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Cloning of cDNA sequences of a progestin-regulated mRNA from MCF7 human breast cancer cells

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ABSTRACT

A cDNA clone corresponding to an mRNA regulated by the progestin R5020, has been isolated by differential screening of a cDNA library from the MCF7 breast cancer cell line, which contains estrogen and progesterone receptors. This probe hybridized with a single species of poly A + RNA of 8-kb molecular weight as shown by Northern blot analysis and could also be used to total RNA preparation. This recombinant clone hybridized specifically to an mRNA coding for a 250,000 daltons protein when tranlated **in vitro**. This protein was identical to the 250 kDa progestin-regulated protein that we previously described (Biochem. Biophys. Res. Commun. **121**, 421-427, 1984) as shown by immunoprecipitation with specific rabbit polyclonal antibodies. Dose-response curve and specificity studies show that the accumulation of the Pg8 mRNA and that of the 250-kDa protein was increased by 5 to 30-fold following progestin treatment and that this effect was mediated by the progesterone receptor. Time course of induction indicated that the accumulation of mRNA was rapid and preceded that of the protein. This is the first report on a cloned cDNA probe of progestin-regulated mRNA in human cell lines.

INTRODUCTION

MCF7 and T47D cell lines, which have been established from human metastatic mammary cancers, contain estrogen and progesterone receptors (1). In these cell lines estrogens stimulate cell proliferation (2,3) and accumulation of certain proteins and mRNA (4,5), whereas progestins decrease protein secretion (6) and inhibit cell proliferation (7,8). The cloning of cDNA sequences corresponding to specific steroid-regulated mRNA is a crucial step toward understanding the mechanism by which steroids act on these cells and is particularly interesting since this regulation can be studied in permanent cell lines. A ds-cDNA clone corresponding to an estrogen-

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stimulated MCF7 RNA has recently been isolated (9). The sequence (10) and the distribution and hormonal regulation of the mRNA have been studied (11,12,13). In contrast to estrogens, no progestin-regulated sequences have yet been isolated and consequently the steps which are regulated by progestin in breast cancer cannot be specified. Nevertheless, we have recently described several proteins specifically regulated by progestins in these cells. A protein (48 kDa) is released into the medium by the R5020 treated T47D cells, but it is present in small amounts (6). Another protein (250 kDa) is not secreted, more abundant, and regulated in both MCF7 and T47D cells (14). In an attempt to clone the cDNA corresponding to this protein, we prepared a cDNA library from MCF7 cells stimulated by R5020, which is a synthetic progestin, and screened it by hybridization with cDNAs prepared from cells which had or had not been treated by progestin. We report on the isolation and characterization of a cDNA clone corresponding to the 250-kDa mRNA. This is to our knowledge the first description of a ds-cDNA clone corresponding to mRNA sequences whose level is specifically increased by progestins in human cell lines.

MATERIALS AND METHODS

Cell Culture

MCF7 cells obtained from Dr M. Lippman (NCI, Bethesda) were maintained in Ham's F12 medium/Dulbecco's modified Eagle's medium (1/1) supplemented with 10 % fetal calf serum (FCS) (Flow) and 0.6 μg/ml insulin (Sigma).

To test the effects of progestins, the following protocol was used. Slightly confluent MCF7 cells were plated out in T75 flasks (8-fold dilution) for RNA extraction or in microwells (Nunc, 0.6 cm diameter - 10,000 to 50,000 cells depending on the duration of hormonal treatment) for protein analysis. The cells were stripped of steroid hormones by culturing in medium containing 10 % serum treated with dextran-coated charcoal (FCS-DCC) as previously described (4). The medium was changed every two days. Six days later, 10 nM R5020 (17,21-dimethyl-19-nor-4,9 pregnadiene-3,20-dione, kindly donated by Roussel-

Uclaf (Romainville, France), was added to the medium in an ethanol solution (final concentration of ethanol 0.1 %) and solvent was added to control cells.

Cellular proteins analysis

After hormonal stimulation, the cells were labeled for 4 h with 200 µCi/ml L-|^{35}S|methionine (specific activity 800 Ci/mmole). They were washed twice with phosphate saline buffer and twice with 10 mM 1,4 piperazinediethane sulfonic acid (pipes) pH: 6.8, 100 mM KCl, 300 mM sucrose, 2.5 mM $MgCl_2$ and 1 mM phenylmethyl-sulphonyl fluoride. The soluble fractions, obtained by incubation for 2 min with the same buffer plus 1 % Triton x100, were removed from the dish and analyzed on 10 % SDS-polyacrylamide gel (4). Gels were fixed in methanol (45 %) acetic acid (10 %), dried and exposed for 3 days to Kodak X-Omat Films were scanned using a Vernon densitometer (Paris, France) and the amount of 250-kDa protein estimated from the traces. Proteins were silver stained using a Kit from Biorad (Richmon, CA).

