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EFFECTS OF ESTRADIOL ON TRANSLATION ACTIVITY OF POLY (A)-RICH RNA FROM PITUITARY TUMOR WHOSE GROWTH IS INHIBITED BY ESTRADIOL

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Fischer rats bearing s.c. $MtTF_4$ pituitary tumor were treated for 15 days with Silastic implants containing or not 17 β-estradiol. Poly(A)-rich RNA was translated in a rabbit reticulocyte lysate system. The translation products were analysed by one and two-dimensional polyacrylamide gel electrophoresis.

It is shown that estradiol affects the translation activities of a limited number of mRNAs. Some of the effects observed, such as the stimulation of PRL mRNA and the relative inhibition of GH mRNA are similar to those previously reported in normal pituitary. Other modifications are specific of the pituitary tumor : the stimulation of the mRNA activities coding for 26,000 and 33,000 dalton proteins. These results indicate that the inhibition of tumor growth by 17 β-estradiol is accompanied by a specific modulation of mRNA translation activity and is not a non specific toxic effect.

Previous work from this laboratory showed that 17 β-estradiol (E) inhibits the growth of the $MtTF_4$ pituitary tumor (1). Such an inhibition was at first paradoxical. Indeed, this tumor had been induced some years ago by chronic treatment with a synthetic estrogen, the diethylstilbestrol (2) and in addition, the doses of estradiol which inhibit the tumor growth stimulate, in the same animal, the growth of pituitary (1).

The stimulation of pituitary growth by estradiol is well documented (3, 4) and is more pronounced in Fischer rats than in other species of rats (5). On the opposite the inhibition of the $MtTF_4$ tumor growth under E treatment had been briefly mentioned (6). More recently, estradiol was shown to inhibit the growth of the rat 7315a pituitary transplantable tumor (7). The dual effect of estrogens on cell multiplication have been reported in a number of different tissues and species, for example in rat uterus (8), rat mammary tumors (9), rat prostatic tumors (10), human breast tumor (11, 12) and human myelogenous cell line HL 60 (13).

The mechanism(s) by which estradiol modulates tissue growth or cell multiplication are under current investigation in a number of laboratories. It is widely

Abbreviations. E : 17 β-estradiol ; 1D-PAGE : one-dimensional polyacrylamide gel electrophoresis ; 2D-PAGE : Two-dimensional polyacrylamide gel electrophoresis ; poly(A) : poly adenylic acid ; TCA : trichloracetic acid ; SDS : sodium dodecyl sulfate ; K : kilodaltons ; GH : growth hormone ; PRL : prolactin.

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accepted that estradiol controls gene expression via its binding to cytosol receptor and translocation of this complex into the nuclei. However, the relationship between the control of gene expression and the control of cell multiplication remains unknown. One way to delineate whether or not some estrogen sensitive gene products could be linked to the control of cell multiplication is to compare the proteins which are synthetized by tissues whose growth is regulated in two opposite directions by estradiol, for example the pituitary and the $MtTF_4$ pituitary tumor. However, since this tumor contains large zones of necrosis which could alter either the proteins synthetized by explants or the cells during their isolation, we chose to determine at first the effect of estradiol on the translation activity of poly(A)-rich RNA extracted from the tumor. We report here the analysis of the translation products of poly(A)-rich RNA extracted from tumor borne by rats treated or not by estradiol for fifteen days.

METHODS

In vivo treatment. The transplantable pituitary tumor $MtTF_4$ has been maintained in Fischer F334 rats by successive transfers as previously described (1). For the experiments reported here, a suspension of $MtTF_4$ cells was injected s.c. in the dorsal back region of 2-3 months old male Fischer rats. When a tumor became palpable at the point of injection, a Silastic capsule filled with 8 mg E was placed s.c. along the neck dorsal midline in the conditions described previously (1). Empty capsule was placed in control rats. Fifteen days after Silastic implantation, rats were killed, $MtTF_4$ tumors were removed, quickly frozen in liquid nitrogen and stored at $-70°C$ until RNA extraction.

