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Tightly bound nuclear progesterone receptor is not phosphorylated in primary chick oviduct cultures

(steroid receptor/phosphoprotein/receptor transformation)

TERESA GARCIA, INGRID JUNG-TESTAS, AND ETIENNE-EMILE BAULIEU

Institut National de la Santé et de la Recherche Médicale (INSERM U. 33) Laboratoire Hormones, 94275 Bicêtre CEDEX, France

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ABSTRACT **Oviduct cells from estradiol-treated chicks were grown in primary culture. After 3–5 days of culture in medium containing estradiol, 90% of the cellular progesterone binding sites were detected in the cytosol. After exposure to [^{3}H]progesterone at 37°C, 80% of the progesterone binding sites were found in nuclear fractions. Progesterone receptor phosphorylation was assessed after incubating the cells with [^{32}P]orthophosphate. Receptor components were immunoprecipitated with a specific polyclonal antibody (IgG-G_3) and analyzed by NaDodSO$_4$/PAGE and autoradiography. In the cytosol, constant amounts of ^{32}P-labeled 110-kDa subunit (the B subunit, one of the progesterone-binding components of the receptor) and of the non-steroid-binding heat shock protein hsp90 were found, whether cells had been exposed to progesterone or not. No ^{32}P-labeled 79-kDa subunit (the A subunit, another progesterone-binding subunit) was detected. Various procedures were used to solubilize nuclear progesterone receptor (0.5 M KCl, micrococcal nuclease, NaDodSO$_4$), and in no case was ^{32}P-labeled B subunit detected in the extracts. However, nonradioactive B subunit was detected by immunoblot in a nuclear KCl extract of progesterone-treated cells. These results suggest that the fraction of the B subunit that becomes strongly attached to nuclear structures is not phosphorylated upon exposure of cells to progesterone.**

Two progesterone-binding proteins, called B (110 kDa) and A (79 kDa) subunits, have been described as components of the progesterone receptor in the chicken oviduct (1). The nontransformed form of the progesterone receptor also contains a non-progesterone-binding 90-kDa heat shock protein, hsp90 (2–4). These three components of the nontransformed progesterone receptor have been shown to be phosphorylated *in vivo* (2, 5). Purified A and B subunits can be phosphorylated *in vitro* by a cAMP-dependent protein kinase (6), and the B subunit, by the epidermal growth factor receptor tyrosine kinase (7). Purified B subunit and hsp90 can be phosphorylated by an endogenous protein kinase in highly purified progesterone receptor preparations (8).

This paper reports the phosphorylation of progesterone receptor after addition of radioactive phosphate to primary oviduct cell cultures obtained from estradiol-treated chicks. Such cells multiply and respond to estradiol and progesterone *in vitro* (9). We studied ^{32}P-labeling of progesterone receptor components in the cytosol and nuclear fractions from cells treated or not treated with progesterone. The tightly bound nuclear B (110-kDa) subunit, detected by radioactive hormone binding and immunoblot analysis, was not ^{32}P-labeled, unlike the form found in the cytosol, suggesting that the hormone-dependent nuclear binding of the receptor does not depend on its phosphorylation.

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EXPERIMENTAL PROCEDURES

Reagents. [2,4,6,7-^{3}H]Progesterone (99 Ci/mmol; 1 Ci = 37 GBq), [^{32}P]orthophosphate (carrier-free), and Na^{125}I were from the Radiochemical Centre. Nonradioactive steroids were from Roussel-Uclaf (Romainville, France). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were obtained from GIBCO, and calf serum was from Merieux (Lyon, France). High molecular weight standards for gel electrophoresis were from Sigma. Micrococcal nuclease (EC 3.1.31.1) was from Worthington.

Animals. One-day-old female Warren chicks were obtained from commercial sources. After 1 week, the chicks were injected i.m. for 10 days with estradiol benzoate (1 mg per day in sesame oil) and then allowed to withdraw for 3–6 weeks. For secondary stimulation, the chicks were reinjected for 8 days with 1 mg of estradiol benzoate per day.

