SIMULTANEOUS TRANSLATION OF GROWTH HORMONE AND PROLACTIN MESSENGER RNA FROM RAT PITUITARY TUMOR CELLS

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Received 3 September 1976

1. Introduction

Cultured cells derived from transplantable rat pituitary tumors constitute and interesting model system to investigate the various steps involved in the synthesis and secretion of polypeptide hormones [1]. Some of these cell lines (e.g., GH_3) are known to synthesize and to secrete growth hormone (GH) and prolactin (PRL). Other hormones, such as triiodothyronine, thyrotropin releasing hormone, estrogens, and hydrocortisone have been shown to control GH and/or PRL production by GH_3 cells [1–5]. How these hormones effectively regulate specific gene expression in GH_3 cells could be studied more directly if methods were available to quantitate both GH and PRL messenger RNA.

The aim of this study was to attempt the simultaneous translation in the reticulocyte lysate of GH and PRL mRNA from cultured GH_3 cells and from tumors grown in the rat by transplantation with the same cell line. The results show that, while both mRNA activities are present in the cultured cells, only GH mRNA is detected in the transplantable tumor.

2. Materials and methods

GH_3 cells were maintained in culture under standard conditions [6]. Tumors were grown in female rats of the Wistar Furth strain by serial transfers in vivo of the same GH_3 cell line [6]. For RNA extraction, the tumors (2–15 g) were rapidly dissected under ether anesthesia and immediately frozen in liquid nitrogen and stored at −70°C. The tissue was ground in a mortar under liquid nitrogen and the resulting powder was poured in ten volumes of 10 mM Tris–HCl, pH 8.5, 100 mM NaCl, 1 mM EDTA, 100 U/ml Heparin (Schwarz Mann) and 0.5% sodium dodecylsulfate (SDS). Within seconds, an equal volume of a phenol–chloroform mixture (1 vol/vol) was added and the RNA extracted at room temperature until no denatured protein could be observed at the interphase. The ethanol precipitated RNA was washed once with 0.1 M NaCl in 66% ethanol, dissolved in 10 mM Tris–HCl, pH 7.5, and precipitated overnight in 2 M LiCl at 4°C. Following 2 washes with LiCl 2 M and an additional wash in 66% ethanol, the RNA was dissolved in water.

RNA was extracted from cultured cells according to the same procedure except that, following two washes with serumless culture medium, the cells were directly suspended in the extraction buffer and extracted with the phenol–chloroform mixture, without freezing.

Acellular protein synthesis was carried out in rabbit reticulocyte lysates essentially as described by Palmiter [7]. [^{3}H]leucine (Amersham, 46 Ci/mM) was used at a concentration of 170 μCi/ml. Growth hormone and prolactin syntheses in the lysate were measured by a double antibody immunoprecipitation reaction performed in the presence of 1% sodium deoxycholate and 1% Triton X-100 (see legend to table 1). The anti-rat–GH serum was raised in the monkey, while the anti-rat–PRL serum was of rabbit origin. Second antibodies directed against monkey or

Gilbert Vassart is Chargé de Recherche at the Belgian Fonds National de la Recherche Scientifique.

Table 1
Quantitation of GH and PRL mRNA activities in transplantable tumors

RNA added to lysate (μg/ml)	Serum	Radioactivity in immunoprecipitate (mean cpm ± range)	Radioactivity in TCA precipitate (cpm)	Immuno-precipitate (% of TCA)
0	control	1710 ± 375	1 623 400	0.11
0	anti GH	1542 ± 157	1 623 400	0.09
300	control	1868 ± 352	746 500	0.25
300	anti GH	16 461 ± 29	746 500	2.19
0	control	843 ± 118	410 800	0.20
0	anti PRL	747 ± 323	410 800	0.18
300	control	929 ± 400	300 100	0.30
300	anti PRL	872 ± 272	300 100	0.29

Reticulocyte lysates (250 μl) were incubated with or without tumor RNA. At the end of a 60 min incubation at 27°C the samples were diluted to a final concentration of 1% sodium deoxycholate (DOC), 1% Triton X-100 and 0.1% cold L-leucine and spun at 100 000 × *g* for 1 h. Immunoprecipitations were performed in duplicate on 50 μl samples of the supernatant diluted to 100 μl with NaCl 0.15 M, phosphate buffer 0.02 M, pH 7.5, containing the above state concentration of DOC, Triton X-100 and leucine. For anti GH immunoprecipitations, 5 μl of a 1/50 dilution of anti GH serum or normal monkey serum (control) were added. For anti PRL immunoprecipitation, 5 μl of a 1/25 dilution of anti PRL serum or normal rabbit serum (control) were used. After 1 h incubation at room temperature, followed by 16 h at 4°C, 25 μl of goat anti-monkey IgG (GH reactions) or 5 μl of goat anti-rabbit IgG (PRL reactions) were added, and incubation was continued for another 30 min at room temperature and 8 h at 4°C. These protocols were shown to lead to complete precipitation of 1 rg of either GH or PRL. The total acid precipitable radioactivity (TCA) was measured on 1 μl samples as already described [14].

