths of the reaction mixture remaining after removal of the one-tenth used for determination of total protein synthesis. In experiments I and II (see Table 1), 3 units (10 μl) of unlabeled H4 enzyme and, in experiment III, 21 units (70 μl) of unlabeled enzyme were added as carriers, and 10 and 70 μl, respectively, of antiserum were used for the overnight precipitation of phenylalanine hydroxylase at 0°C. Appropriate portions of the extracts of fully induced H4 cells labeled with [^{14}C]leucine in culture were similarly treated. Controls were set up with nonspecific (preimmune) serum plus goat anti-rabbit IgG. The precipitates were sedimented in an Eppendorf microfuge, washed 3 times with 0.1% sodium deoxycholate containing 1 mM L-leucine in phosphate-buffered saline, and suspended in 20 μl of 62.5 mM Tris·HCl buffer, pH 6.8/5% (vol/vol) 2-mercaptoethanol/2% $NaDodSO_4$. The mixture was heated at 100°C for 3 min.

Electrophoresis and Radioautography. To analyze the total translation products and immunoprecipitates by $NaDodSO_4$/polyacrylamide gel electrophoresis, the method of Laemmli (15) was used with a discontinuous buffer system in an apparatus similar to that described by Studier (16). The stacking gel was 4.5% and the running gel was 7.5%. The gel slabs were prepared and dried for radioautography on Kodak XR-P5 films as described by Andersen and Weser (9). The films were developed after 4–6 weeks of exposure at −70°C. The developed films were scanned by using a digitized densitometer (Optronics, Photoscan, Model P-1000), and the data were fed into a Digital Equipment Corporation Model PDP 11/40 computer programmed to display the density profiles directly and to integrate selected regions of the scans to obtain their intensities corrected for background.

Materials. All items for cell culture were obtained from the commercial sources cited earlier (1–3). The purity of the L-[U-^{14}C]leucine (>300 Ci/mol) and L-[3,4,5-^{3}H]leucine (142 Ci/mmol), New England Nuclear, was verified by thin-layer chromatography on preactivated (110°C, 30 min) microcrystalline cellulose plates (Applied Science Laboratories, State College, PA) developed in butanol/acetic acid/water (25:4:1). Oligo(dT)-

† Calculated on the assumption that the specific activity of the pure phenylalanine hydroxylase of H4 cells is similar to that of the rat liver enzyme (i.e., ≈1500 units/mg).

cellulose (T-3) was from Collaborative Research (Waltham, MA); ATP, GTP, creatine phosphate, and creatine phosphokinase were from Boehringer Mannheim; items for electrophoresis and sterile micropipette tips were from Bio-Rad; Hepes, unlabeled amino acids, dithiothreitol, and spermine were from Calbiochem; Ouchterlony immunodiffusion plates were from Hyland Laboratories (Costa Mesa, CA); and heparin, NH_4Cl, LiCl, yeast tRNA, ribonuclease-free sucrose, Tris base, dimethylsulfoxide, 2,5-diphenyloxazole, and Coomassie brilliant blue G-250 and R-250 were from Sigma.

RESULTS

Characterization of the Translation System. The wheat-germ lysates, prepared and supplemented as described in *Methods and Materials* incorporated only small amounts of [^{3}H]leucine into protein in the absence of added RNA. The inclusion in the reaction mixture of the purified poly(A)$^+$RNA from the H4 cells increased general protein synthesis 10- to 20-fold above the levels unprimed by RNA. This stimulation was a linear function of time for at least 60 min (Fig. 1*A*) and RNA concentration up to 16–20 μg/ml. The biphasic nature of the stimulation by mRNA (Fig. 1*B*) with two linear portions of different slopes, is almost identical with the profile reported by Sala-Trepat *et al.* (8) for rat liver poly(A)$^+$RNA. The temperature optimum for the reaction was 28°C (Fig. 1*C*) for either total protein synthesis or the spontaneous release of proteins after chain termination. The optimum concentrations of individual components for protein synthesis in the wheat-germ system were determined in numerous pilot experiments, all carried out at an RNA concentration of 20 μg/ml at 28°C for 60 min. We found that Na^+ ions in the incubation mixtures severely inhibited protein synthesis; hence, all upward adjustments of pH were made with KOH. The concentration of K^+ ions was also critical; 65–70 mM gave the best results.

