Chicken Progesterone Receptor is Phosphorylated by a DNA-Dependent Protein Kinase during *in Vitro* Transcription Assays

Nancy L. Weigel, Timothy H. Carter, William T. Schrader, and Bert W. O'Malley

Department of Cell Biology
Baylor College of Medicine
Houston, Texas 77030

Department of Biological Sciences
St. John's University (T.H.C.)
Jamaica, New York 11439

We have reported previously that chicken progesterone receptor (PR) is phosphorylated *in vivo* in response to progesterone administration. Three phosphorylation sites have been reported, two of which show increased phosphorylation in response to hormone and one which is phosphorylated only in response to hormone administration. We found previously that PR lacking the hormone-dependent phosphorylation is active in an *in vitro* transcription assay. Since the source of general transcription factors is a HeLa nuclear extract which contains many kinases, we have analyzed the receptor for phosphorylation during the *in vitro* transcription assay. We report here that the receptor is rapidly and efficiently phosphorylated on new sites, causing a change in receptor mobility on sodium dodecyl sulfate-gels. This phosphorylation is strictly dependent upon the presence of double stranded DNA. A DNA-activated protein kinase with similar properties has been isolated previously from HeLa cell nuclei. We find that phosphorylation of PR with this purified enzyme mimics the phosphorylation observed in the transcription assay. These data suggest that a previously undetected additional series of DNA-dependent phosphorylations may be required for activation of the PR. (Molecular Endocrinology 6: 8–14, 1992)

INTRODUCTION

The chicken progesterone receptor (PR) is a member of a superfamily of ligand-activated transcription factors (1). Chick PR is expressed in two forms [PRb (mol wt, 86,000) and PRa (mol wt, 72,000)] which are produced from one gene by alternate initiation of translation from the same message (2, 3). Thus, PRa is a truncated version of PRb; they have identical hormone- and DNA-binding domains, but PRa lacks a portion of the amino-terminal sequence of PRb. Both forms are active *in vivo*, but appear to have different promoter specificities (4, 5).

We and others have shown that both of these forms undergo hormone-dependent phosphorylation concomitant with activation of the receptor's transcriptional activity *in vivo* (6, 7). We have reported the identification of three phosphorylation sites in chick PR isolated from chicken oviduct cytosol (8). One site, Ser^{530} in PRb (Ser^{402} in PRa[1]) is not phosphorylated detectably until the cells are exposed to progesterone. This site is in the region between the DNA-binding and hormone-binding domains. Mutation of this site (Ser to Gly) diminishes, but does not eliminate, the ability of PR to stimulate transcription in transient transfection studies (9).

Phosphorylation plays a major role in receptor activation, since the hormone requirement can be bypassed by enhancing kinase activity or inhibiting protein phosphatases (10). Those studies also suggested that there are additional phosphorylation sites that are involved at least in the ligand-independent pathway. We have developed a receptor-dependent *in vitro* transcription system using HeLa cell nuclear extracts as a source of general transcription factors (11). Both PRa and PRb were active in the assay.

The purified receptor used in the initial studies was isolated from cytoplasmic extracts of oviducts not treated with progesterone. As we have shown (8), the PR was not phosphorylated on Ser^{530}, the known hormone-dependent site. If additional receptor phosphor-

[1] Because PRa is a truncated form of PRb, Ser^{530} corresponds to Ser^{402} in PRa. To be consistent with the paper identifying the sites, we will refer to this phosphorylation site as Ser^{530} in this paper.

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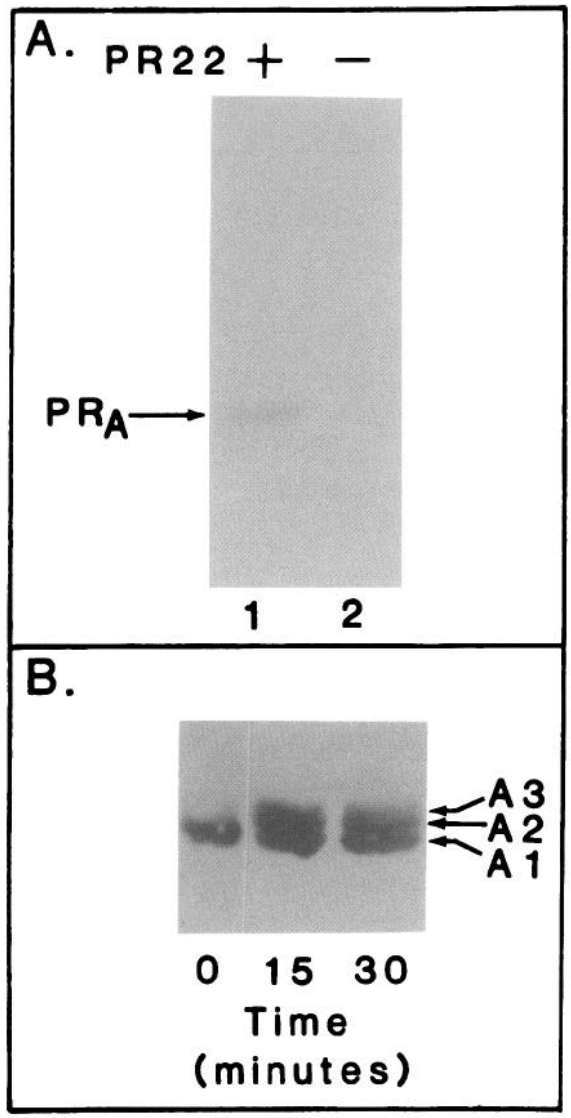