Obtention of immunserum

Immunogen was prepared from MCF7 cells treated for 6 days with 10 nM R5020. The 1 % Triton X100 cellular extract obtained as described above was loaded onto SDS-6 % polyacrylamide gels of 2 mm thickness. The 250 kDa protein was localized by staining part of the gel with coomassie brillant blue R250 and was recovered from the unstained part of the gel by extraction with 0.9 % NaCl in a glass potter. Male New-Zealand rabbits were given three subcutaneous injections at 3-4 weeks intervals of 200 µg 250 kDa protein, emulsified with Freund's incomplete adjuvent. Immunsera were collected 10 days following the third injection. Preimmune sera were obtained from the same animals before the first injection. All sera were aliquoted in 1 ml fraction and stored at -80°C until use.

RNA Preparation, Northern Blots

Total RNA was extracted from MCF7 cells by the LiCl-urea procedure of Auffray and Rougeon (15) and Poly A+ RNA was selected in two cycles of oligo-d(T)-cellulose chromatography. The material was reprecipitated twice with ethanol before

loading on 1 % agarose containing formaldehyde (16). To ensure that equal amounts of each sample of RNA were loaded and also that the RNA was intact, the gel was stained with ethidium bromide before transfer to nitrocellulose membrane. Transfer to nitrocellulose membrane was carried out after partial alkaline hydrolysis to improve the transfer of high molecular weight RNA (17). RNA samples were prepared for dot-blots as described elsewhere (18). Plasmid ds-cDNA were ^{32}P-labeled by nick translation using an Amersham kit. Filters were prehybridized for 24 h at 20°C and then hybridized in 50 % formamide for 3 days at 37°C ($2x10^6$ cpm/ml ; specific activity $2-5x10^8$ cpm/µg). Hybridisation solutions were prepared as previously described (19). Filters were then washed in 2xSSC, 0.1 % SDS twice for 15 min at room temperature, twice for 1 h at 65°C and finally once for 15 min at room temperature. The dry blotted filters were exposed to preflashed Kodak X-Omat for 1-2 days at 80°C.

Preparation and Screening of the cDNA Library

All manipulations were carried out in a category 2 containment laboratory as adviced by the French Genetic Manipulation Advisory Group. Poly A+ RNA was prepared from confluent MCF7 cells grown in 10 % FCS-DCC plus 10 nM R5020. cDNA was synthesized by AMV reverse-transcriptase and ds DNA by the Klenow fragment of E. Coli polymerase I (20). After S_1 digestion, ds-cDNA was size- selected by Sepharose CL4B chromatography. ds-cDNA was then C tailed and annealed to plasmid PUC9 G-tailed at its Pst1 site (21). Plasmid recombinants were used to transform E. Coli HB101. Ampicillin-resistant colonies were transferred to microwells containing freezing medium as described (22) and incubated overnight at 37°C. They were then inoculated on two nitrocellulose filters and placed on agar petri plates containing ampicillin (22) using a transferring plate with 96 iron pins. The filters were incubated overnight at 37°C and the microtitration plate was kept at -20°C. After growth, the colonies were lyzed and the DNA was fixed to the filters as described (23). Filters were prehybridized for 24 h at room

temperature. They were then hybridized in batches of 10 for 40 h in 15 ml of a 50 % formamide hybridization solution (19) with $3x10^7$ cpm of ^{32}P-cDNA (specific activity = 2-3 10^7 cpm/µg) synthesized by reverse transcription of poly A+ RNA prepared from control or R5020-stimulated MCF7 cells. The filters were washed and exposed to X-ray films as described above.

Hybrid-selection of mRNA

Plasmid DNA from clone Pg8 was prepared by an alkaline cleared-lysate procedure (24). 15 µg were bound to a nitrocellulose filter (7 mm x 7 mm) and hybridized with poly A+ RNA (1 mg/ml) in 0.1 ml hybridization buffer for 12 h at 37°C as described (17). Filters were washed extensively and bound RNA was eluted and ethanol precipitated (17). The precipitates were washed twice in 70 % ethanol and resuspended in 5 µl sterile water for **in vitro** translation.