Comparison of Translation of Poly(A)$^+$RNA (mRNA) from Induced and Basal H4 Cells. After the optimum conditions for the translation of mRNA from H4 cells were determined, we carried out three sets of experiments to compare the translation of the mRNAs isolated from fully induced and basal H4 cells. In each set, 30 or 32 confluent cultures were maintained in growth medium [containing 15% (vol/vol) serum] supplemented with 1 μM hydrocortisone and the same number were incubated in serum-free (i.e., basal) medium without the hormone. After harvesting and pooling the cells from identically maintained cultures, we set aside a portion of the cells for the preparation of extracts for assaying the phenylalanine hydroxylase level (1) and used the remainder for the preparation and purification of the polysomal poly(A)$^+$RNAs. The latter were then used for translation in replicate incubations. After samples were taken for measuring incorporation of label into total proteins, the remainders of the replicate incubations were pooled; a measured amount was taken for analysis of the total translation products by $NaDodSO_4$/polyacrylamide gel electrophoresis and the rest was used for immunoprecipitation (see Fig. 2).

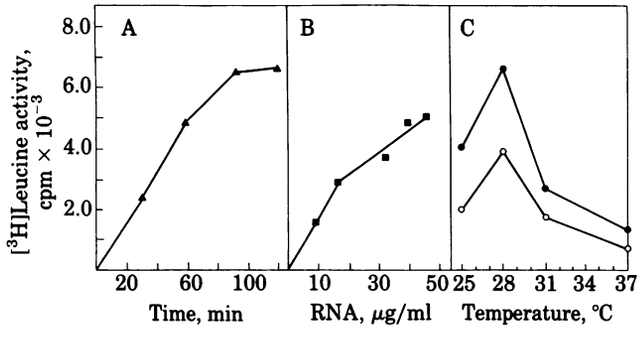

FIG. 1. Characteristics of the translation of poly(A)$^+$RNA isolated from fully induced H4-II-E-C3 hepatoma cells by wheat-germ lysates. All three sets of experiments were carried out with the same wheat-germ lysate (WG 1) in 25-μl incubation mixtures with Mg^{2+} at 2.5 mM and K^+ at 71 mM. (*A*) Effect of incubation time on net incorporation of [^{3}H]leucine into total protein. Room temperature (21 ± 1°C); mRNA at 20 μg/ml. (*B*) Effect of RNA concentration on net incorporation of [^{3}H]leucine into total protein. Room temperature; 30 min of incubation. (*C*) Effect of incubation temperature on [^{3}H]leucine incorporation into total protein (●) and into spontaneously released protein (○) in the 165,000 × *g* × 1 hr supernatant of the reaction mixture. RNA at 8.4 μg/ml; 90 min of incubation. Unprimed (endogenous) values were 298–497 cpm.

The results of the three experiments are shown in Table 1 and Fig. 2. There was no significant difference in the total protein synthesis directed by the purified mRNA from basal or induced H4 cells, as judged by the radioactivity present in proteins precipitated by trichloroacetic acid. Examination of the total translation products by $NaDodSO_4$/polyacrylamide gel electrophoresis and radioautography of the gels also showed similar discrete radioactive bands in the two preparations, mostly in the molecular weight range of and below 50,000, although, in the specimen from the translation of the mRNA from the induced cells, there were also a few bands at higher molecular weights (see Fig. 2*B*, lanes 5 and 6).

When the antiphenylalanine hydroxylase serum was used for immunoprecipitation of either the spontaneously released or the total translation products and the immunoprecipitates were similarly analyzed, radioactive bands were seen mainly at a migration corresponding to molecules of ≈50,000 daltons (see Fig. 2 *A*, lanes 3 and 4, and *B*, lanes 2 and 3). The same 50,000-dalton species was also immunoprecipitated from extracts of fully induced H4 cells grown in the presence of [^{14}C]leucine (see Fig. 2 *A*, lanes 1, 2, and 5, and *B*, lane 1). We believe that the translation product of the phenylalanine hydroxylase-specific mRNA ($mRNA_{phx}$) is represented by the incorporation of radioactive amino acid exclusively into the 50,000-dalton species; the subunit of rat liver phenylalanine hydroxylase has a mass of 50,000 to 55,000 daltons [refs. 13, 17, and our observations; the most recent value is 49,000 ± 2000 (18)], and this molecular species is the only one coded by the hepatoma cell message that is markedly concentrated by precipitation with the specific antiserum. Moreover, the labeled products of smaller molecular size, present in lesser amounts in the immunoprecipitates, are probably not related to the hydroxylase; they were also seen in the immune complex formed between control (preimmune) serum and goat anti-rabbit IgG (see Fig. 2 *A*, lane 6, and *B*, lane 4).