Cell Culture. Details are described in ref. 9. In brief, cells were dissociated and plated in DMEM supplemented with 15% FBS, 50 nM estradiol, and insulin (0.12 unit/ml). After 3–5 days of culture, FBS was replaced by charcoal-treated serum 24 hr before measurement of progesterone receptor.

Labeling of Intact Cells with [^{3}H]Progesterone. Cells ($5–7 \times 10^7$) were harvested by scraping culture dishes and resuspended in 2 ml of phosphate-free phosphorylation buffer (0.9% NaCl/glucose (1 mg/ml)/1.3 mM $MgCl_2$/2.8 mM $CaCl_2$/1% essential amino acids/25 mM Hepes, pH 7.4 at 37°C) containing 10 nM [^{3}H]progesterone with or without 1 μM unlabeled progesterone. Cells were incubated at 37°C for 10 min, then centrifuged and washed twice with 3 ml of ice-cold Dulbecco's phosphate-buffered saline (Ca^{2+}- and Mg^{2+}-free). All subsequent steps were performed at 0°C.

All measurements were done using two groups of cells, labeled with [^{3}H]progesterone alone or in the presence of nonradioactive hormone in excess. Progesterone binding is expressed as the difference between total binding and nonspecific binding determined in the presence of unlabeled progesterone.

Cell Fractionation. *Cytosol preparation.* Cells were resuspended in 1 ml of TET buffer (10 mM Tris·HCl, pH 7.4/1 mM EDTA/1 mM dithioerythritol/10 mM NaF) containing 20 mM sodium molybdate and were homogenized using a glass/glass Potter–Elvejehm homogenizer. The homogenate was centrifuged at 800 × *g* for 20 min. The supernatant was then centrifuged at 105,000 × *g* for 1 hr; the resultant supernatant is referred to as "cytosol."

Nuclear KCl extraction. The 800 × *g* pellet was washed once with 1 ml of TET buffer containing 0.33 M sucrose, 3 mM $MgCl_2$, and 1% (vol/vol) Triton X-100 and twice with the same buffer without Triton. The pellet was resuspended in 0.5 ml of TET buffer plus 0.5 M KCl and extracted for 1 hr. Soluble nuclear proteins, referred to as "KCl extract," were obtained by centrifugation of the nuclear suspension at

Abbreviation: hsp90, 90-kDa heat shock protein.

105,000 × *g* for 45 min, and the nuclear pellet containing insoluble proteins is referred to as the "NP fraction."

Micrococcal nuclease digestion. Digestion of the nuclei with micrococcal nuclease was performed as described (10). In brief, the 800 × *g* nuclear pellet was washed, resuspended at a concentration of 1 mg of DNA per ml, and digested for 10 min at 20°C with micrococcal nuclease (500 units/mg of DNA) in the presence of 1 mM $CaCl_2$. The reaction was stopped by adding EDTA to 2 mM. The preparation was centrifuged to yield the "nuclease supernatant;" the pellet was resuspended, lysed in hypotonic buffer, and centrifuged to obtain "soluble chromatin" and "chromatin pellet."

$NaDodSO_4$ extraction of nuclei. This extraction was performed as described (11). The washed 800 × *g* pellet was resuspended in 200 μl of TET buffer containing 1 mM phenylmethylsulfonyl fluoride and 4% $NaDodSO_4$, boiled for 4 min, and centrifuged at 1200 × *g* for 5 min. The pellet was resuspended in an equal volume of water and centrifuged. The two supernatants were pooled and diluted with four volumes of Triton buffer [190 mM NaCl/6 mM EDTA/50 mM Tris·HCl, pH 7.4/1 mM phenylmethylsulfonyl fluoride/10 mM NaF/2.5% (vol/vol) Triton X-100].