In vitro cell free translation of poly A+ and Pg8 mRNAs

Cell free translation in a rabbit reticulocyte lysate (Amersham, nuclease free) of Poly A+ mRNA was carried out at 30°C for 120 min as described by the supplier except that the tRNA and aminoacids concentrations were increased to 40 µg/ml and 30 µM respectively. The mixture was centrifuged (10,000 g x 20 min at 4°C) and the supernatant diluted to a final volume of 100 µl in immunoprecipitation buffer (100 mM Tris PH 8, 100 mM NaCl, 0.5 % NP40, 2 mM phenylmethyl-sulphonyl fluoride, 0.5 % w/v bovine serum albumine). It was then incubated with 100 µl of protein A-Sepharose (6 % w/v in the same buffer) for 4 h at 4°C under agitation. After centrifugation (10,000 g x 10 min at 4°C) the supernatant was incubated with polyclonal antibodies 1 h at 20°C then overnight at 4°C. Protein A-Sepharose (100 µl) was then added for 4 h at 4°C. The protein A-Sepharose gel was washed 5 times with 1.2 ml Tris LiCl buffer (100 mM Tris PH 8.6, 500 mM LiCl, 0.5 % NP40) and 1 time with 50 mM Tris HCl PH 6.8. The antigen immunoglobuline complex was dissociated in sample buffer and analyzed in SDS/polyacrylamide gel as described above.

RESULTS

Cloning and Differential Screening of Progestin-Stimulated ds-cDNA

In order to clone ds-cDNA sequences corresponding to progestin-regulated transcripts, poly A+ RNA was prepared from R5020-treated MCF7 cells, and ds-cDNA was synthesized as described above. After integration in PUC9 plasmid vector, the material was used to give E. Coli HB101 cells ampicillin-resistance. About 2,000 ampicillin-resistant colonies were obtained. Preliminary experiments showed that at least 80 % of the antibiotic-resistant colonies failed to promote a complementation of ß-galactosidase activity in JM103 bacteria, suggesting that at least 80 % of the colonies contained recombinant plasmids. Colonies were then transferred to 96-well microtiter plates and inoculated on two nitrocellulose membranes, as described in Materials and Methods. After growth, 2-3 mm diameter colonies were lyzed and their DNA fixed to filters as described (23). DNA was then hybridized in situ with ^{32}P-labeled cDNA synthesized from two poly A+ mRNA populations obtained from MCF7 cells maintained in FCS-DCC (control probe) and from MCF7 cells treated for 2 days with 10 nM R5020 (R5020 probe). Out of a total of 2,000 colonies, 10 colonies hybridized more strongly with the control probe than with the R5020 probe (an example is given Fig 1A). These colonies should contain recombinant plasmids harboring a ds-cDNA sequence corresponding to an mRNA whose accumulation is decreased by progestins. They have not been studied further. 36 colonies hybridized more with the R5020 probe than with the control probe, suggesting that they contained a ds-cDNA insert corresponding to a progestin-stimulated transcript. An example is given Fig. 1B.

Since the difference in hybridization could be explained by variations in the yield of transfer to nitrocellulose filters, we used the alkaline method to purify the 36 plasmids possibly containing the R5020-stimulated sequences. Plasmid DNA was applied in duplicate to a nitrocellulose sheet, using a minifold apparatus, and screened by differential hybridization as described above. Out of the 36 plasmids tested, only 4

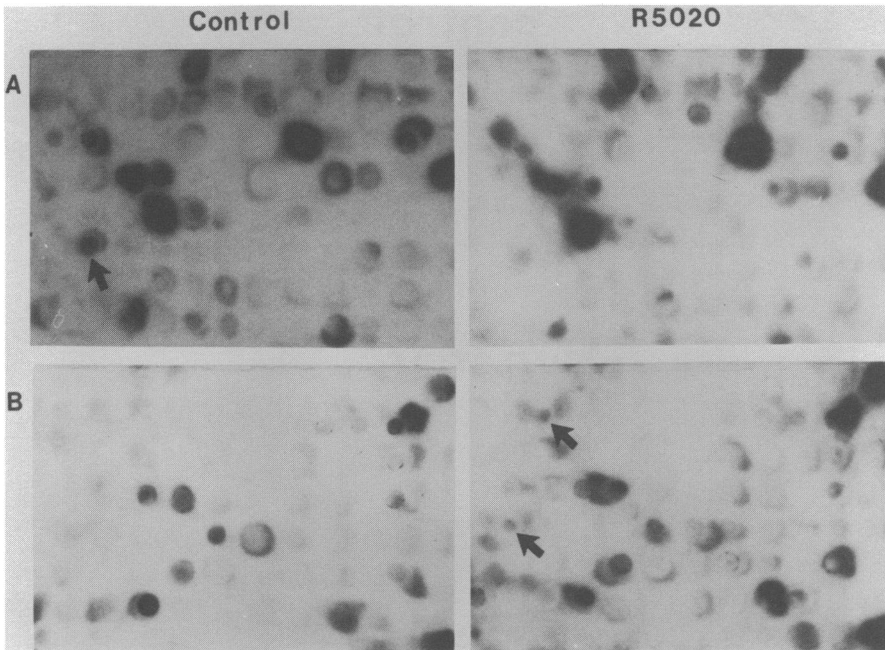


Figure 1. Initial screening of the ds-cDNA library with cDNA prepared from control and R5020-treated MCF7 cells.

