# Sequence and Expression of a Functional Chicken Progesterone Receptor

**Orla M. Conneely, Alan D. W. Dobson, Ming-Jer Tsai, Wanda G. Beattie, David O. Toft, Clark S. Huckaby*, Tanya Zarucki, William T. Schrader, and Bert W. O'Malley**

Department of Cell Biology (B.W.O.)
Baylor College of Medicine
Texas Medical Center
Houston, Texas 77030

Department of Biochemistry and Molecular Biology (D.O.T.)
Mayo Medical School
Rochester, Minnesota 55905

**We have cloned and sequenced 4.5 kilobases (Kb) of cDNA encoding the chicken progesterone receptor. The complete cDNA contains an open reading frame of 2361 nucleotides in length and encodes a polypeptide of 787 amino acids with a mol wt of 85.9 K. At least four mRNA species have been detected in chick oviduct cells. Direct sequencing of variant cDNAs has suggested that two of the mRNAs (4.5 Kb and 3.6 Kb) differ only in the length of their 3′-untranslated regions. A third mRNA (1.8 Kb) produces a truncated polypeptide which encodes the immunoreactive $NH_2$ terminal sequence of the receptor but lacks the hormone binding regional and half of the DNA-binding domain. The polypeptide expressed from the receptor cDNA in progesterone receptor negative Cos M-6 cells is indistinguishable from oviduct progesterone receptor in terms of hormone binding and antibody reactivity. Furthermore, the cloned receptor is capable of activating transcription of a target gene. This activation is progesterone dependent (with half-maximal stimulation at ~$3.3 \times 10^{-10}$ M) and specific for the target gene. (Molecular Endocrinology 1: 517–525, 1987)**

## INTRODUCTION

Steroid hormones regulate gene expression in eucaryotes by binding to specific intracellular receptors which in turn modulate the transcription of target genes (1–3). The recent cloning of the glucocorticoid (4, 5), estrogen (6–8), thyroid hormone (9, 10), progesterone (11–14), and vitamin D receptors (15) has now facilitated examination of the structure-function relationships of these receptors. Amino acid homologies among these proteins suggests that they belong to a family of hormone receptors (16).

0888-8809/87/0517-0525$02.00/0
Molecular Endocrinology


We have used the chick oviduct as a model system to examine the molecular mechanisms involved in regulation of gene transcription by progesterone. In the chick oviduct the interaction of progesterone with its receptor results in increased transcription of a defined set of genes coding for egg-white proteins (17). We have reported previously that the chicken progesterone receptor (cPR) is composed of two hormone-binding proteins (18). The A protein binds to DNA with high affinity while the B protein binds less well to DNA but interacts with certain nonhistone chromosomal proteins (19). In order to examine the primary structure of the receptor and to establish the origin and functional significance of the A and B proteins, we have isolated previously partial cDNA clones encoding the cPR (11). The partial cDNA sequence we reported was in agreement with the partial cDNA sequence presented by Jeltsch *et al.* (12).

In the present paper, we report the sequence of the entire cPR cDNA and examine the homology between cPR and other steroid receptors. In addition, we demonstrate that the polypeptide product expressed from the cPR cDNA displays the functional activities inherent in the authentic progesterone receptor (PR) from chick oviduct.

## RESULTS

### Chicken PR cDNA and Protein Sequence

We have reported previously the isolation of partial cDNA clones encoding the cPR (11). In order to obtain the sequence of the complete cPR cDNA, the longest of these clones [cPR2, 1.3 kilobases (kb)] which encodes the immunoreactive domain of the receptor protein was used as a $^{32}$p-labeled probe to screen two independent λgt11 cDNA libraries and a pCD cDNA library prepared from chicken oviduct poly(A)$^+$ RNA. A number of the overlapping cDNA clones obtained were sequenced by the M13 dideoxy chain termination

method (20) or by chemical degradation according to the method of Maxam and Gilbert (21). The sequencing strategy is shown in Fig. 1. The sequence was confirmed by comparison with that of genomic clones covering the entire cDNA (Huckaby, C. S., O. M. Conneely, W. G. Beattie, A. D. W. Dobson, M.-J. Tsai, and B. W. O'Malley, 1987, in preparation).