Fig. 1. A, Phosphorylation of Chicken PR during *in Vitro* Transcription Assays

Each sample of PRa (0.6 μg) was preincubated, as described in *Materials and Methods*, with 18 μl HeLa nuclear extract (see *Materials and Methods*), 3 μg herring sperm DNA, and 0.3 μg test template PRE 2+pLovTATA (11) in a final volume of 69 μl. After the preincubation, 21 μl of mix, including 15 μCi [^{32}P] ATP were added and incubated as usual. Ten microliters of monoclonal antibody PR22 (lane 1 only), 20 μl 2 mg/ml rabbit antimouse IgG, and 25 μl 5 × IPB [2.5 M NaCl, 0.25 M Tris (pH 7.5), 1% sodium azide, and 1% Triton X-100] were added to the samples, which were then incubated on ice for 30 min. A portion (0.1 ml) of a 1:1 slurry of protein-A-Sepharose in IPB was added to each sample, and the samples were rocked for 30 min at room temperature. The samples were centrifuged 1 min in an Eppendorf centrifuge, and the supernatants were discarded. The pellets were resuspended in 0.5 ml IPB, rocked for 5 min, and centrifuged, and the supernatants were discarded. The samples were washed two more times with IPB, followed by three water washes and eluted with 0.5 ml 1 M acetic acid. The acetic acid eluates were dried in a Savant Speed-Vac (Hicksville, NY), suspended in SDS sample buffer, and run on a 7.5% SDS-gel, as previously described (8). The ^{32}P was detected by autoradiography of the wet gel. Lane 1, Complete reaction; lane 2, no PR22. B, Effect of phosphorylation of PR during the *in vitro* transcription assay on receptor mobility. PRa was incubated with assay components exactly as described in A, except that [^{32}P]ATP was omitted. At the indicated times after the preincubation, aliquots containing 0.12 μg PRa were removed, and the reaction was stopped by the addition of SDS sample buffer. The samples were run on SDS-gels and detected by immunoblot, as described in *Materials and Methods*.

ylation is required for activity, these results suggest that the HeLa nuclear extract might contain a kinase that phosphorylates the PR. We demonstrate in these studies that the receptor is phosphorylated in the *in vitro* transcription assay and that the phosphorylation is due to a DNA-dependent kinase previously shown to be active in nuclear transcription extracts (12). This suggests that a heretofore unsuspected additional series of phosphorylations may be required for full activation of the receptor.

RESULTS

To determine whether PRa is phosphorylated during the *in vitro* transcription assay, a transcription assay was performed, as reported previously (see *Materials and Methods*), except that [^{32}P]ATP was added in addition to the usual amounts of unlabeled ATP. Figure 1A shows the autoradiogram of a sodium dodecyl sulfate (SDS)-gel of receptor immunopurified from the transcription assay. There is a band of phosphorylated receptor in the sample purified using PR22, the receptor antibody, but no signal in the control sample. Because the incubation contains an ATP-generating system (see *Materials and Methods*), it was not possible to determine directly the stoichiometry of receptor phosphorylation from the ^{32}P in this experiment.

We and others have shown that hormone-dependent phosphorylation of PR *in vivo* results in a change in mobility on SDS-gels (6, 7, 13, 14). We used this fact to test PR incubations prepared with nonradioactive ATP for alterations in receptor gel mobility. Figure 1B shows the results of such an experiment. At the indicated times after the addition of ATP, SDS sample buffer was added to stop the reaction. The samples were then analyzed by immunoblotting, as described in *Materials and Methods*. The receptor migrates as a single band at the beginning of the reaction, but two new bands with slower mobility are present at 15 min and persist throughout the incubation. Typically, the slowest band (A3) is much less abundant than the control (A1) and middle (A2) bands. In samples containing less receptor (*e.g.* 2C), A3 is frequently not detected. Additional experiments indicated that phosphorylation reaches a steady state level between 5–10 min after the addition of ATP (data not shown). Since more than half of the receptor exhibits altered mobility, it is clear that the receptor is efficiently phosphorylated

during the *in vitro* transcription assay. Moreover, since the phosphorylation occurs early in the incubation, the transcriptional activity previously measured (11) is not that of control receptor, but, rather, that of a mixture of the input receptor form and a majority of multiply phosphorylated receptor.