Ampicillin-resistant colonies were transferred to duplicate nitrocellulose filters and hybridized **in situ** with ^{32}P-cDNA synthesized from the poly A+ RNA of steroid-withdrawn MCF7 cells treated (R5020 probe) or not (control probe) with the synthetic progestin R5020. A total of 20 pairs of nitrocellulose sheets were screened. Two pairs are shown as examples. Pair A : the arrow indicates a colony which hybridized more strongly with the control probe than with the R5020 probe. Pair B : arrows indicates 2 colonies which hybridized more strongly with the R5020 probe than with the control probe.

showed a 1.5 to 3-fold stronger hybridization with the R5020 probe than with the control probe (Fig. 2). The same results were obtained in a second independent experiment. A recombinant plasmid (Pg8) harboring the largest insert was retained for further studies. One of the plasmids (C3) displaying the same hybridization with the control and the R5020 probe was chosen as a control.

Identification of the Pg8 ds-cDNA

In order to confirm that the Pg8 plasmid contained a

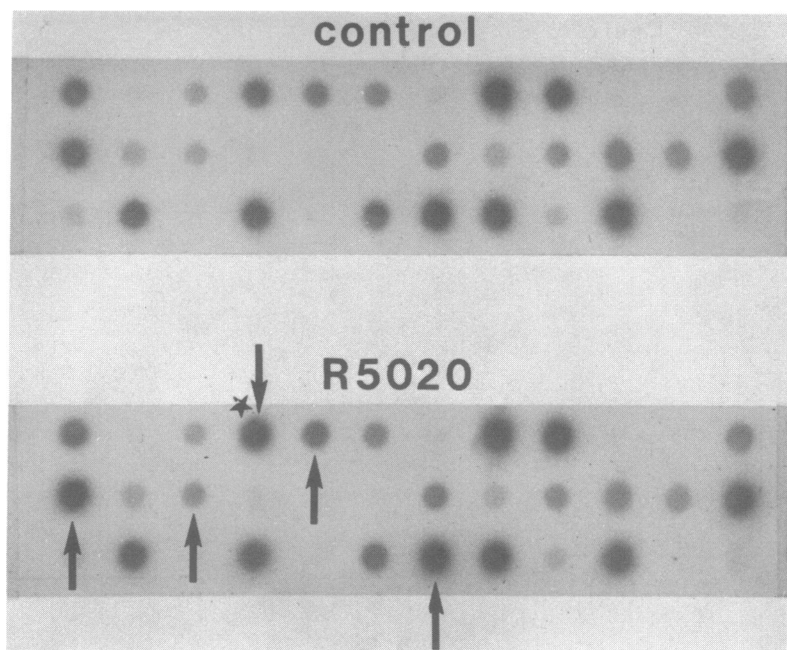


Figure 2. Second screening of the ds-cDNA library by differential hybridization of purified plasmids.
200 ng of each plasmid was filtered, in duplicate, through 2 separate nitrocellulose membranes using a hybridot manifold (Bethesda Research Laboratories). Nitrocellulose sheets containing the DNA samples were then hybridized separately with ^{32}P-cDNA prepared from control or R5020-treated cells and autoradiographed. The 5 plasmids that hybridized more strongly with the R5020 probe are indicated by arrows (* = Pg8 plasmid).

ds-cDNA insert corresponding to a progestin-induced transcript, it was ^{32}P-labeled by nick translation and used to hybridize equal amounts of poly A+ mRNA from control or R5020 treated cells transferred to a nitrocellulose sheet by Northern blot (Fig. 3 A et B). Duplicate experiments were done with 2 independent preparations of mRNA. As expected, the nick-translated probe hybridized more strongly with Poly A+ RNA when prepared from R5020-treated cells than from control cells. Out of 10 experiments, this difference of hybridization varied from 5 to 10-fold. The hybridization corresponded to a single species of poly A+ RNA of at least 8,000 nucleotides, and we thus designated the cDNA probe as Pg8. We verified that the same