rabbit IgG were raised in the goat. One μg of cold rat PRL (N.I.H. reference preparation 1) was routinely added to the GH immunoprecipitation mixtures in order to eliminate coprecipitation of labeled PRL. Immunoprecipitates were washed by centrifugation through 1 M sucrose [8] and neither dissolved in Soluene 350 (Packard) for counting, or dissociated with SDS, according to Palmiter et al. [9], prior to electrophoresis in 15% polyacrylamide gels containing SDS [10].

3. Results and discussion

3.1. *Translation of RNA from tumors*

When added to a reticulocyte lysate, the total RNA extracted from the tumors clearly stimulated the incorporation of [^{3}H]leucine only into a material immunologically related to GH (table 1). Synthesis of PRL related material could not be detected. As noted by others [7], the RNA decreased the total protein synthesis in the lysate.

The ^{3}H-labeled material translated in the lysate system was compared to authentic ^{14}C-labeled GH by means of co-electrophoresis in SDS polyacrylamide gels (fig.1). The latter material was prepared by immunoprecipitation of GH secreted in the media by the same rat pituitary cell line grown in tissue culture in the presence of [^{14}C]leucine. A single major ^{3}H-labeled peak was obtained from immunoprecipitates of reticulocyte lysates incubated with anti GH serum (fig.1A), while immunoprecipitates of the same material in the presence of normal monkey serum yielded only background radioactivity (fig.1B). The GH related material, synthesized in the lysate, migrated slightly but reproducibly slower than authentic ^{14}C-labeled GH secreted by the GH_3 cells (fig.1A). This observation confirms in another assay system results

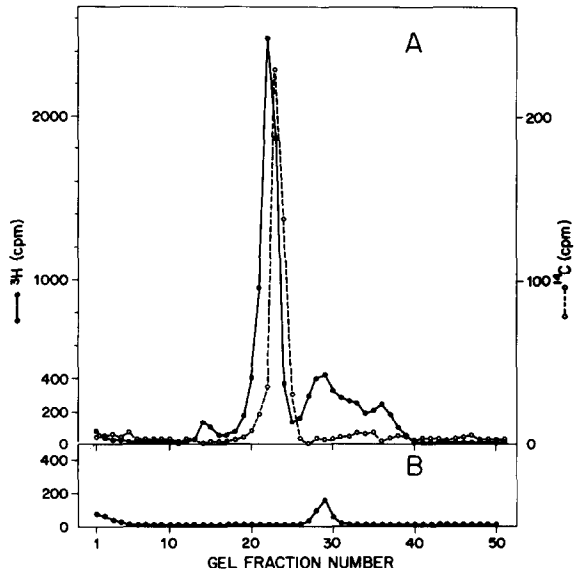

Fig.1. SDS polyacrylamide gel electrophoresis of immunoreactive GH synthesized in the lysate programmed with tumor RNA. ^{3}H-labeled specific and control immunoprecipitates were prepared from lysates programmed with total tumor RNA as described in the legend of table 1. ^{14}C-labeled GH was immunoprecipitated from the medium of GH_3 cells cultured for 2 h in the presence of [^{14}C]leucine (324 mCi/mM; 50 μCi/ml). A: ^{3}H-labeled and ^{14}C-labelled precipitates were mixed, dissociated and subjected to co-electrophoresis in polyacrylamide gel (see methods). B: electrophoretic pattern of ^{3}H-labeled material present in an immunoprecipitate carried out in the presence of normal monkey serum, without GH antiserum.