Jackson *et al.* (15) reported that another transcription factor, Sp1, is quantitatively phosphorylated upon addition to an *in vitro* transcription incubation that contains HeLa nuclear extract. The change in Sp1 mobility on SDS-gels mimics the change observed *in vivo*. They found that the kinase responsible for the phosphorylation required DNA for activity and that a purified DNA-dependent protein kinase isolated from HeLa cells (15–17) phosphorylated Sp1 *in vitro*.

We partially characterized the kinase activity in nuclear extracts that was responsible for receptor phosphorylation. Creatine phosphate and ribonucleotides other than ATP could be eliminated from the incubations without diminishing phosphorylation (Fig. 2), showing that the phosphorylation is not a cotranscriptional event. However, the phosphorylation was highly DNA dependent. Figure 2A shows the results of incubations with [^{32}P]ATP in the presence or absence of DNA. Only the sample containing DNA showed significant incorporation of label. The wet gel autoradiography used to detect the ^{32}P-labeled receptor does not resolve the various forms, so PRa includes A1, A2, and A3 forms. Figure 2B shows a direct immunoblot of comparable unlabeled incubations. Only in the sample containing DNA was a significant fraction of PR shifted to the A2 and A3 forms.

The DNA-dependent protein kinase described by Carter's (16, 18) and by Anderson's (17–19) groups requires double stranded DNA, but is not activated by single stranded DNA. To determine whether the kinase that phosphorylates PR has similar properties, the effects of various DNAs on receptor phosphorylation were tested, as shown in Fig. 2C. Again, there was no mobility shift in the absence of DNA, and addition of double stranded herring sperm DNA resulted in receptor phosphorylation. In contrast, addition of an equivalent amount of single stranded plasmid DNA did not stimulate phosphorylation. Addition of both the herring sperm and the progesterone response element (PRE) template resulted in only slightly enhanced phosphorylation over that seen with the herring sperm DNA alone. These properties are consistent with those reported for the HeLa cell DNA-dependent kinase, termed DNA-dependent protein kinase (DNA-PK), which requires double stranded DNA for activity.

We next tested the ability of purified DNA-PK to phosphorylate PR. The kinase was purified from HeLa cell nuclear extracts as previously described (16). The kinase was determined to be more than 80% of the total protein based on SDS-gel analysis followed by silver staining. No other protein kinase activity was detected using casein as a substrate, as previously described (16). Figure 3A shows that in the absence of added DNA-PK, there was very little phosphorylation of