The complete cDNA sequence encoded by the longest cPR mRNA (4.5 Kb), together with the deduced amino acid sequence of the receptor protein is shown in Fig. 2. The longest open reading frame is 2361 nucleotides in length and contains an initiation methionine signal at nucleotide 315. The open reading frame closes with a TGA stop signal at nucleotide 2676 in the sequence. Upstream at nucleotide 156 and in frame with the initiation ATG is a TGA stop signal which suggests that translation cannot initiate 5′ of this ATG. The authenticity of this TGA stop signal has been confirmed in two distinct cDNA clones and in the genomic DNA itself. In fact, this region has been crossed five times from each direction using both the dideoxy and Maxam and Gilbert methods. The 3′-end of the open reading frame is followed by a long 3′-untranslated region which contains a consensus polyadenylation signal (AATAAA) 32 nucleotides upstream from the poly(A) addition site.

The longest open reading frame encodes a protein of 787 amino acids in length with a predicted polypeptide mol wt of 85.9 K. Examination of the receptor sequence reveals a second methionine codon 385 nucleotides downstream of the most 5′-initiation signal in the same translation reading frame. This internal ATG is flanked by a consensus sequence consistent with that reported optimal for initiation of translation in eucaryotes (22).

## Receptor cDNA Variants

Three variant cDNAs were isolated during screening of the cDNA libraries. These variants with the corresponding oviduct mRNAs are shown in Fig. 3. Two of the cDNAs (4.5 kb and 3.6 kb) contain identical coding sequences and appear to differ only in the length of their 3′-untranslated regions. The third cDNA variant (1.8 kb) which was isolated from three independent libraries produces a truncated polypeptide which contains the entire immunoreactive domain of the receptor but lacks the hormone binding domain and half of the DNA binding domain of the protein. Northern blot hybridization analysis shows that the variant cDNAs may correspond to three mRNA species detected using chick oviduct poly(A)$^+$ RNA. For these analyses, a $^{32}$P-labeled antisense RNA probe was used which is derived from cDNA which encodes only the immunogenic region of the receptor protein. This region is not conserved among the steroid receptors thus minimizing the possibility of cross-hybridization of the probe with mRNAs encoding other steroid receptors. An additional mRNA was detected in lower concentration at approximately 8.0 kb in size. Although no cDNA clones have been isolated to date corresponding to this band, it may derive from alternate polyadenylation at a site further downstream from the poly(A) site of the 4.5 kb mRNA.

## Chicken PR Structural Homology with the Steroid and Thyroid Hormone Receptors

The amino acid sequence of the cPR was deduced from the nucleotide sequence of the complete cDNA and