Comparison of lanes 3 and 4 in Fig. 2*A* and of lanes 2 and 3 in Fig. 2*B* shows that the $mRNA_{phx}$ activity of the fully induced cultures was substantially greater than that of the basal cultures. This visual impression was also borne out by the computerized densitometric scanning of the 50,000-dalton bands in the radioautographic films. The integrated values of the densities of these bands, giving relative estimates of the radioactivities they contained, are shown in Table 1. In experiment I, the immunoprecipitates were made from the spontaneously released proteins in the 165,000 × *g* 1-hr supernatant of the incubation mixtures but, in the other two experiments, the total reaction mixtures were used for immunoprecipitation. The ratio of the values from incubations a and b of experiment I was almost identical with the ratio of the enzyme levels measured in the induced and basal cultures. In the other two experiments, the ratios of the *in vitro* translations of the $mRNA_{phx}$ were also the same order of magnitude as the ratios of the enzyme levels in the cultures from which the mRNAs were prepared. These obser-

Table 1. Translation of poly(A)$^+$RNA (mRNA) from induced and basal H4 cells and phenylalanine hydroxylase content of the cultures

Experiment (wheat-germ lysate)	Type of culture	*In vitro* translation: Incubation vol, μl	mRNA, μg/ml	Total protein synthesis,* cpm × 10^{-3}	$mRNA_{phx}$ activity†	a/b	Phenylalanine hydroxylase content: Enzyme units/g of fresh cells	a/b
I (WG 1)‡	Induced (a)	100	12.5	63 ± 10	1700		770	
	Basal (b)	100	12.5	67 ± 7	110	15.5	49	15.7
II (WG 5a)§	Induced (a)	100	12.8	317 ± 6	1092		490	
	Basal (b)	100	12.8	325 ± 9	75	14.6	61	8.0
III (WG 6-1)‡	Induced (a)	154	12.5	432 ± 87	249		717	
	Basal (b)	154	12.5	332 ± 19	32	7.8	122	5.9

In vitro translation incubations were for 60 min.
* Mean ± SD.
† Expressed as density of 50,000-dalton band in autoradiogram in arbitrary units.
‡ $n = 3$.
§ $n = 2$.

vations are consistent with the conclusion that the combined stimulatory effect of serum plus hydrocortisone on the phenylalanine hydroxylase content of the H4 cells was mediated solely by an increase in polysome-associated $mRNA_{phx}$ activity. Analogous results were recently reported by Olson *et al.* with respect to the induction of tyrosine aminotransferase by glucocorticoid hormone in the HTC rat hepatoma cell line (19).

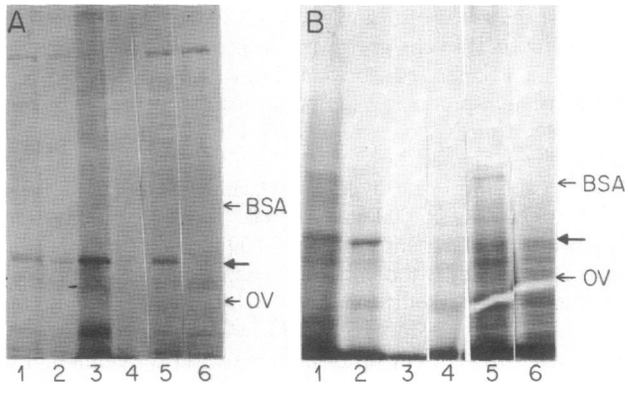

FIG. 2. Analysis of immunoprecipitates and total mRNA-translation products by $NaDodSO_4$/polyacrylamide gel electrophoresis and radioautography. (*A*) Lanes: 1, 2, and 5, proteins precipitated by antiphenylalanine hydroxylase serum from extracts of fully induced H4 cells grown in the presence of [^{14}C]leucine in culture; 3 and 4, identical volumes of spontaneously released proteins precipitated by antiphenylalanine hydroxylase serum from translations of poly(A)$^+$RNAs isolated from fully induced and basal H4 cells, respectively [material from incubations a and b, respectively, of experiment I (see Table 1)]; 6, same material as in lanes 1, 2, and 5 except that precipitation was by control (preimmune) serum plus goat anti-rabbit IgG. Film exposed for 3 weeks. (*B*) Lanes 1, same as lanes 1, 2, and 5 in *A*; 2 and 3, material similar to lanes 3 and 4 in (*A*) except that the samples were from incubations a and b, respectively, of experiment II (see Table 1); 4, same as lane 1 except that precipitation was by control (preimmune) rabbit serum plus goat anti-rabbit IgG; 5 and 6, total translation products from incubations a and b of experiment II. Heavily labeled bands at the bottom of each lane represent residual free radioactive amino acids that were incompletely removed even from washed immunoprecipitates. The white swathe in lanes 5 and 6 resulted from a crack in the gel during drying. Film exposed for 4 weeks. Thick arrows indicate M_r 50,000 regions (in lane 4 of *A* and lane 3 of *B*, these bands are barely perceptible, although they were easily detected on the original films). OV, ovalbumin; BSA, bovine serum albumin.