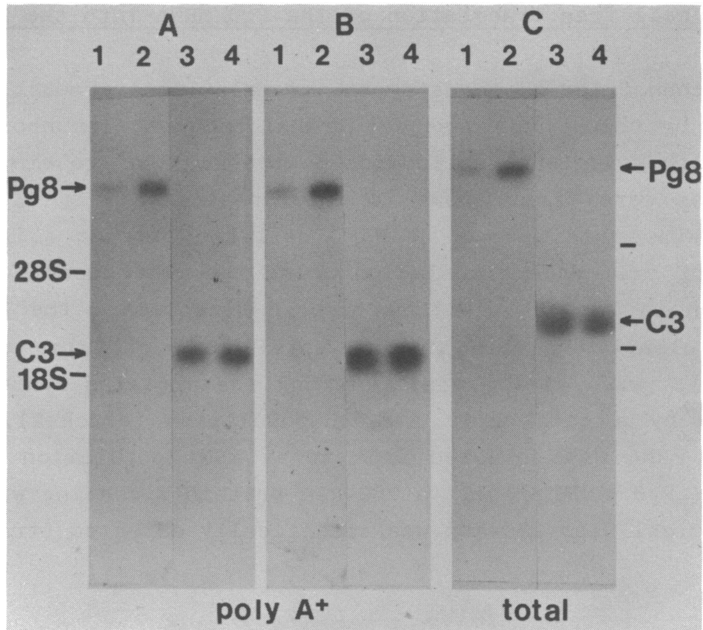


Figure 3. Evidence for a cDNA clone corresponding to an mRNA whose level is increased by progestin in MCF7 cells.

MCF7 cells (one T175 confluent flask per treatment) were stripped of steroids, as described in Materials and Methods, and treated (2,4) or not (1,3) with 10 nM R5020 for 2 days. Total RNA was extracted from these cells and part of the total RNA was subjected to two cycles of oligo d(T) cellulose chromatography. 2 µg of poly A+ RNA (A,B) or 40 µg of total RNA (C) were then electrophoresed on a 1 % agarose denaturating gel. Northern blots were probed with plasmid Pg8 and C3, as described in Material and Methods and transferred to nitrocellulose membrane before hybridization with the ^{32}P nick-translated Pg8 (1,2) and C3 (3,4) plasmids.

A and B : poly A+ RNA from 2 independant experiments.

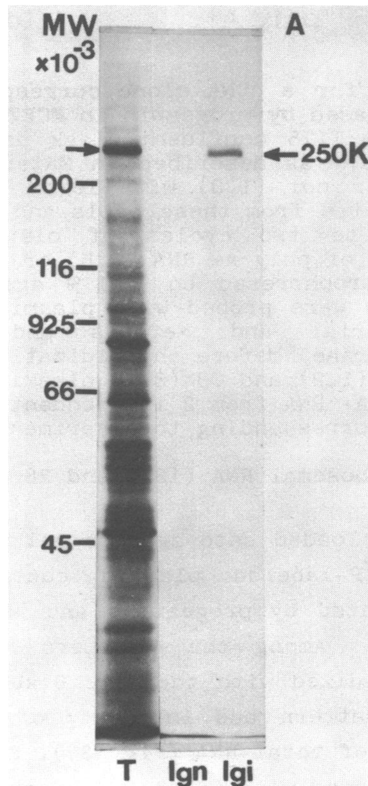
C : total RNA corresponding to experiment B with different electrophoresis.

Migration of ribosomal RNA (18 S and 28 S) is indicated.

amounts of RNA were loaded onto agarose gel by hybridizing the filters with a ^{32}P-labeled plasmid containing a ds-cDNA insertion not regulated by progestins and corresponding to an RNA of 3 kb (C3). Among the 4 others "R5020 stimulated" clones, three hybridized with the same 8 kb mRNA species (not shown). The same pattern and intensity of hybridization was observed with 40 µg of total RNA (Fig. 3C). Further experiments were therefore performed with total RNA preparations.

In vitro cell free translation of the Pg8 mRNA into the 250 kDa protein

We then tried to identify the translational product of the Pg8 mRNA by using hybrid-select translation and immunoprecipitation with polyclonal antibodies to the 250-kDa protein. These antibodies were first shown to immunoprecipitate specifically the 250-kDa protein from an MCF_7 cellular extract (Fig. 4A). Results of cell free translation using the reticulocyte lysate are shown in Fig. 4B. Without hybrid selection, the 250-kDa protein clearly visible in the total MCF_7 cellular extract (track 1), was faintly visible among the proteins synthesized **in vitro** by a total poly A+ RNA population (track 2). When specific mRNA was selected from total mRNA population by the insert of Pg8 cDNA probe, a 250-kDa protein migrating with the authentic cellular 250-kDa was specifically detected (track 4).