Preparation of poly(A)-rich RNA. Frozen tissue was pulverized with a stainless-morter and pestle in liquid nitrogen and homogenized in solution A (16 ml/g) : urea (6M), LiCl (3M), Heparin (100 U/ml). The homogenate was kept overnight at 0°C and centrifuged at 16,000 g for 20 min. at 4°C. The pellet was dissolved in 5 ml of buffer B : Tris 10 mM HCl, pH 7.6, sodium lauryl sarcosinate 0.5 %, and incubated overnight at 0°C with 25 ml of solution A. After centrifugation at 16,000 g for 20 min. at 4°C, the pellet was dissolved in 8 ml of buffer B and incubated with protease K (100 µg/ml) at 37°C for 30 min. The RNA was extracted three times with an equal volume of redistilled phenol-chloroform (v/v) saturated with buffer B. The RNA was precipitated from the final aqueous phase by 2 volumes of : ethanol, sodium acetate (0.1M, pH 5) overnight at −20°C. The RNA was pelleted by centrifugation at 70,000 g for 20 min. and dissolved in sterile water. Poly(A)-rich RNA was obtained after two passages through oligo(dT) cellulose (type T-3, Collaborative Research), according to the method of Aviv and Leder (14). The ratio between the absorption at 260 nm to that at 280 nm was over 2.

Cell-free translation of poly(A)-rich RNA. Poly(A)-rich RNA was translated in a rabbit reticulocyte system treated with micrococcal nuclease as described by Pelham and Jackson (15). Incubations were performed during 90 min at 30°C in a final volume of 100 µl containing poly(A)-rich RNA (0.5-3 µg), (^{35}S)-methionine from Amersham (75 µCi, specific activity : 1150 Ci/mM), KCl (50 mM), $MgCl_2$ (1.5 mM), lysate (40 %), aminoacids (30 µM) without methionine, spermidine (0.5 mM), ATP (1 mM), GTP (0.2 mM), dithioerythritol (2 mM), creatinine phosphate (8 mM), creatine phosphokinase (7 U/ml), hemin (25 µM) and Yeast tRNA (50 µg/ml). The newly synthetized [^{35}S] labelled-proteins were precipitated by TCA 10 %, and collected on filters. The radioactivity was counted in the Packard emulsifier Scintillator 299. The

proteins specifically directed by tumor RNA were determined from the difference between the proteins synthetized in assays containing or not tumor RNA. Immunoprecipitation was performed according to Maurer et al (16) using either anti-PRL antiserum (M. Dubois, Paris, final dilution 1:250) or anti-GH antibody (NIAMDD, A.F. Parlow, final dilution : 1/300). In every case, tumor RNA from E-treated and control rats were analyzed simultaneously.

Electrophoresis of translation products. For comparing the translation products of RNA from E-treated and control rats, the same amounts of TCA-precipitable radioactivity were analyzed by one and two-dimensional PAGE. The Laemmli's system was used (17) and polyacrylamide concentration was 12.5 %. Isoelectrofocalisation was done according to O'Farrel (18) using a mixture of 4 % ampholine LKB, pH ranges : 5-8 and 3.5-10 (4:1). Autoradiography was done after treatment with salicylate using a pre-exposed Kodak X-Omat film (19).

RNA was measured in tumor extracts according to the orcinol method (20) and after purification by absorption measurements at 260 nm.

RESULTS

The RNA and the translation system. The amount of RNA contained per gramme of tumor was 5.16 mg $\pm$ 0.63 in control rats and 5.77 mg $\pm$ 1.67 in treated rats (mean $\pm$ SD from 4 experiments in each group). The yield of extraction was slightly higher after E treatment ($\simeq$ 85 %) than in control rats ($\simeq$ 60 %). The percentages of poly(A) rich RNA recovered after two passages through an oligo(dT) cellulose column were 2.6 % in control and 2.7 % in E-treated rats.