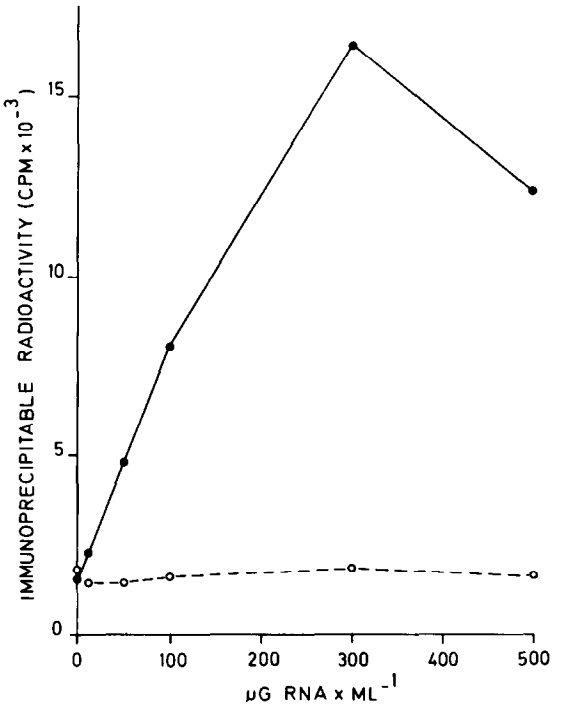

Fig.2. Immunoreactive GH synthesis in the lysates programmed with increasing amounts of tumor RNA. Tumor RNA was added to lysates to yield the final concentrations indicated on the abscissa. Immunoprecipitations were performed as described in the legend of table 1. Anti GH serum (•————•); normal monkey serum (o- - - - -o).

recently published by Sussman et al. [11] showing that RNA from a subclone of the GH_3 cell line is translated in the wheat germ system into a pre-growth hormone about 20% larger than the mature polypeptide.

In order to test the possibility of using the reticulocyte lysate as a method of dosing the GH mRNA activity, we have programmed lysates with increasing amounts of tumor RNA and performed precipitations with either normal monkey serum or monkey anti-rat–GH serum. The results on fig.2 show that, with RNA levels below 300 μg/ml, a fairly good linearity exists between radioactivity in specific immunoprecipitates and RNA concentration in the lysate. Thus, this method could be used to investigate the transcriptional control of GH synthesis in the tumor-bearing rats or in cultured GH_3 cells.

An estimation of the size of GH mRNA is provided by the results of experiments where GH mRNA activity was measured in fractions collected after sucrose density gradient centrifugation of the total tumor RNA. The results, illustrated on fig.3, reveal that GH mRNA sediments between 11 S and 18 S.

3.2. *Translation of RNA from cultured cells*

Translation of RNA from cultured GH_3 cells resulted in the synthesis of immunoreactive GH and PRL (table 2). However, the yield in mRNA activities was lower than that observed with tumor RNA. Five times larger assay mixtures were required to obtain significant specific syntheses. Nevertheless, the labeled materials in the immunoprecipitates were shown to be specific since they migrate as single peaks in SDS polyacrylamide gels (fig.4) while control samples yielded only polydisperse background radioactivity.

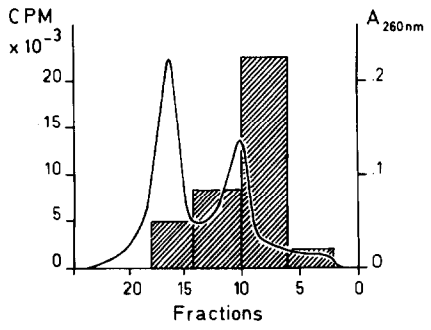

Fig.3. Sedimentation pattern of GH mRNA activity. Tumor RNA was dissolved in 10 mM Tris–HCl, pH 7.5, 10 mM NaCl, 1 mM EDTA and 200 μg were layered on 5–30% linear sucrose gradients prepared in the same buffer. After centrifugation for 19 h at 24 000 rpm (Beckman Rotor SW 27), fractions were collected while the absorbance at 260 mm was monitored [14]. The RNA in fractions corresponding to the hatched areas was ethanol precipitated, dissolved in 80 μl H_2O and added to reticulocyte lysates. Incubations and immunoprecipitations were as described in the legend to table 1. The height of the columns represents the immunoprecipitable radioactivity. Immunoprecipitates from lysates without added RNA contained 1.89 ± 0.03 cpm × 10^{-3}.