DISCUSSION

We have translated the polysomal poly(A)$^+$RNA of cultured rat hepatoma cells to assess in an uncommitted heterologous cell-free protein-synthesizing system the functional status of the phenylalanine hydroxylase-specific message undergoing active translation in the intact cell. Recognizing that the gene transcript of this cytoplasmic enzyme was likely to be only a small fraction of the total cellular message, we chose wheat germ rather than rabbit reticulocytes as the source of the cell-free system because of the low levels of endogenous message in the former (12) and its superior protein-synthetic power under the direction of exogenous poly(A)$^+$RNA (our observations; see also ref. 20, figure 6).

From the results, we conclude the following: (*i*) wheat-germ lysates are able to translate $mRNA_{phx}$ with fidelity, as judged by the serologic and physical-chemical properties of the phenylalanine hydroxylase subunit synthesized (see Fig. 2) and (*ii*) elevation of cellular hydroxylase levels after exposure of intact cells to both glucocorticoid and serum is accompanied by a marked increase in polysomal $mRNA_{phx}$ template activity without measurable change in total mRNA content or translational capability.

The overall template activity of a specific polysome-associated mRNA could be elevated by any of several different mechanisms, the possibilities for at least some of which have already been established in other systems. $mRNA_{phx}$ could accumulate throughout the entire cytoplasm as the result of some extranuclear event, such as a decrease in the rate of $mRNA_{phx}$ degradation (21). Alternatively, this accumulation of specific mRNA could be brought about by changes in the nucleus, such as a stimulation of gene transcription (i.e., the rate of synthesis of heterogeneous nuclear RNA, the primary gene product) (22), an inhibition of degradation of heterogeneous nuclear RNA, or an increase in the efficiency of processing of heterogeneous nuclear RNA into poly(A)$^+$RNA or in the rate of export of the latter into the cytoplasm (23). Conversely, the total amount of $mRNA_{phx}$ might remain constant, with stimulation of phenylalanine hydroxylase synthesis being mediated by the recruitment of potentially translatable, but previously sequestered, $mRNA_{phx}$ into polysomes from an extrapolysomal site (20, 24, 25). Finally, it is possible to envision an increase in the rate of specific enzyme synthesis as occurring through a fully reversible alteration in mRNA structure, analogous to enzymic activation through an induced conformational change resulting from al-

losteric interaction with a specific ligand. Although we are unaware of any precedent for this last possibility,‡ such a mechanism would not be distinguished from specific mRNA accumulation by a cell-free translation assay but rather would require for its detection the technique of molecular hybridization. With regard to these various possibilities, it is likely, through analogy with other systems (22), that the pretranslational effect of glucocorticoid is mediated by an increase in specific gene transcription; but whether the mechanisms of action of glucocorticoids and the nonsteroidal serum factor in elevating $mRNA_{phx}$ activity are identical or not is not known at present.

We are much indebted to Mr. Herb Sanguinetti of General Mills for the generous gift of wheat germ and to Dr. Robert Andersen for instructing us in the preparation of wheat-germ lysate and in the optimization of the translation reaction, as well as for assistance with the initial analyses of our results by radioautography and densitometry. In addition, we wish to thank Dr. James McGinnis for the temporary use of electrophoresis equipment and for fruitful discussions during the course of the work and Dr. James Bonner for his interest and helpful suggestions. We also thank Dr. J. Michael Anchors for his assistance in our early attempts at characterizing the protein-synthesizing systems from wheat germ and rabbit reticulocytes and Dr. Jasim Al-Janabi for the preparation of the rabbit antiphenylalanine hydroxylase serum. The computerized densitometric scanner was the property of the UCLA Molecular Biology Institute. This work was supported by U.S. Public Health Services Grant HD 06576 from the National Institute of Child Health and Human Development.

‡ The most relevant example appears to be the alteration in mRNA structure proposed by Farmer *et al.* (20) as one possible explanation for the diminished translatability in two heterologous *in vitro* protein-synthesizing systems observed with the poly(A)+ RNA derived from quiescent anchorage-dependent 3T6 cells after their prolonged cultivation in suspension.

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