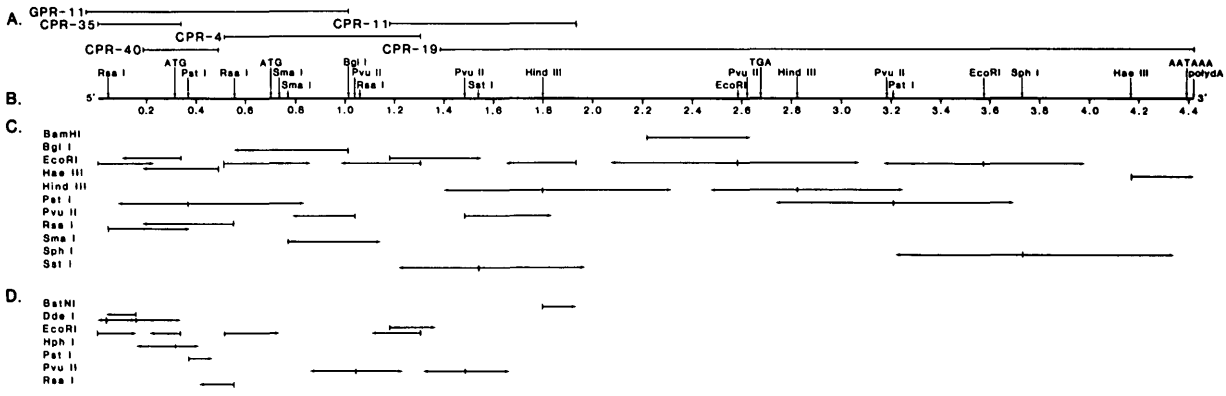


**Fig. 1.** Strategy for Sequencing of the cDNA

A, Placement of some of the cDNA clones which were used to determine the sequence of the gene. GPR-11 is a fragment from a genomic DNA clone. B, A composite restriction map line shows the placement of relevant restriction sites. The locations of the possible initiation codons for the A and B proteins, the stop codon and the polyadenylation signal are also shown. Numbers below the line refer to kilobase pairs of fragment length from the 5′-end of the gene. C, D, The *vertical lines* for each enzyme represent the position of restriction sites where fragments were generated for (C) ligation into the multiple cloning site region of a M13 vector (33), or (D) labeling with [$\gamma$-$^{32}$P] ATP and $T_4$ polynucleotide kinase. The sequence of the fragments was determined by (C) the dideoxy chain termination method (20) on templates derived from M13 phages, or (D) chemical degradation according to the method of Maxam and Gilbert (21). The *arrows* indicate the direction and extent of sequence from these restriction sites. Eighty one and three-tenths percent of the final sequence was determined from both strands. Ten and a half percent was determined at least twice on one strand from overlapping restriction sites, and the remaining 8.3% was determined at least twice on one strand from the same restriction site.

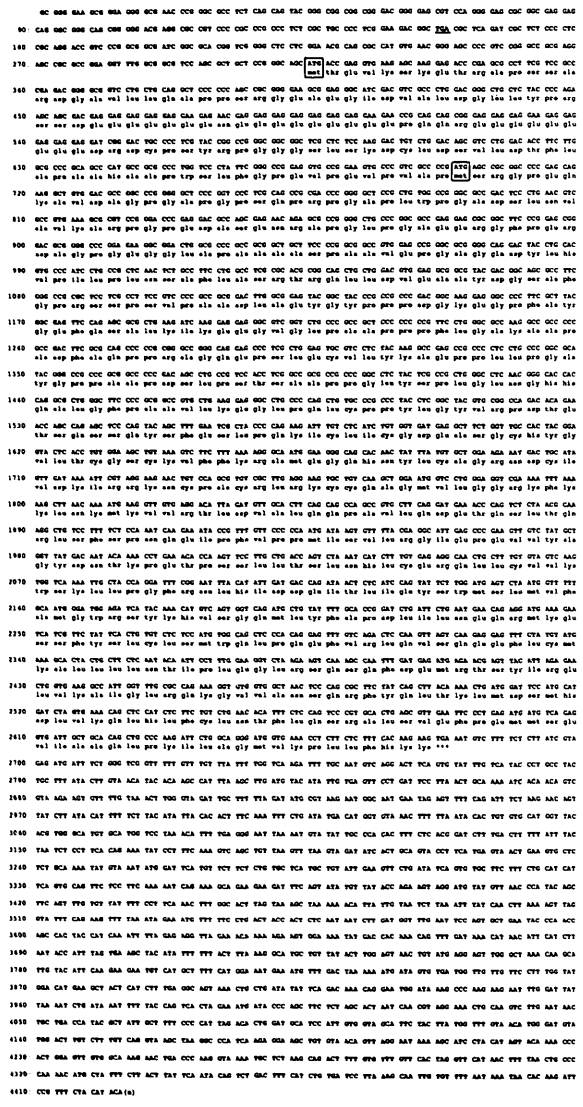

**Fig. 2.** Nucleotide Sequence of the cPR cDNA and Predicted Amino Acid Sequence of the Receptor Protein

The longest open reading frame begins at nucleotide 315 in the sequence (MET 1) and closes with a TGA signal at nucleotide position 2676. The Met 1 codon and an additional ATG used for internal translation initiation are *blocked*. The in-phase TGA stop signal upstream of MET 1 is *underlined*.

was compared to that of the rabbit PR (rbPR) (13) and human (14) PR (hPR), rat (5) and human (4) glucocorticoid receptors (GR), chicken (8) and human (6, 7) estrogen receptors, chicken vitamin D receptor (15), and chicken (10) and human (9) thyroid hormone receptors. In Fig. 4, the receptors are aligned on the basis of structural similarities produced by conserved amino acid sequences. Two major functional regions are conserved between cPR and all of the hormone receptors. They comprise the DNA-binding region and a part of the hormone-binding domains and are contained within a 350 amino acid stretch extending from the COOH-terminus of the receptor proteins. The region of greatest diversity among the receptors is found in the amino terminal region. This domain is hypervariable in terms of both receptor size and amino acid sequence. Comparison of PRs between species in this domain showed an overall amino acid homology of 24% between cPR and rbPR and 23% between cPR and hPR. Both hPR and rbPR contain an insertion of 146 and 143 amino acids, respectively, which are not contained in cPR.