Progesterone Binding Assay in Subcellular Fractions. Cell fractionation was performed as described. The NP fraction was resuspended in 0.5 ml of TET buffer and aliquots of all fractions were incubated for 3 hr at 0°C with 10 nM [^{3}H]progesterone in the presence or absence of 1 μM progesterone. At the end of the incubation, an equal volume of charcoal suspension [0.4% (wt/vol) charcoal/0.04 (wt/vol) dextran in TET buffer] was added to the cytosol and to the nuclear extract, and they were incubated for 20 min at 0°C and then centrifuged at 2000 × *g* for 10 min. The resuspended NP fraction was washed in TET buffer and extracted with 0.5 ml of acetone. Radioactivity was determined in all fractions. Progesterone binding is expressed as the difference between total and nonspecific binding determined in the presence of excess unlabeled progesterone.

[^{32}P]Orthophosphate Labeling. Cells (5–7 × 10^7) were harvested, suspended in 2 ml of phosphate-free phosphorylation buffer and incubated with 1 mCi of [^{32}P]orthophosphate for 1 hr at 37°C. Where indicated, 10 nM progesterone was added to the mixture after 50 min of incubation. Cells were centrifuged and washed twice with 3 ml of ice-cold phosphate-buffered saline prior to homogenization.

Immunoprecipitation of ^{32}P-Labeled Progesterone Receptor. *Antibodies.* IgG-G_3 is the immunoglobulin fraction of a goat antiserum against the nontransformed, molybdate-stabilized 8S progesterone receptor. This antibody recognizes all the constituents of the native and transformed forms of the receptor—i.e., the A and B subunits and the 90-kDa protein (hsp90). IgG-G_0 is the corresponding fraction of preimmune goat serum. The preparation and specificity controls of these immunoglobulins have been described (12). Anti-goat IgG rabbit antibodies were prepared (13), and the equivalent point for precipitation was determined by using [^{3}H]progesterone-labeled cytosol, so that antibody was in large excess.

Immunoprecipitation. Aliquots (100–200 μl) of ^{32}P-labeled cytosols and nuclear soluble extracts were incubated with IgG-G_3 (50–100 μg) for 16 hr at 0°C. The second antibody (400–800 μg) was then added and the mixture was incubated for 4 hr at 0°C. The immunoprecipitates were centrifuged twice at 16,000 × *g* for 10 min through a 700-μl cushion of 1 M sucrose in TET buffer containing 1% sodium deoxycholate. The final precipitate was resuspended in sample buffer for electrophoresis (14) and boiled for 1–2 min.

Gel Electrophoresis. $NaDodSO_4$/polyacrylamide gel electrophoresis (14) was done using 7.5–15% polyacrylamide gradient slab gels. Gels were fixed, stained with Coomassie blue G-250 (Sigma; 0.4 g/liter), and destained overnight. For autoradiography, gels were dried and exposed under Kodak AR X-Omat films, using an intensifier screen, at −70°C. Films were scanned with a Beckman DU-8 spectrophotometer.

Immunoblot Analysis. After electrophoresis, proteins were transferred to nitrocellulose sheets (Schleicher & Schuell; 0.45-μM pore size) according to the method described (15, 16). The nitrocellulose was then saturated with bovine serum albumin (30 mg/ml) and incubated with IgG-G_3 (5–10 mg/ml) at room temperature for 16 hr. ^{125}I-labeled anti-goat IgG rabbit antibody (10^6 cpm/ml, 10–50 μCi/μg) was used to detect anti-receptor antibodies. The nitrocellulose sheet was extensively washed, dried, and exposed under AR X-Omat Kodak film at −70°C. The film was scanned as above.

RESULTS

Specific Progesterone Binding Sites in Subcellular Fractions of Oviduct Cells. The amount and distribution of progesterone binding sites in cytosol and nuclear fractions were determined in cells not exposed to hormone and in cells exposed to [^{3}H]progesterone for 5–45 min at 37°C. The total number of progesterone binding sites per cell detected by both labeling procedures was comparable (7698 ± 908, or 10,630 ± 2705), but their distribution was different. In cells not exposed to hormone, 90% of specific progesterone binding sites were found in the cytosol, whereas after exposure to progesterone, ≈80% of specific progesterone binding activity was recovered in the nuclear fractions, with about one-fifth of the nuclear activity in the 0.5 M KCl extract (Fig. 1). Routinely the cells were incubated with [^{3}H]progesterone at 37°C for 10 min, and the distribution pattern was the same in many experiments using distinct primary cell cultures.