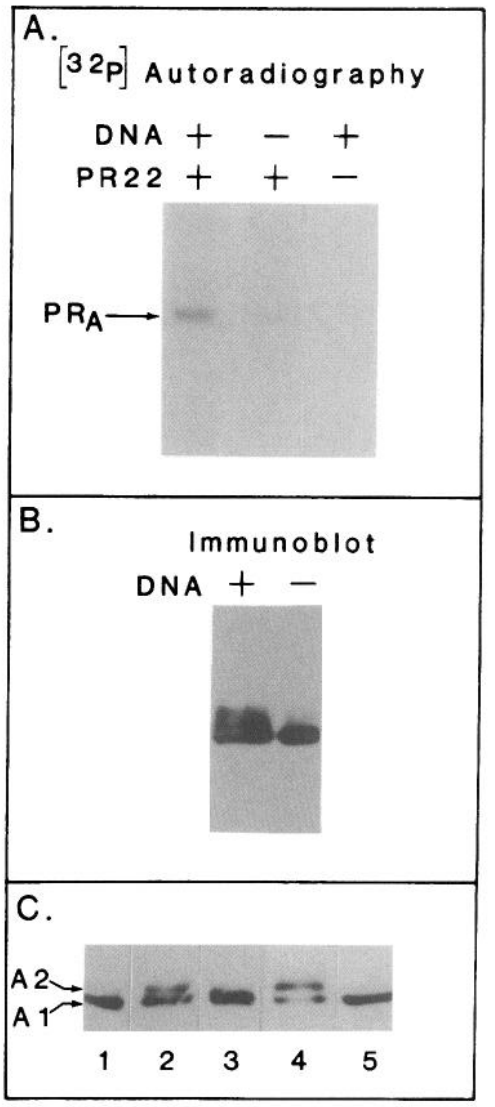

Fig. 2. DNA Dependence of Receptor Phosphorylation

A, PRa (0.6 μg) was incubated with 10 μl HeLa nuclear extract using the incomplete transcription assay conditions (see *Materials and Methods*), either with or without 3 μg herring sperm DNA and 0.3 μg test template PRE 2+pLovTATA. [^{32}P]ATP (5 μCi) was added to each incubation with the cold ATP. Samples were immunopurified and analyzed as described in Fig. 1. B, Samples incubated as described in the *upper panel* were incubated without [^{32}P]ATP and analyzed by immunoblotting. C, Specificity for double stranded DNA. PRa (0.06 μg) was incubated in the incomplete transcription mix containing 3 μl HeLa nuclear extract containing the indicated DNA in a final volume of 30 μl. Ten microliters of 4-fold concentrated sample buffer was added at the end of the incubation, and samples were analyzed by immunoblotting. Lane 1, No added DNA; lane 2, 1.2 μg double stranded herring sperm DNA; lane 3, 1.2 μg single stranded M6 DNA; lane 4, 2.4 μg double stranded herring sperm DNA and 0.24 μg PRE 2+pLov; lane 5, identical to lane 4, except SDS sample buffer was added to the initial incubation before the addition of receptor.

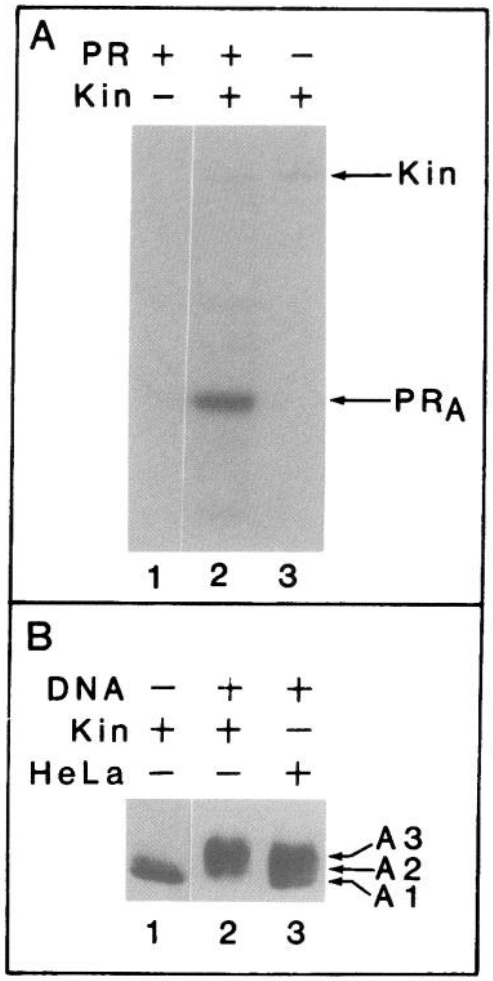

Fig. 3. Phosphorylation of PRa with Purified DNA-PK

A, Receptor (0.6 μg) was incubated for 15 min at 37 C in 30 μl containing the components of the incomplete transcription assay, 1.2 μg double stranded herring sperm DNA, 0.12 μg PRE 2+pLov, and 20 μCi [^{32}P]ATP. The samples were run on SDS-gels, and ^{32}P was detected by wet gel autoradiography (exposure time, 1 min). Kin, DNA-PK. Lane 1, PRa alone; lane 2, PRa plus 2 μl purified DNA-PK; lane 3, 2 μl purified DNA-PK alone. B, Effect of phosphorylation by DNA-PK on PR mobility. Receptor (0.06 μg) was incubated for 15 min at 37 C with incomplete transcription components and the indicated amounts of DNA and kinase. Samples were run on SDS-gels, and receptor was detected by immunoblot. Lane 1, No DNA; lane 2, 1.6 μg PRE 2+pLov DNA and 4 μl DNA-PK; lane 3, 1.6 μg PRE 2+pLov DNA and 3 μl HeLa extract.

PR. Addition of purified DNA-PK together with DNA resulted in phosphorylation of PRa as well as in the characteristic autophosphorylation of the 300-kilodalton kinase itself (16).

The immunoblot shown in Fig. 3B shows that DNA-PK-catalyzed phosphorylation also resulted in altered receptor mobility similar to that observed in the HeLa extract incubations (lane 3). The reaction was highly dependent on added DNA (see lane 1 *vs.* lane 2). Although some kinases are stimulated by polyanions, such as heparin, RNA, or DNA, DNA-PK is the only enzyme that has been reported to be stimulated exclusively by double stranded DNA (16). Thus, it appears that it is the DNA-PK that is responsible for the receptor phosphorylation.