The most striking area of amino acid homology (C1) is contained in the DNA binding domain of the receptors. The domain consists of an 85 amino acid stretch in the central portion of cPR and is characterized by a high content of cysteines and basic amino acid residues. The amino acid conservation with cPR in this region is highest for the rbPR and hPR (100%) and rat and human GR (87%). The homology between cPR and the remaining receptors ranges from 38–53% in this domain.

The second most conserved region (C2) is contained within the hormone-binding domain of the receptors. The amino terminal portion of this domain is characterized by an hydrophobic region which is conserved between all of the receptors. The degree of homology is highest between the PR and GR in this region and throughout the hormone-binding domain. Comparison of the amino acid sequence of cPR with the remaining receptors shows significant amino acid homologies (22–25%) in the hydrophobic region and no significant amino acid conservation in the remainder of the hormone-binding domain. The remarkable structural similarity between the PR and GR throughout the two major functional domains is not surprising since there is considerable similarity between the steroid ligands and the DNA-binding specificities of the two receptors (23).

## Expression of cPR cDNA in Heterologous Cells

In order to examine the hormone-binding properties of polypeptides expressed from the receptor cDNA, we used a 3.1 Kb cDNA construct, termed $cPR_A$, which contains 190 nucleotides upstream from the internal ATG (highlighted in Fig. 2) and the remainder of the open reading frame sequence including 1.0 kb of 3′-untranslated region. The cDNA was inserted into the eucaryotic expression vector, p91023(B) at the *Eco*RI site downstream from the adenovirus major late promoter. The salient features of this vector system have been described elsewhere (24). The p91023(B) $cPR_A$ construct was then transfercted into PR negative COS M-6 monkey kidney cells. The cells were administered with 20 nM $^3$H-labeled progesterone overnight before harvesting. High salt (0.4 M KCl) nuclear extracts were prepared from the cells 48 h after transfection and incubated in the presence and absence of the $\alpha$cPR monoclonal antibody, PR22 (25). The extracts were then subjected to sedimentation analysis in sucrose gradients containing 10–30% sucrose and 0.3 M KCl. The sedimentation profiles of the [$^3$H]PR complex are shown in Fig. 5. In the absence of PR22, the [$^3$H]PR complex sedimented at 3–4 S in the gradient. This position corresponds to the sedimentation behavior of authentic cPR from oviduct cytosol (18). The addition