Since only 20% of the nuclear binding sites were solubilized by 0.5 M KCl, micrococcal nuclease digestion of the nuclei was performed. After digestion of the nuclei, 40–50% of nuclear progesterone binding sites were solubilized and recovered in the nuclease supernatant and soluble chromatin fractions. Finally, when nuclei were extracted with $NaDodSO_4$, the proportion of solubilized nuclear receptor binding sites was increased to about 70%.

Phosphorylation of Progesterone Receptor in Subcellular Fractions of Oviduct Cells. Cells were incubated with [^{32}P]orthophosphate for 1 hr at 37°C, in the presence or

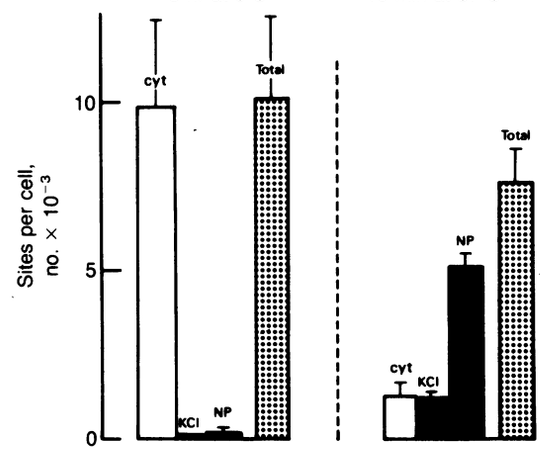

FIG. 1. Specific progesterone binding sites measured in subcellular fractions. Cells were fractionated and labeled for 3 hr at 0°C with 10 nM [^{3}H]progesterone (*Left*) or were incubated for 10 min at 37°C with 10 nM [^{3}H]progesterone and then fractionated (*Right*). Specific progesterone binding sites were measured in cell fractions as described in *Experimental Procedures*. Cyt, cytosol; KCl, 0.5 M KCl nuclear extract; NP, nuclear pellet. Error bars represent SEM for 5 determinations.

absence of 10 nM progesterone. Equal amounts of protein (100 μg) from cytosol and nuclear KCl extract were immunoprecipitated with IgG-G_3, subjected to $NaDodSO_4$/PAGE, and autoradiographed. A major radioactive band at 110 kDa and a minor band at 90 kDa were immunoprecipitated from cytosol. There was no obvious difference in the pattern and in the amount of radioactive protein whether cells were exposed to progesterone for 5, 10, or 20 min (data not shown). In the nuclear KCl extract, no ^{32}P-labeled 110-kDa band was detected. The ^{32}P labeling of the 90-kDa protein was not affected by progesterone treatment and was lower in the nuclear fraction than in the cytosol (Fig. 2).

As a control for the specificity of IgG-G_3 immunoprecipitation, the corresponding fraction of preimmune goat serum, IgG-G_0, was used (Fig. 3*A*). Only IgG-G_3 immunoprecipitated ^{32}P-labeled 110-kDa and 90-kDa proteins, whereas other phosphoproteins were immunoprecipitated with the preimmune antibody IgG-G_0. Another control was performed by immunoprecipitation of 50 μl of ^{32}P-labeled cytosol with IgG-G_3 antibodies in the presence or absence of 0.65 μg of nontransformed (8S) purified progesterone receptor. The intensity of the 110-kDa band decreased by 55% and the 90-kDa band was no longer observed (Fig. 3*B*). An identical control was performed in the presence of 0.8 μg of purified B (110-kDa) subunit (17), leading to a decrease of ≈60% of the 110-kDa band (Fig. 3*C*).