The efficiency of translation of the poly(A)-rich RNA from E-treated and control rats was determined in varying concentrations of K^+, Mg^{2+} and RNA. For both RNA, the optimal efficiency was obtained in the presence of 50 mM K^+ and 1 mM Mg^{2+} in the reaction medium (results not shown). In these optimal conditions, the amounts of proteins synthetized were proportional to the concentration of the RNA added up to 20 µg/ml (Fig. 1). In two out of four RNA preparations, even in using RNA concentrations lower than 20 µg/ml and the optimal ionic conditions, the efficiencies of the translation of poly(A)-rich RNA of E-treated and control rats were different. This difference disappeared after heating RNA at 60°C for 5 min. before the assay.

Electrophoresis of the translation products. Figure 2 shows the autoradiographs and the densitometry scannings of the cell-free translation products of poly(A) rich RNA from control and E-treated rats. A limited number of differences was observed notably an increase in the intensity of the bands corresponding to the 24 K, 26 K and 33 K proteins. A similar observation has been reproduced twice and confirmed by the more resolutive 2D electrophoresis (Fig. 3). These proteins represent respectively 5.5, 3.8 and 2.8 % of the total proteins synthetized in E-treated rats ; the percentages were calculated by dividing the peak area by the total surface of the scan.

Figure 3 (upper pannels) shows the autoradiography of 2D-PAGE electrophoresis of the translation products. It illustrates the differences in the number or the intensity of several spots induced by E treatment. The most evident modifications

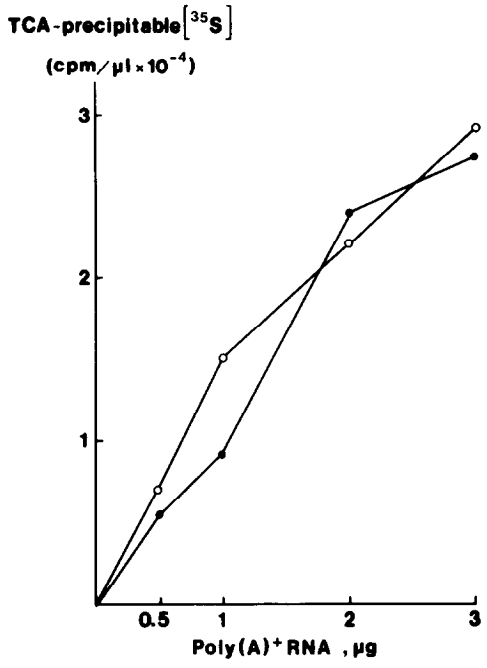

Figure 1. Protein synthesis directed by increasing concentrations of poly(A)-rich RNA

Poly(A)-rich RNAs were prepared from tumor borne by either control (●) or estradiol-treated rats (O) and increasing concentrations were translated in a reticulocyte lysate system in the conditions described in Material and Methods. The volume of one assay was 0.1 ml. The ^{35}S-methionine incorporated in proteins specifically directed by tumor RNA was determined after precipitation with TCA.

observed after E treatment are : 1°) The dramatic increase of two proteins which have the same pI (≃ 5.4) but two different molecular weights, 26 K and 33 K ; 2°) The increase of two 24 K proteins (spots c and d) whose pI are respectively 5.7 and 5.6 and 3°) The decrease of two 24 K proteins (spots a and b) whose pI are respectively 6.1 and 5.9. These three kinds of modifications have been reproduced four times by comparing the translation products of different poly(A) rich RNAs extracted from tumors borne by control or E treated rats. However, the 33 K protein has been found increased in two experiments only. Figure 3 (middle and bottom pannels) shows the autoradiography of 2D-PAGE of the translation products after immunoprecipitation either by anti-rPRL or anti-rGH sera. The anti-rPRL serum precipitated preferentially proteins c and d. The E treatment increases the amount of these two proteins. The anti-rGH serum precipitated mostly proteins a and b which were decreased by E treatment. No other spots were observed outside the area represented and especially the 26 K and 33 K proteins are not precipitated by these antisera.