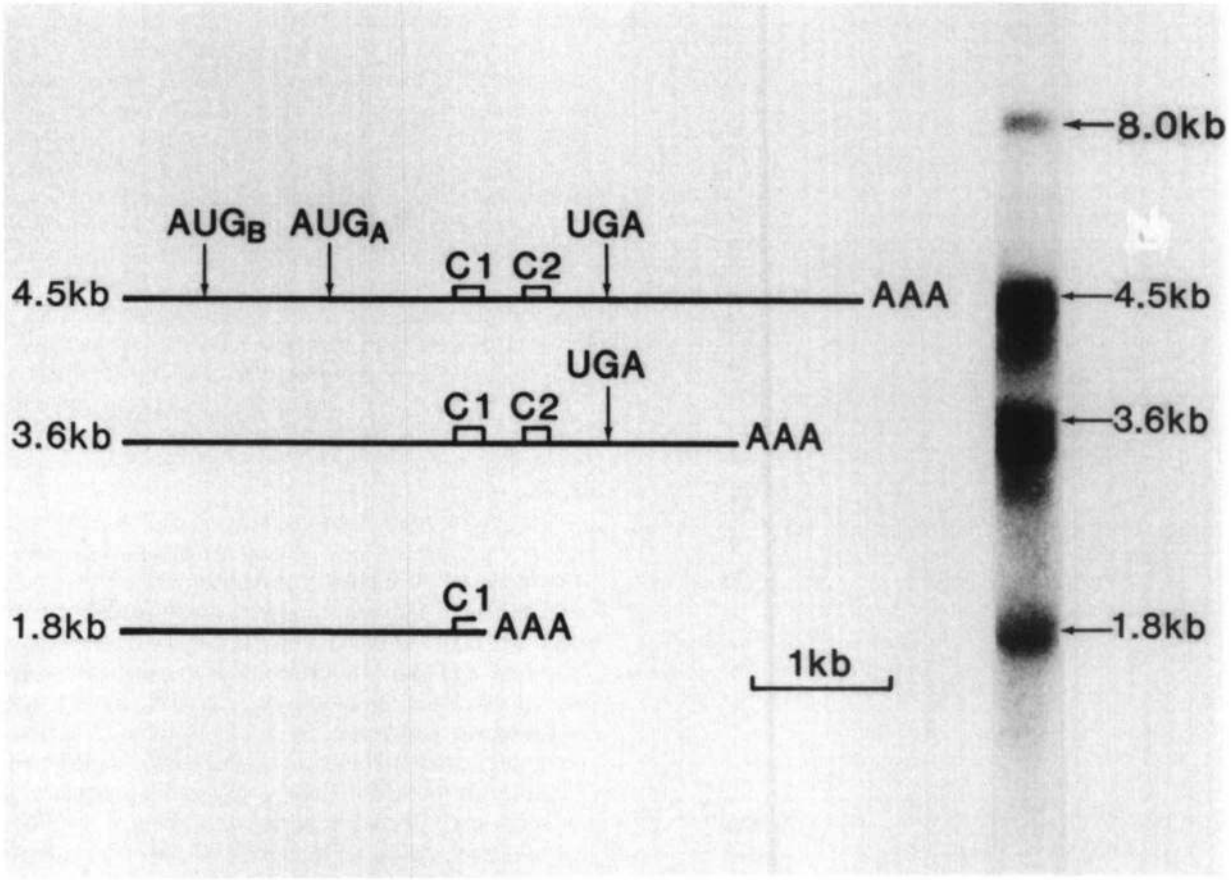


**Fig. 3.** Schematic Representation of cPR Variant cDNAs Together with Corresponding mRNAs from Chick Oviduct

Northern blot analysis was carried out using total RNA (10 μg) from oviducts of estrogen-treated (8 h) chickens as described in *Materials and Methods*.

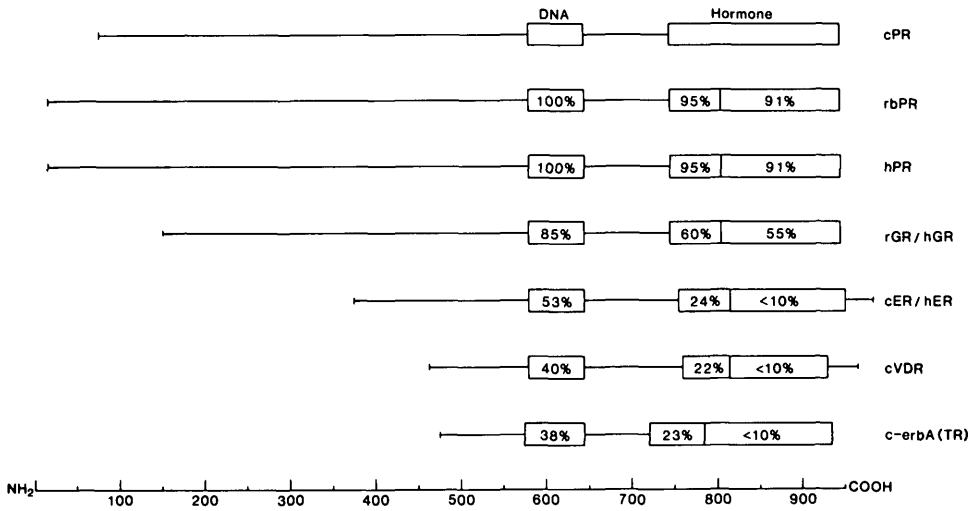


**Fig. 4.** Structural Homology between the Steroid and Thyroid Hormone Receptors (TR)

The receptors were aligned on the basis of amino acid homology with the cPR. The *blocked regions* represent the highly conserved DNA- and hormone-binding domains. The *numbers* contained *within* the *blocked areas* refer to the regional homology for each receptor relative to the cPR. cER, Chicken estrogen receptor; hER, human estrogen receptor; cVDR, chicken vitamin D receptor.