Since progesterone treatment induced a decrease of the number of progesterone binding sites in the cytosol, the ^{32}P labeling of the 110-kDa and 90-kDa bands was compared in aliquots of cytosol containing an equal amount of progesterone binding sites obtained from untreated or hormone-treated cells (Fig. 4). $NaDodSO_4$/PAGE and autoradiography showed a 2.6-fold increase in the signal for the 110-kDa protein in the cytosol of progesterone-treated cells. In spite

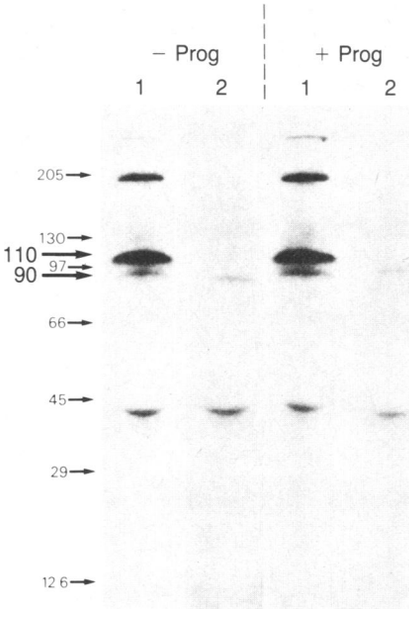

FIG. 2. Autoradiography of phosphorylated progesterone receptor components. Cells were labeled for 1 hr at 37°C with [^{32}P]orthophosphate, in the absence (−Prog) or presence (+ Prog) of 10 nM progesterone, added 10 min before the end of the labeling. Cytosol (100 μl, lanes 1) and nuclear KCl extract (200 μl, lanes 2) were immunoprecipitated using the IgG-G_3 antibody. The immunoprecipitates were analyzed by $NaDodSO_4$/PAGE and autoradiography. Standards run in parallel were myosin heavy chain (205 kDa), β-galactosidase (130 kDa), phosphorylase *b* (97 kDa), serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), and cytochrome *c* (12.6 kDa). Large arrows indicate bands at 110 and 90 kDa representing B subunit and hsp90, respectively.

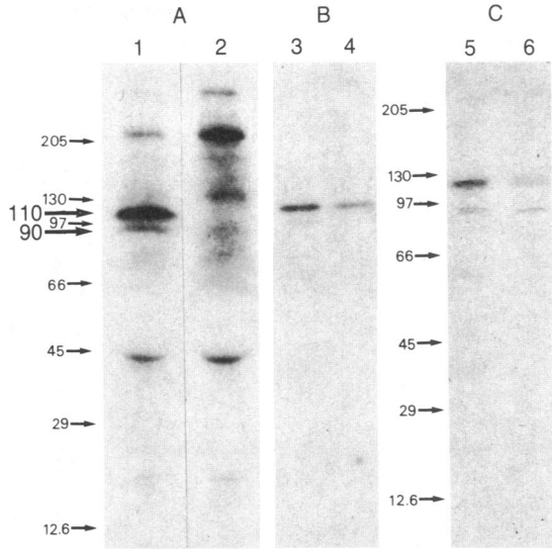

FIG. 3. Specificity of immunoprecipitation of phosphorylated progesterone receptor components. (*A*) Aliquots (100 μl) of ^{32}P-labeled cytosol were immunoprecipitated either with IgG-G_3 (lane 1) or with the IgG fraction of a preimmune antiserum (IgG-G_0, lane 2). (*B*) Equal amounts (50 μl) of ^{32}P-labeled cytosol were incubated with IgG-G_3 (15 μg) in the absence (lane 3) or presence (lane 4) of an excess of unlabeled purified nontransformed 8S progesterone receptor (0.65 μg based on specific progesterone binding activity). (*C*) Equal amounts (50 μl) of ^{32}P-labeled cytosol were incubated with IgG-G_3 (15 μg) in the absence (lane 5) or presence (lane 6) of an excess of unlabeled purified progesterone receptor (0.8 μg of B subunit). The immunoprecipitates were analyzed by $NaDodSO_4$/PAGE and autoradiographed. Standards are the same as in Fig. 2.

of the different volumes of cytosol used in this experiment, a similar amount of ^{32}P-labeled 90-kDa protein was immunoprecipitated. This was probably not due to a difference in specific activity of the protein but to the saturation of the antibodies by an excess of 90-kDa antigen (see *Discussion*).