Hormone-dependent PR phosphorylation results in only a single new receptor species (20). The results in Figs. 1–3 show that the DNA-dependent enzyme must be phosphorylating at least one additional site, since two slower bands (A2 and A3) can be seen. To examine the ability of this kinase to phosphorylate receptor that has been previously phosphorylated on Ser^{530}, receptor from progesterone-treated oviducts was isolated. We have shown previously that the altered mobility of receptor isolated from progesterone-treated chickens is due to phosphorylation, because treatment with alkaline phosphatase produces the A1 form (8). Figure 4 shows the results of the DNA-dependent phosphorylation of the receptor from progesterone-treated chickens. Control receptor phosphorylated with the HeLa nuclear extract shows the usual change in mobility. The receptor from injected animals exists as a doublet, with mobilities corresponding to A1 and A2, due to the presence of a substantial fraction of phospho-Ser^{530}. As shown in the *extreme right lane*, the mobility of the progesterone-treated sample is further retarded by DNA-dependent phosphorylation, and an additional upper band (A3) appears. Comparison of the relative intensities of the bands in lanes with and without DNA indicates that at least some of the receptor phosphorylated on Ser^{530} has been additionally phosphorylated and shows reduced mobility. Thus, we conclude that both control and Ser^{530}-phosphorylated receptor are substrates for the kinase.

DISCUSSION

These studies show that DNA-dependent phosphorylation of PR occurs readily during cell-free transcription assays. Based on the change in mobility on SDS-gels, it is clear that a majority of the receptor is phosphorylated rapidly on at least one site, and it is the activity of this mixture of receptor forms that we have measured in previous studies.

Phosphorylation *in vitro* does not always mimic phosphorylation *in vivo*. Frequently, purified kinases will show some ability to phosphorylate purified proteins in the absence of competing substrates *in vitro*, when there is no evidence that such a reaction occurs *in vivo*. However, in the transcription assays, the kinase is in the presence of its normal milieu of proteins, and the added progesterone receptor is as little as 0.1% of the total protein. Moreover, the change in receptor mobility indicates that a substantial fraction of the receptor is phosphorylated on at least one site. Thus, the receptor appears to be a very good substrate for this enzyme.

We have reported the identification of three PR phosphorylation sites (8). The receptor used in those experiments was isolated from the cytosol of progesterone-

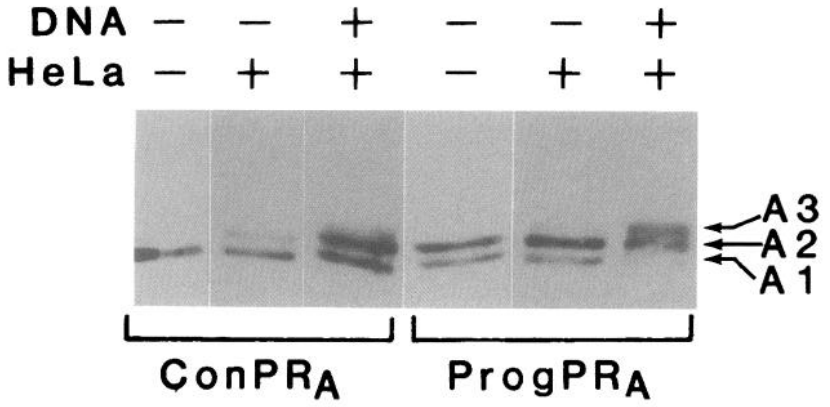

Fig. 4. Phosphorylation of PR from Progesterone-Treated Oviducts

Receptor (control or from progesterone-treated oviducts) was incubated with the incomplete transcription assay components with or without 3 μl HeLa and/or 2.4 μg double stranded herring sperm DNA and 0.24 μg PRE 2+pLov, as indicated. Receptor was analyzed by SDS-gel electrophoresis and immunoblotting.

treated oviduct tissue slices. If DNA-dependent phosphorylation occurs subsequent to binding to DNA in the nucleus, only receptor isolated from nuclei would be phosphorylated on these sites. Our recent peptide-mapping studies using purified DNA-PK indicate that the two most prominent DNA-PK phosphopeptides coelute with peptides containing previously identified sites (data not shown), suggesting that we may simply not have detected a small amount of additional nuclear phosphopeptides in the tryptic digests. Washburn *et al.* (21) have reported that estrogen receptor isolated from the nuclear, but not the cytosolic, extract of estrogen-treated mouse uteri shows a reduced mobility form with enhanced incorporation of ^{32}P. These data suggest that DNA-dependent phosphorylation may be a common step in the activation of members of this family of transcription factors. Clearly, further analysis of receptor isolated from chicken nuclear extracts will be necessary to determine whether DNA-dependent phosphorylation occurs in chicken oviduct.