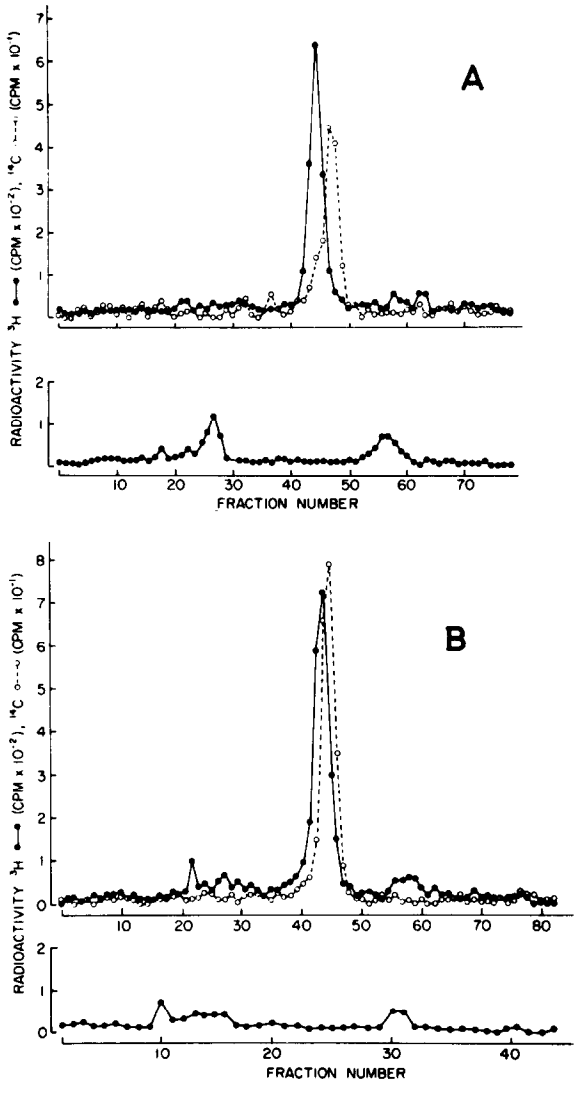

Fig.4. SDS polyacrylamide gel electrophoresis of immunoreactive GH and PRL synthesized in the lysate programmed with RNA from cultured cells. Co-electrophoresis of authentic ^{14}C-labeled (○- - - - -○) GH (pannel A) or PRL (pannel B) with ^{3}H-labeled material (●————●) immunoprecipitated by anti GH (pannel A) or anti PRL serum (pannel B) from lysates programmed with RNA from cultured GH_3 cells were performed as described in the legend of fig.1. The electrophoretic pattern of the material immunoprecipitated from lysates with no added RNA is shown at the bottom of each pannel.

Table 2
Quantitation of GH and PRL mRNA activities in cultured GH_3 cells

RNA added to lysate (μg/ml)	Serum	Radioactivity in immunoprecipitate (mean cpm ± range)	Radioactivity in TCA precipitate (cpm)	Immunoprecipitate (% of TCA)
0	anti GH	4914 ± 512	19 233 10^6	0.026
200	anti GH	12 390 ± 160	8050 10^6	0.154
0	anti PRL	4891 ± 88	19 233 10^6	0.025
200	anti PRL	13 004 ± 642	8050 10^6	0.161

Incubations and immunoprecipitations were performed as described in the legend of table 1 except that 200 μl lysate were used for each immunoprecipitation.

Comparison of the translation products with authentic rat GH and PRL (fig.4) showed the same small discrepancy in size as that observed with GH mRNA from tumors. These results confirm that PRL as well as GH syntheses must involve the transient existence of slightly larger precursors [12,13].

Comparison of the results obtained with RNA from tumors or from cultured cells suggests that PRL gene must be repressed in GH_3 cells under in vivo environment while the GH gene would be actively transcribed. This observation is in agreement with recent results showing suppression of PRL in plasma, pituitary and tumors from rats transplanted with the same tumor line [6].

The simultaneous quantitation of GH and PRL mRNA in GH_3 cultured cells exposed to various regulatory agents such as T_3 or TRH will allow best understanding of the mode of action of these agents.

Acknowledgements

We wish to thank Dr A. H. Parlow for supplying the antisera directed against rat GH and PRL, Dr Armen H. Tashjian, Jr for providing us with the GH_3 cell line, and Dr Victor D. Fang for invaluable technical advice in the maintenance and propagation of this pituitary tumor cell line.

This work was supported in part by a US Public Health Service Grant AM 15 070, and in part by a Contract of the Ministère de la Politique Scientifique (Actions Concertées) within the framework of the Association Euratom–University of Brussels–University of Pisa.

We also wish to acknowledge the gift from Mr and Mrs Harry Katz in support of the travel expenses of one of us (C.V.). The continuing support and stimulating discussions with Professor Jacques E. Dumont are also acknowledged.

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