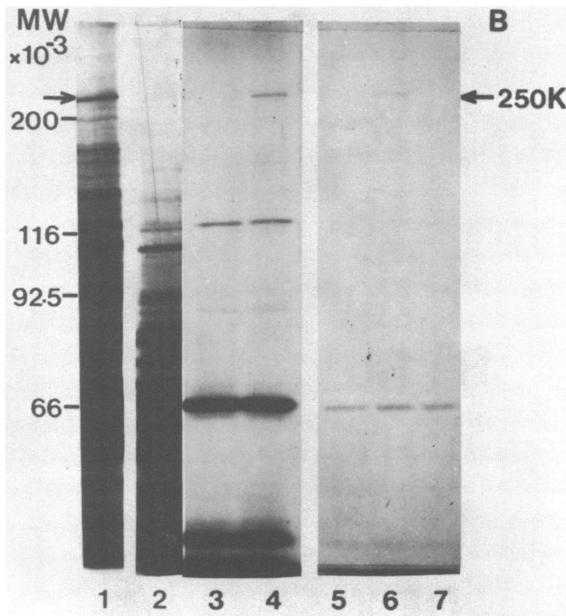


Figure 4 - A and B.

A. Specificity of the antiserum directed against the 250-kDa protein.

SDS-polyacrylamide 10 % gel analysis of $|^{35}S|$methionine labeled proteins extracted from MCF_7 cells :

Track 1 : Total extract (T) obtained as previously described (14) from MCF_7 cells.

Track 2 : Proteins immunoprecipitated by non immune rabbit serum (Ign).

Track 3 : Proteins immunoprecipitated by rabbit immunserum to the 250-kDa protein (Igi).

B. In vitro translation of Pg8 mRNA into 250-kDa protein.

SDS-polyacrylamide 10 % gel analysis of proteins labeled by $|^{35}S|$methionine and synthesized **in vivo** (track 1) or **in vitro** using the reticulocyte lysate (track 2 to 7) as described in Material and Methods.

Track 1 : Total cellular extract.

Track 2 : Translational products of total poly A+ RNA.

Track 3 : Translational products in the absence of added mRNA.

Track 4 : Translational products of poly A+ RNA hybrid-selected with Pg8 plasmid.

Track 5 : Translational products in the absence of added mRNA after immunoprecipitation with 250-kDa antiserum.

Track 6 : Translational products of the hybrid select after immunoprecipitation with 250-kDa antiserum.

Track 7 : The same after immunoprecipitation with non immune rabbit serum.

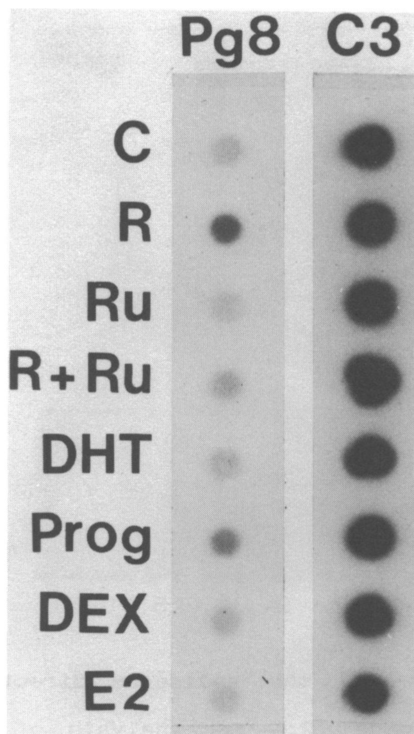


Figure 5. Steroid specificity of the accumulation of the Pg8 transcript.
MCF7 cells were stripped of steroids as described in Material and Methods, and then treated for 2 days by 1 nM of the indicated steroids (10 nM for RU486). C = no added hormone ; R = R5020 , Ru = RU486 ; R+Ru = R5020 plus RU486 ; DHT = 5α-dihydrotestosterone ; Prog = progesterone ; DEX = dexamethasone ; E_2 = 17ß-estradiol. Total RNA was prepared from the cells and 40 µg was dot-spotted in duplicate on 2 separate filters. They were then separately probed with ^{32}P nick-translated Pg8 and C3 plasmids, as described in Materials and Methods.

It was absent in the pattern obtained with the reticulocyte lysate without added mRNA (track 3). This 250-kDa protein synthesized **in vitro** from the Pg8 mRNA was specifically immunoprecipitated by the polyclonal antibodies (track 6). The second protein of 66-kDa also precipitated was likely a protein of the reticulocyte lysate, since it was also observed when non-immune rabbit serum was used (track 7) and when the translation product, obtained without added mRNA, was immuno-

precipitated by the immunserum (track 5).