Lees-Miller and Anderson (19) mapped the major DNA-PK phosphorylation sites in the human heat shock protein and determined that both contain a threonine followed by one or more glutamine residues in conjunction with glutamic acid or aspartic acid residues. On that basis, possible phosphorylation sites (postulated to contain SQ or TQ sequences imbedded in a relatively acidic environment) were identified in a variety of DNA-binding proteins, the PR among them (18). It is interesting that Takimoto *et al.* (22) reported that mutations in the DNA-binding domain of human PR compromise the phosphorylation of the receptor. This group has suggested that binding to DNA is necessary for some of the human PR phosphorylation observed *in vivo*. Jackson *et al.* (15) demonstrated that the transcription factor Sp1 undergoes a similar DNA-dependent phosphorylation *in vitro*. The phosphorylation does not alter DNA binding, but a similar phosphorylation occurs *in vivo* and correlates with the level of transcriptional activity. These observations suggest that many transcription factors can bind to DNA in the absence of phosphorylation, but they may be subsequently phosphorylated by the DNA-dependent kinase with a resulting change in transcriptional activity. Since DNA-PK must bind to DNA to be active, it is possible that the transcription factors do not need to bind to DNA to be phosphorylated, but binding would enhance proximity to the enzyme and, therefore, enhance the chances of phosphorylation.

We, therefore, propose the following model (shown in Fig. 5) for the role of phosphorylation in receptor activation. In the absence of hormone, the receptor is associated with heat shock protein-90 and other non-hormone-binding proteins (23, 24) and is phosphorylated only on constitutive sites. Phosphorylation of these sites does not alter receptor mobility on SDS-gels, and this form has the mobility of A1. Binding of hormone causes a conformational change that allows phosphorylation of Ser^{530}, which favors dissociation of the heat shock complex, receptor dimerization, and binding to PRE sequences. This form has the mobility of A2 and may be inactive or partially active. A subsequent round of DNA-dependent phosphorylation then produces the fully active receptor.

All members of the steroid/thyroid hormone superfamily that have been examined for phosphorylation have been shown to be phosphorylated (13, 24–29). Progesterone (6, 7, 13, 14), glucocorticoid (30, 31), and estrogen (21, 32) receptors exhibit enhanced phosphorylation in response to their cognate hormones. This suggests that hormone-dependent and perhaps also DNA-dependent phosphorylations may be common mechanisms for regulating the function of this family of transcription factors.

MATERIALS AND METHODS

Materials

[^{32}P]ATP was obtained from ICN (Costa Mesa, CA). 1-Thioglycerol and 2-mercaptoethanol were purchased from Sigma

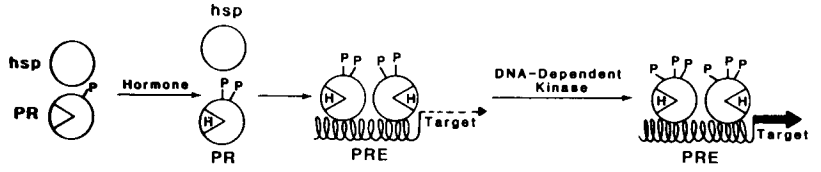

Fig. 5. A Model for Transcriptional Activation of PR by Phosphorylation

hsp, Heat shock protein-90 and other nonsteroid-binding proteins. Each P represents a class of phosphorylation sites rather than an individual site.

(St. Louis, MO). The monoclonal antichicken PR antibody (PR22) was kindly provided by Dr. David O. Toft (Mayo Clinic, Rochester, MN) (33). Rabbit antimouse immunoglobulin G (IgG) was obtained from Zymed (San Francisco, CA). Protein-A-Sepharose was purchased from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ). Acrylamide was obtained from Serva Fine Biochemicals, Inc (Paramus, NJ). All other gel electrophoresis supplies were obtained from Bio-Rad (Richmond, CA). The ECL immunoblot detection kit and rainbow markers were purchased from Amersham (Arlington Heights, IL).

Purified DNA-dependent protein kinase was purified as previously described (16).

Purification of Chicken PR

Chicken PRa was purified as previously described (11). Receptor purity typically ranged between 30–80%.