DISCUSSION

We report the analysis of the translation products of poly(A)-rich mRNA extracted from pituitary $MtTF_4$ tumor. We emphasize that 2D-PAGE coupled to cell-

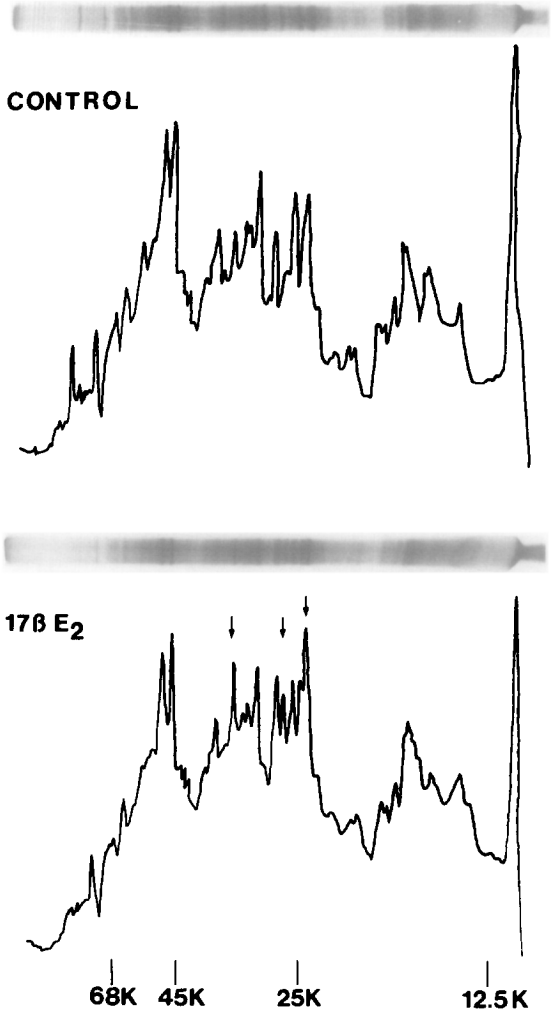

Figure 2. One-dimensional gel electrophoresis of poly(A)-rich RNA translation products

Poly(A)-rich RNAs were prepared from $MtTF_4$ tumor borne by either control rats or by estradiol-treated rats (17 β-E_2) and translated in a reticulocyte lysate system in the presence of (^{35}S) methionine. 85 000 cpm of each TCA-precipitable products were analysed by SDS-PAGE, as described in Material and Methods. The autoradiographs and densitometric tracings are shown. The bottom line indicates the molecular weight of ^{14}C standard proteins : bovine serum albumine (68 K), ovalbumine (45 K), chymotrypsinogene (25 K), beef cytochrome C (12.5 K). Arrows point to E_2 stimulated bands.

free translation assays is a powerfull tool to analyze the effect of estradiol on mRNA activities. We would like to outline that $MtTF_4$ tumor contains different populations of mRNA translation activities if taking the normal pituitary as reference. Some of them are found both in tumor and pituitary and are regulated in the same way in both tissues by estradiol treatment. Other ones are detected and stimulated by E mostly or exclusively in tumors.