Since the nuclear KCl extract contained only 20% of the nuclear progesterone binding sites, a search for ^{32}P-labeled 110-kDa protein was performed after digestion of the nuclei with micrococcal nuclease or after extraction of the nuclei with 4% $NaDodSO_4$. Both methods allowed the solubilization of a higher proportion (40–70%) of nuclear progesterone receptor. No radioactive 110-kDa subunit was immunoprecipitated from these soluble nuclear fractions (Figs. 5 and 6).

To exclude the possibility that the absence of ^{32}P-labeled 110-kDa subunit in nuclear immunoprecipitates was due to an artifact, two control experiments were performed. First, we confirmed that the ^{32}P-labeled 110-kDa subunit could be detected when added to a nuclear extract. Immunoprecipitation of a mixture of cytosol containing ^{32}P-labeled 110-kDa subunit and of a nuclear $NaDodSO_4$ extract showed that the phosphorylated protein could be detected under these conditions (Fig. 6). Then, the immunoprecipitates from nuclear KCl extracts were analyzed by immunoblotting with IgG-G_3 antibodies. Densitometric scanning of the autoradiograms indicated that the 110-kDa subunit was present only in nuclei of cells exposed to progesterone (Fig. 7). The strong signal at 66 kDa corresponds to the heavy chain of the goat IgG used for the immunoprecipitation, which was recognized by the iodinated second antibody.

No radioactive phosphorylated form of the A (79-kDa) subunit of the progesterone receptor was seen either in cytosol or in nuclear extracts, although this subunit was detected in the cytosol by immunoblotting (data not shown). For the nucleus, however, data are ambiguous, since IgG-G_3 recognized not only the A subunit but also other proteins of ≈80 kDa (3). Therefore, no conclusion concerning the A

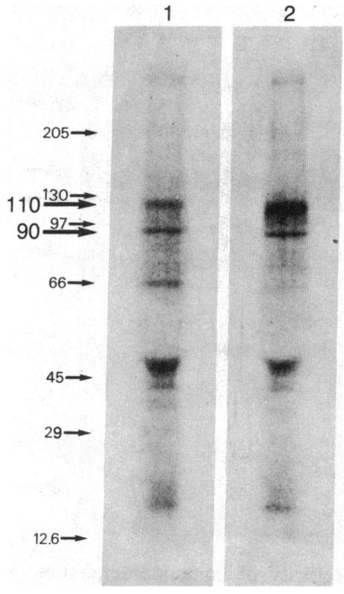

FIG. 4. Autoradiography of phosphorylated receptor components after immunoprecipitation of equal amounts of cytosolic specific progesterone binding sites. Two groups of cells ($5–7 \times 10^7$) were each divided in half. One half was labeled with [^{32}P]orthophosphate in the absence or presence of 10 nM progesterone. The other half was incubated in the presence or absence of the hormone (without phosphate) and used for determination of specific cytosolic progesterone binding sites. Aliquots of ^{32}P-labeled cytosol containing an equivalent amount of specific progesterone binding sites (≈0.02 pmol of receptor) were immunoprecipitated with IgG-G_3 and analyzed by NaDodSO$_4$/PAGE and autoradiography. Lane 1: 20 μl of cytosol from untreated cells. Lane 2: 100 μl of cytosol from progesterone-treated cells.

subunit can be drawn from the peak indicated by an unlabeled arrowhead in Fig. 7.