Purification of Chicken PR from Progesterone-Treated Chickens Diethylstilbestrol-treated chicks (34) used for the preparation described above were each injected sc with 5 mg progesterone dissolved in sesame oil. Oviducts were collected after 30 min, and cytosol was prepared, as previously described (2), using buffer A [10 mM Tris (pH 7.4), 1 mM EDTA, and 12 mM thioglycerol] containing 50 mM K_xPO_4, pH 7.5, and 50 mM NaF. The cytosol (30 ml) was incubated with 30 nM [^{3}H]progesterone (43 Ci/mmol) overnight at 4 C. The receptor was precipitated with 32% ammonium sulfate, the sample was centrifuged for 30 min at 8000 × *g*, and the pellet was resuspended in 20 mM K_xPO_4 in buffer A. The sample was diluted to a conductivity equivalent to 0.15 M NaCl and loaded on a 7-ml diethylaminoethyl column equilibrated with 0.15 M NaCl in buffer A. The column was washed with 15 ml 0.15 M NaCl in buffer A. The flow-through and wash were combined, diluted to 0.1 M NaCl, and loaded on a 0.2-ml DNA cellulose column (Sigma calf thymus DNA cellulose). The column was washed with 20 mM HEPES (pH 7.9), 0.1 mM EDTA, 10 mM dithiothreitol, and 20% glycerol, and the receptor was eluted in the same buffer containing 0.3 M NaCl. Receptor was stored frozen at −20 C. This procedure yields a receptor preparation that is not more than 1% pure.

In Vitro Transcription Assay Incubations

Nuclear extracts were prepared by the method of Dignam *et al.* (35). The complete reactions (Fig. 1) were prepared and incubated essentially as previously described (11). Each reaction contained 20 mM HEPES-KOH (pH 7.9), 8% (vol/vol) glycerol, 60 mM NaCl, and the indicated amounts of HeLa extract, DNA, and receptor. The samples were incubated for 15 min at room temperature, and an additional mix was added to the incubation to give final concentrations of 5 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM creatine phosphate, 0.5 mM ATP, 0.5 mM CTP, 20 μM UTP, and 0.5 mM O-methyl-GTP. The samples were incubated at 30 C for the indicated times and either immunopurified (Fig. 1A) or loaded directly onto SDS-gels (Fig. 1B).

Incomplete Transcription Assay Incubations Each reaction contained 20 mM HEPES-KOH (pH 7.9), 12% (vol/vol) glycerol, 60 mM NaCl, and the indicated amounts of HeLa extract, DNA, and receptor. The samples were incubated for 15 min at room temperature, and an additional mix was added to the incubation to give final concentrations of 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.5 mM ATP. The samples were incubated at 30 C for the indicated times and either immunopurified or loaded directly onto SDS-gels.

Detection of PR Forms by Immunoblot Analysis Samples were run on 6.5% SDS-gels, using rainbow standards for markers. The gels were run until the BSA was at the bottom of the resolving gel (~12 cm), the proteins were transferred to nitrocellulose using a Bio-Rad Semi-Dry Transblot apparatus following the manufacturer's directions, and the receptor was detected using monoclonal antibody PR22 (which is specific for chicken PR), rabbit antimouse IgG, and the Amersham ECL chemiluminescent detection kit.

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Address requests for reprints to: Dr. Nancy Weigel, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

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REFERENCES

1. Evans RM 1988 The steroid and thyroid hormone receptor superfamily. Science 240:889–895
2. Schrader WT, O'Malley BW 1972 Progesterone-binding components of chick oviduct. IV. Characterization of purified subunits. J Biol Chem 247:51–59
3. Conneely OM, Maxwell BL, Toft DO, Schrader WT, O'Malley BW 1987 The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. Biochem Biophys Res Commun 149:493–501
4. Carson MA, Tsai M-J, Conneely OM, Toft DO, Schrader WT, O'Malley BW 1987 Analysis of the functional domains of the chicken progesterone A subunit. Mol Endocrinol 1:791–801
5. Tora L, Gronemeyer H, Turcotte B, Gaub MP, Chambon P 1988 The N-terminal region of the chicken progesterone receptor specifies target gene activation. Nature 333:185–188
6. Denner LA, Bingman WE, Greene GL, Weigel NL 1987