Effect of progestins on 250-kDa mRNA and protein levels

To ascertain the hormonal specificity of the Pg8 mRNA, we then tested the effect of several steroids at 1 nM concentrations (or 10 nM in the case of the antiprogestin RU486). Dot-blot hybridization (Fig. 5) clearly showed that the level of Pg8 mRNA was increased after the cells were treated with the 2 progestins, R5020 and progesterone. Progesterone was less active than R5020 but its efficiency could be increased by changing the medium more frequently. This is also observed for the induction of others progestins responses (48-kDa, 250-kDa proteins) and was consistent with the extensive metabolism of progesterone in these cells (25) while R5020 was not metabolized **in vitro** (26). RU486, DHT, dexamethasone, and estradiol did not significantly increase its level compared to the control. Moreover, the antiprogestin RU486 which displays a high affinity for the progesterone receptor inhibited the effect of R5020 on transcript accumulation. Control C3 was constant under these different conditions. The same hormonal specificity was therefore observed for the mRNA and for the protein (14). Furthermore, the antiprogestin RU486 was unable to stimulate the accumulation of this specific progestin regulated mRNA. Fig. 6 shows the dose-response curves of the accumulation of the 250-kDa mRNA and protein. The two curves are practically super-imposable, the slight differences observed being possibly explained by the two different hormone stimulated cell populations which have been analysed. The concentration of R5020 giving half-maximal stimulation (ED50 $\simeq$0.6 nM) was approximatively 10-fold lower than the K_D of the R5020-progesterone receptor interaction (26). Similar difference between ED50 and K_D was observed in $T_{47}D$ cells for induction of the 48-kDa progesterone regulated protein (6). The R5020 concentration giving maximal response (10 nM) was also similar for the RNA and the protein.

The time course of the 250-kDa mRNA accumulation was rapid (Fig. 7). The level of this mRNA was very low at time zero and increased after 1 h of hormonal treatment, reaching a plateau after 5-6 h that lasted until 48 h corresponding to a 5-fold

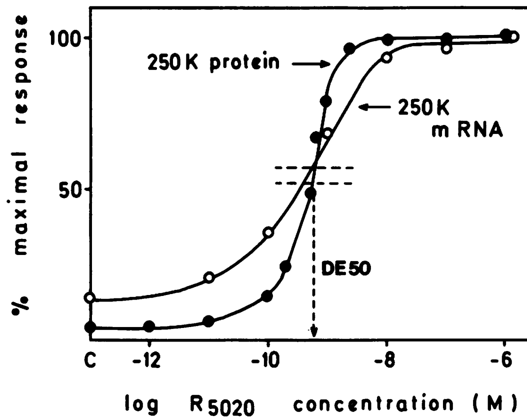


Figure 6. Effect of increasing concentrations of R5020 on the level of 250-kDa mRNA and protein.

Steroid stripped MCF_7 cells were treated by the indicated concentrations of R5020 for 2 days. For each concentration, one T75 flask, for RNA extraction and one microwell for protein analysis were used.

(O) Total RNA (40 µg) was electrophoresed in a denaturating 1 % agarose gel. After transfer to a nitrocellulose filter, RNA was hybridized with ^{32}P- labeled Pg8 plasmid, then with ^{32}P-labeled C3 plasmid, as described above (Fig. 3).

Relative amounts of the 250-kDa mRNA was measured by scanning the autoradiograph. The densitometric absorption in bands obtained after hybridization with Pg8 probe was corrected for RNA content as evaluated by hybridization of the same filter with the constant non regulated C3 probe.

(●) Cellular proteins were extracted from MCF_7 cells labeled for 4 h with |^{35}S|methionine. After electrophoresis in a 10 % SDS-polyacrylamide gel, proteins were revealed by autoradiography. The film was then scanned and the amount of the 250-kDa protein was estimated from the traces in arbitrary units.