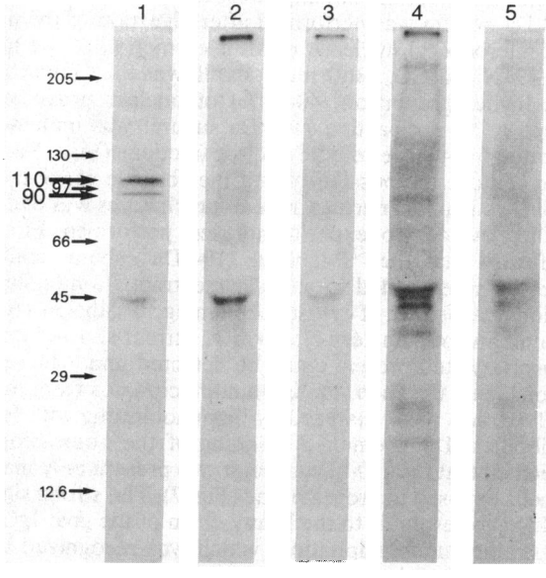

FIG. 5. Autoradiography of phosphorylated progesterone receptor components after micrococcal nuclease digestion of nuclei. Cells were labeled for 1 hr at 37°C with 1 mCi of [^{32}P]orthophosphate in the presence of 10 nM progesterone. Aliquots (100 μl) of cytosol (lane 1), nuclease supernatant (lanes 2 and 3), and soluble chromatin (lanes 4 and 5) were immunoprecipitated either with IgG-G_3 (lanes 1, 2, and 4) or with preimmune IgG-G_0 (lanes 3 and 5) and analyzed by NaDodSO$_4$/PAGE and autoradiography.

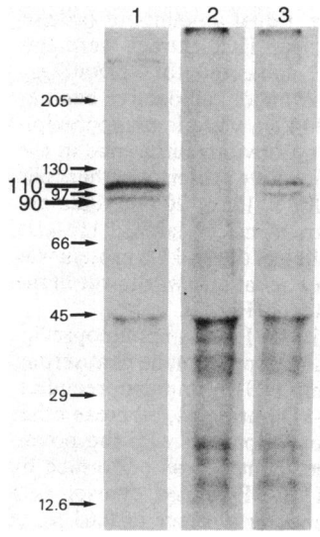

FIG. 6. Autoradiography of phosphorylated progesterone receptor components after NaDodSO$_4$ extraction of nuclei. Cells were labeled for 1 hr at 37°C with 1 mCi of [^{32}P]orthophosphate in the presence of 10 nM progesterone. Cytosol (250 μl, lane 1), the soluble nuclear extract (500 μl, lane 2), or a mixture of both (lane 3) were immunoprecipitated with IgG-G_3 and analyzed by NaDodSO$_4$/PAGE and autoradiography.

DISCUSSION

We have studied the subcellular distribution of chick oviduct progesterone receptor and its phosphorylation, using a cultured cell system more convenient for labeling of proteins than *in vivo* experiments. Since no established avian cell line expressing the progesterone receptor is available, we used primary cultures of chick oviduct cells. After 4–5 days of culture in the presence of estradiol, such cells are still responsive to hormone stimulation (9).

The cells contained 7000–10,000 specific progesterone binding sites per cell; these values are lower than those measured in fresh tissue (18, 19), as commonly observed in primary tissue culture systems. The distribution of the receptor in subcellular fractions was changed radically by exposure of the cells to progesterone. In cells incubated with [^{3}H]progesterone, about 80% of the specific binding sites

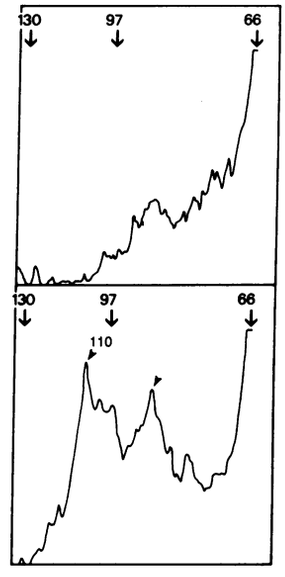

FIG. 7. Detection of nuclear progesterone receptor by immunoblot analysis of immunoprecipitated 0.5 M KCl extracts. Cells were incubated in the absence (*Upper*) or presence (*Lower*) of 10 nM progesterone at 37°C. Nuclear KCl extracts (100 μl) were immunoprecipitated and then subjected to NaDodSO$_4$/PAGE and immunoblot analysis using IgG-G_3 and iodinated anti-goat IgG rabbit antibodies. Nitrocellulose sheets were autoradiographed, and the film was scanned from 130 kDa to 66 kDa. Vertical arrows indicate positions of standards (130, 97, and 66 kDa). Peaks corresponding to B subunit (110 kDa) and A subunit are indicated by arrowheads.

were found in the nuclear fractions, whereas in cells not exposed to the hormone, >90% of the sites were present in the cytosol. It has been reported (20, 21) that the distribution of progesterone receptor between the biochemically defined cytosol and nuclear fractions should not be interpreted as being representative of its actual intracellular localization but might reflect only the easier extraction of the nuclear nontransformed receptor in the absence of hormone.