Phosphorylation of the chicken progesterone receptor. J Steroid Biochem 27:235–243
7. Sullivan WP, Smith DF, Beito TG, Krco CJ, Toft DO 1988 Hormone-dependent processing of the avian progesterone receptor. J Cell Biochem 36:103–119
8. Denner LA, Schrader WT, O'Malley BW, Weigel NL 1990 Hormonal regulation and identification of chicken progesterone receptor phosphorylation sites. J Biol Chem 265:16548–16555
9. Denner LA, Conneely OM, Schrader WT, Weigel NL, Functional regulation of chicken progesterone receptor transcriptional activity by phosphorylation. 73rd Annual Meeting of The Endocrine Society, Washington DC, 1991, p 32 (Abstract)
10. Denner LA, Weigel NL, Maxwell BL, Schrader WT, O'Malley BW 1990 Regulation of progesterone receptor-mediated transcription by phosphorylation. Science 250:1740–1743
11. Klein-Hitpass L, Tsai SY, Weigel NL, Allen GF, Riley D, Rodriguez R, Schrader WT, Tsai M-J, O'Malley BW 1990 The progesterone receptor stimulates cell-free transcription by enhancing the formation of a stable preinitiation complex. Cell 60:247–257
12. Carter TH, Kopman CR, James CBL 1988 DNA-stimulated protein phosphorylation in HeLa whole cell and nuclear extracts. Biochem Biophys Res Commun 157:535–540
13. Logeat F, Le-Cunff M, Pamphile R, Milgrom E 1985 The nuclear-bound form of the progesterone receptor is generated through a hormone-dependent phosphorylation. Biochem Biophys Res Commun 131:421–427
14. Horwitz KB, Wei LL, Francis MD, 1986 Structural analyses of progesterone receptor. J Steroid Biochem 24:109–117
15. Jackson SP, MacDonald JJ, Lees-Miller S, Tjian R 1990 GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. Cell 63:155–165
16. Carter T, Vancurova I, Sun I, Lou W, DeLeon S, 1990 A DNA-activated protein kinase from HeLa cell nuclei. Mol Cell Biol 10:6460–6471
17. Lees-Miller SP, Chen YR, Anderson CW 1990 Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, p53, and other regulatory proteins. Mol Cell Biol 10:6472–6481
18. Carter TH, Anderson CW 1991 The DNA-activated protein kinase, DNA-PK. Prog Mol Subcell Biol 12:37–58
19. Lees-Miller SP, Anderson CW 1989 The human double stranded DNA-dependent protein kinase phosphorylates the 90 kilodalton heat shock protein hsp90 at two amino terminal threonine residues. J Biol Chem 264:17275–17280
20. Denner LA, Weigel NL, Schrader WT, O'Malley BW 1989 Hormone-dependent regulation of chicken progesterone receptor deoxyribonucleic acid binding and phosphorylation. Endocrinology 125:3051–3058
21. Washburn T, Hocutt A, Brautigan DL, Korach KS 1991 Uterine estrogen receptor *in vivo*: phosphorylation of nuclear specific forms on serine residues. Mol Endocrinol 5:235–242
22. Takimoto GS, Tasset DM, Eppert AC, Horwitz KB, Transcription is regulated by phosphorylation of human progesterone receptors (h PR) following DNA binding. 73rd Annual Meeting of The Endocrine Society, Washington DC, 1991, p 308 (Abstract)
23. Dougherty JJ, Puri RK, Toft DO 1984 Polypeptide components of two 8S forms of chicken oviduct progesterone receptors. J Biol Chem 259:8004–8009
24. Smith DF, Faber LE, Toft DO 1990 Purification of unactivated progesterone receptor and identification of novel receptor-associated proteins. J Biol Chem 265:3996–4003
25. Dougherty JJ, Puri RK, Toft DO 1982 Phosphorylation *in vivo* of chicken oviduct progesterone receptor. J Biol Chem 257:14226–14230
26. Housley PR, Pratt WB 1983 Direct demonstration of glucocorticoid receptor phosphorylation by intact L-cells. J Biol Chem 258:4630–4635
27. Grandics P, Miller A, Schmidt TJ, Litwack G 1984 Phosphorylation *in vivo* of rat hepatic glucocorticoid receptor. Biochem Biophys Res Commun 120:59–65
28. Pike WJ, Sleator NM 1985 Hormone-dependent phosphorylation of the 1,25-dihydroxyvitamin D3 receptor in mouse fibroblasts. Biochem Biophys Res Commun 131:378–385
29. Goldberg Y, Glineur C, Gesquiere J-C, Ricouar A, Sap J, Vennstrom B, Ghysdael J 1988 Activation of protein kinase C or cAMP-dependent protein kinase increases phosphorylation of the c-*erb*A encoded thyroid hormone receptor and of the v-*erb*A encoded protein. EMBO J 7:2425–2433
30. Hoeck W, Rusconi S, Groner B 1989 Hormone dependent phosphorylation of the glucocorticoid receptor occurs mainly in the amino terminal transactivation domain. J Biol Chem 264:14396–14402
31. Orti E, Mendel DB, Smith LI, Munck A 1989 Agonist-dependent phosphorylation and nuclear dephosphorylation of glucocorticoid receptors in intact cells. J Biol Chem 264:9728–9731
32. Denton RR, Koszewski NJ, Carter TH, Notides AC, Phosphorylation of the estrogen receptor increases its affinity for estrogen specific DNA response elements. 73rd Annual Meeting of The Endocrine Society, Washington DC, 1991, p 312 (Abstract)
33. Sullivan WP, Beito TG, Proper J, Krco CJ, Toft Do 1986 Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 119:1549–1557
34. Coty WA, Schrader WT, O'Malley BW 1979 Purification and characterization of the chick oviduct progesterone receptor A subunit. J Steroid Biochem 10:1–12
35. Dignam JD, Lebovitz RM, Roeder RG 1983 Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11:1475–1489