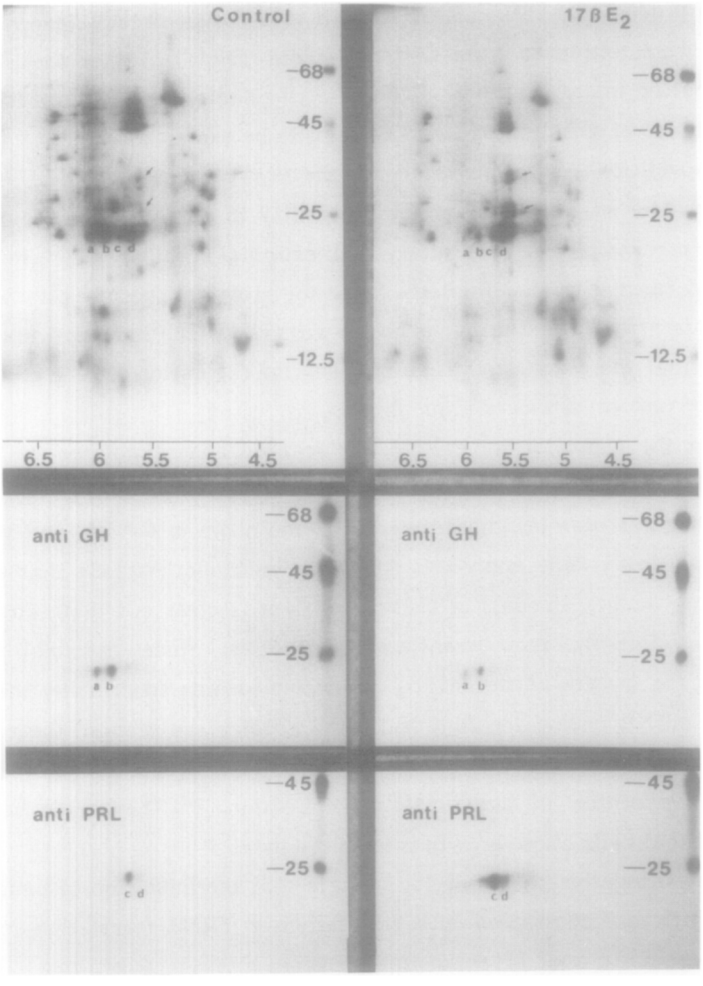

Figure 3. Two-dimensional gel electrophoresis of poly(A)-rich RNA translation products.

Poly(A)-rich RNAs were prepared from $MtTF_4$ tumors borne either by control rats (left pannels) or by E-treated rats (right panels) and translated into a reticulocyte lysate system in the presence of (^{35}S) methionine. The same amounts of labelled proteins (290,000 cpm) were analysed by 2D-SDS-PAGE, either without previous immunoprecipitation (top panels) or after immunoprecipitation by anti-rGH (middle pannels) or by anti-rPRL sera (bottom pannels). The autoradiogramms correspond to 8 days (top pannels) or 22 days (middle and bottom pannels) exposure. The molecular weight of the standard markers – which are the same as in Fig. 2 – are indicated at the right of each pannel. The pH values which were measured in a parallel gel rod are indicated in the top pannels. Arrows point to the major spots which are increased by estradiol treatment.

It is known that the $MtTF_4$ tumor retains some properties of the pituitary tissue from which this tumor was obtained. For example it secretes ACTH, GH and PRL (21). Our results support these data. Indeed, among the proteins whose synthesis is directed by $MtTF_4$ RNA, some are precipitated by anti-GH (spots a and b, Fig. 3) and others by anti-PRL (spots c, d, Fig. 3) sera. They displayed together a molecular weight of ≃ 24 K. These results strongly suggest that spots c and d correspond to pre-PRL and that spots a and b correspond to pre-GH. Such an heterogeneity of the

translation products of PRL mRNA and GH mRNA has already been reported when the RNAs were extracted from GH_3 cells (22). Taken together these observations indicate that no major degradation occurs during the preparation of $MtTF_4$ RNA but the significance of this heterogeneity remains to be explained.

In addition we demonstrated, for the first time, that some of the translation mRNA activities of the tumor retain the ability to be controlled by estradiol as in pituitary. For example, as in pituitary (23), estradiol stimulates PRL mRNA activity while it decreases that of GH mRNA. Since the growth of normal pituitary gland and $MtTF_4$ tumor are affected in two opposite ways (1) by the same estradiol treatment, we suggest that there is no tight link between the regulation of PRL and GH and that of normal or tumoral pituitary tissue growth.

On the opposite, some RNA are located and stimulated by E, in $MtTF_4$ tumor and not in the pituitary gland (results not shown). This is the case of two mRNA coding for proteins characterized by the same pI ($\sim$ 5.4) and two different molecular weights : 26 K and 33 K. Although we cannot exclude that the pituitary could contain minute amounts of these mRNA, it is clear cut that estradiol did not increase significantly their translation activities. Since estradiol speed down specifically the growth of the $MtTF_4$ tumor and affects specifically the translation activity of mRNA coding for 26 K and 33 K proteins, we propose the two following hypotheses : 1°) These tumor specific mRNAs or their products could be involved in the E-negative control of tumor cell multiplication. 2°) They could be markers of pituitary tumour cells whose growth is inhibited by E.

Taken together these results indicate that the inhibition of tumor growth by estradiol which is accompanied by a modulation of mRNA translation activity is not due to a non specific toxic effect of this hormone.

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