After incubation of oviduct cells with [^{32}P]orthophosphate, the radioactive receptor components were analyzed in the cytosol and nuclear extracts. A 110-kDa phosphoprotein was immunoprecipitated only from the cytosol and identified as the B subunit of the progesterone receptor. The specificity of the immunoprecipitation was shown by the negative result obtained with the preimmune goat serum and by competition studies using an excess of purified progesterone receptor.

As already stated, 80% of the progesterone binding sites were recovered in the nuclear fractions after incubation of the cells with progesterone. However, when [^{32}P]orthophosphate labeling of receptor was assessed in the presence of progesterone, no change of ^{32}P-labeled 110-kDa subunit in the cytosol was observed, and the presence of nonphosphorylated 110-kDa subunit in a nuclear KCl extract was detected by immunoblotting. These observations could be explained by the following hypothesis. The progesterone-binding B (110-kDa) subunit exists in a phosphorylated and a nonphosphorylated form. In the absence of progesterone, both forms are recovered in the cytosol. In the presence of the hormone, only the nonphosphorylated form becomes tightly bound to DNA and its level decreases in the cytosol, therefore increasing the ratio of ^{32}P-labeled B subunit to hormone-binding activity in this fraction.

Recent experiments have demonstrated preferential binding of the purified B subunit to the 5′ flanking region of the lysozyme gene (22). Phosphorylation can significantly alter the binding mode of certain proteins to nucleoprotein complexes. This has been reported for the DNA-binding proteins of adenovirus (23, 24) and herpes simplex virus (25). It has also been suggested that dephosphorylated glucocorticoid receptors have a higher DNA-binding ability than the phosphorylated forms (26). This observation supports the hypothesis that the ^{32}P-labeled form of progesterone receptor in our experimental conditions might not be permissive for hormonal activation. However, we cannot exclude that the difference observed in ^{32}P incorporation in the B subunit reflects changes in the turnover of protein phosphate groups or that the small fraction of nuclear progesterone receptor not solubilized by the extraction procedures is phosphorylated.

Since the preliminary announcement of this work,* the presence of phosphorylated rabbit progesterone receptor in cytosol and nuclear fractions after ^{32}P labeling of uterine slices has been reported (27). These different results might be due to differences between species, hormonal treatment, and ^{32}P-labeling protocols. In contrast, W. Tienrungroj and W. B. Pratt (personal communication) have not been able to demonstrate ^{32}P in DNA-bound glucocorticoid receptors from ^{32}P-labeled mouse L cells after transforming receptors in cell-free lysate and assaying their binding to DNA-cellulose.

Phosphorylation/dephosphorylation of steroid receptors has been suggested as a mechanism of modulation of receptor steroid-binding activity. The binding capacity of calf uterus estrogen receptor is restored by an enzyme that can utilize ATP (28, 29). The steroid binding capacity of unoccupied cytosolic glucocorticoid receptor is inactivated by a dephosphorylation mechanism (30, 31). However, a recent report (32) on the partially purified chick oviduct progesterone receptor indicates that high-affinity steroid binding sites are not affected by alkaline phosphatase treatment. Furthermore, under our experimental conditions, the nuclear progesterone binding activity corresponded to the presence of a nonphosphorylated form of the B subunit.

The role of phosphorylation of hsp90 (which is a component of the progesterone receptor) remains to be investigated, since IgG-G_3 antibodies recognize this heat shock protein, which is present in large stoichiometric excess over the progesterone-binding subunits (4).

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