Results are expressed as percent of the maximal amount of 250-kDa mRNA (o) or protein (●) obtained after hormone stimulation.

stimulation. The constant level of the non-regulated probe C3 indicated that the amount of transferred RNA was similar. The time-course of synthesis of the 250-kDa protein, as measured by |^{35}S|methionine incorporation and of its accumulation, as measured by silver staining, were retarded compared to the accumulation of its mRNA. R5020 increased the labeling of the 250 kDa protein within 3 h. Maximal stimulation was reached after 12 h and no decrease was observed following longer

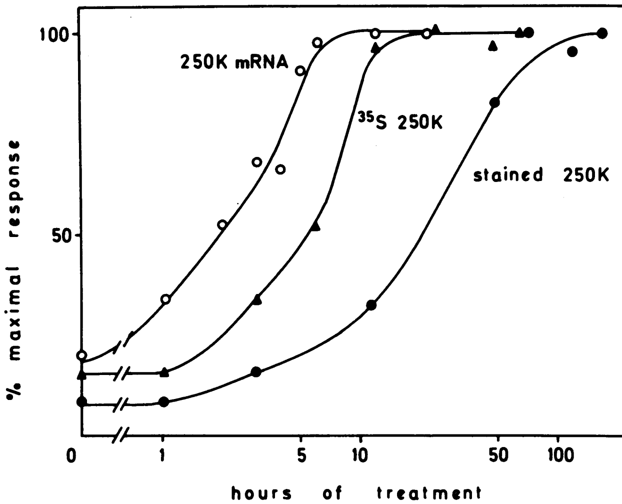


Figure 7. Time course of the effect of R5020 on the accumulation of the 250-kDa mRNA and protein.

Steroid-stripped MCF_7 cells were treated for the indicated periods of time with 10 nM R5020 as described in Fig. 6. Treatments were started sequentially 6 days after the incubation of cells with FCS-DCC, and all cultures were stopped at the same time when the cells were confluent. The titration of 250-kDa mRNA and of |^{35}S|labeled protein was in Fig. 6. The accumulation of 250-kDa protein was evaluated by silver staining the gel as described elsewhere (6).

periods of treatment. The effect of R5020 on the accumulation of the 250 kDa protein was detectable after 3 h and was maximal after 3 days. Similar lag between the effects on mRNA accumulation and protein synthesis has been reported for other steroid regulated proteins (27).

DISCUSSION

The 250-kDa protein is a cellular protein specifically induced by progestins in MCF_7 and $T_{47}D$ human breast cancer cells. It is abundant (14) and its induction by R5020 can be inhibited by the antibiotics actinomycin D and cordycepin that inhibit RNA synthesis (unpublished experiment). In an attempt to clone the corresponding cDNA, we have screened by differential hybridization a cDNA library constructed with poly A+ RNA extracted from MCF_7 cells treated by R5020. Analysis of 2,000

recombinant plasmids allowed us to isolate 5 cDNA clones corresponding to R5020 stimulated mRNAs. Among them, 4 clones contained an insertion hybridizing with an mRNA of 8 kb as shown by Northern blot analysis. The Pg8 recombinant harboring the largest insertion (≃2 kb) was used for further studies. Other clones appeared to contain sequences corresponding to mRNA inhibited by R5020. This is consistent with the inhibition of some proteins synthesis by progestins also described in these cell lines (6)(8). The Pg8 plasmid hybridized with a single Poly A+ RNA species of ≃8 kb which could also be detected among total RNA populations suggesting its relative abundance. Analysis of the translation products obtained with mRNA selected by Pg8 plasmid, showed essentially a protein which comigrated with the 250-kDa protein of the progestin stimulated MCF_7 cells. This protein was specifically immunoprecipitated by monospecific antiserum directed against the purified 250-kDa protein of MCF_7 cells. We thus conclude that Pg8 plasmid contains an insertion corresponding to the 250-kDa mRNA.

The hormonal specificity, the dose-response curve and the time-course of the Pg8 mRNA accumulation, are in total agreement with previous characterization of the 250-kDa protein (14). The 250-kDa mRNA accumulation appears to be the product of progesterone receptor stimulation, since physiological concentrations of androgens, glucocorticoids, estrogens or an antiprogestin, are unable to stimulate its accumulation. This increased accumulation of the 250-kDa mRNA was detectable within 1 h and was followed 3 h thereafter by an increased accumulation of the protein. These results suggested that progestin could successively stimulate the accumulation of the mRNA and subsequently that of the protein by an additional post-transcriptional mechanism which is currently not specified. Such dual effect of progesterone was observed in the rabbit endometrium for induction of uteroglobin and its mRNA (25).

To our knowledge, this is the first isolated cDNA clone corresponding to an mRNA induced by progestins in continuous cell lines. This cloned probe should therefore prove to be very

useful in studying the regulation of gene expression by progestin. Since it is found in human mammary cell lines, it may also be usefull to monitor the treatment of breast cancer. It should also enable us to specify the mRNA sequence and that of the corresponding gene, its distribution in different hormone responsive and unresponsive systems, and its possible role in the mechanism by which progestins modulate cell proliferation and/or differentiation in breast cancer.

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