of PR22 to the nuclear extracts resulted in an increase in the sedimentation rate of all of the bound [$^3$H]progesterone. These results indicate that all of the complexed [$^3$H]progesterone was bound specifically to the PR. No progesterone binding was observed in the cytosolic extracts from these cells or in untransfected COS M-6 cells (data not shown). These results provide direct evidence that the cloned cPR cDNA sequence encodes the PR.

To examine the functional activity of the expressed

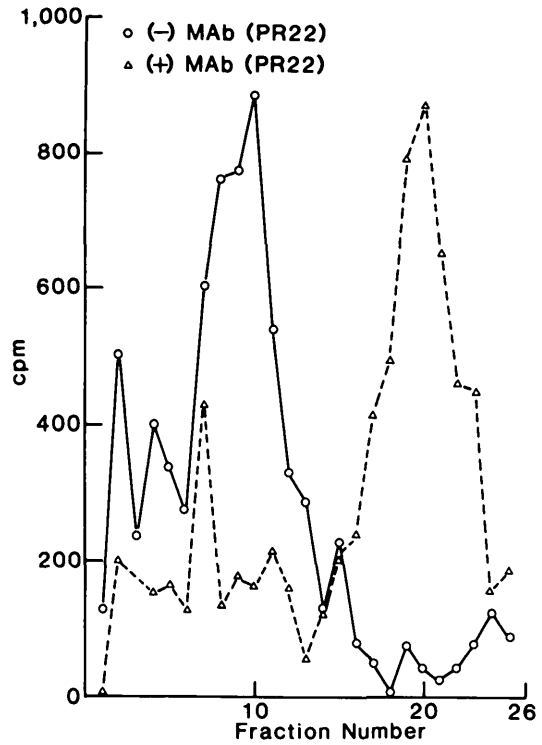


**Fig. 5.** Sedimentation Analysis of cPR in Nuclear Extracts from COS M-6 Cells Transfected with the p91023(B) $cPR_A$ Receptor Expression Plasmid

A 3.1 Kb cDNA containing sequences from nucleotide 501 to 3676 was subcloned into the EcoRI site of p91023(B) 5′ to the adenovirus major late promoter, p91023(B) $cPR_A$. Forty micrograms of the expression plasmid was used to transfect COS M-6 cells. The cells were administered 20 nM [$^3$H]progesterone overnight before harvesting. At 47 h post transfection, the cells were harvested and nuclear extracts were sedimented in 10–30% sucrose gradients containing 0.3 M KCl as previously described (18) in the absence of $\alpha$cPR antibody $\alpha$PR22 and after addition of 10 $\mu$g $\alpha$PR22. The *open circles* represent the sedimentation profile of [$^3$H]cPR in the absence of $\alpha$PR22 (10 $\mu$g); the *triangles* represent [$^3$H]cPR sedimentation in the presence of $\alpha$PR22. Mab, Monoclonal antibody.

polypeptide we cotransfected with the receptor expression construct, p91023(B) $cPR_A$,a plasmid containing a progesterone responsive promoter-enhancer element fused to a reporter gene into CV-1 cells. The CV-1 cell line is derived from monkey kidney cells and does not express SV40 T antigen. These cells do not contain PR. The transfection was carried out in the presence of progesterone. For these experiments, the MMTV-LTR element was used as the progesterone responsive element (23). The reporter gene sequences fused to this hormone-responsive element code for bacterial chloramphenicol acetyl transferase (CAT). The results of such a cotransfection assay are shown in Fig. 6A. The MMTV-CAT construct, pAHCAT, was not expressed in CV-1 cells in the absence of the PR. However, upon cotransfection of the expression vector containing the receptor cDNA sequence together with the pAHCAT construct, the expression vector provided

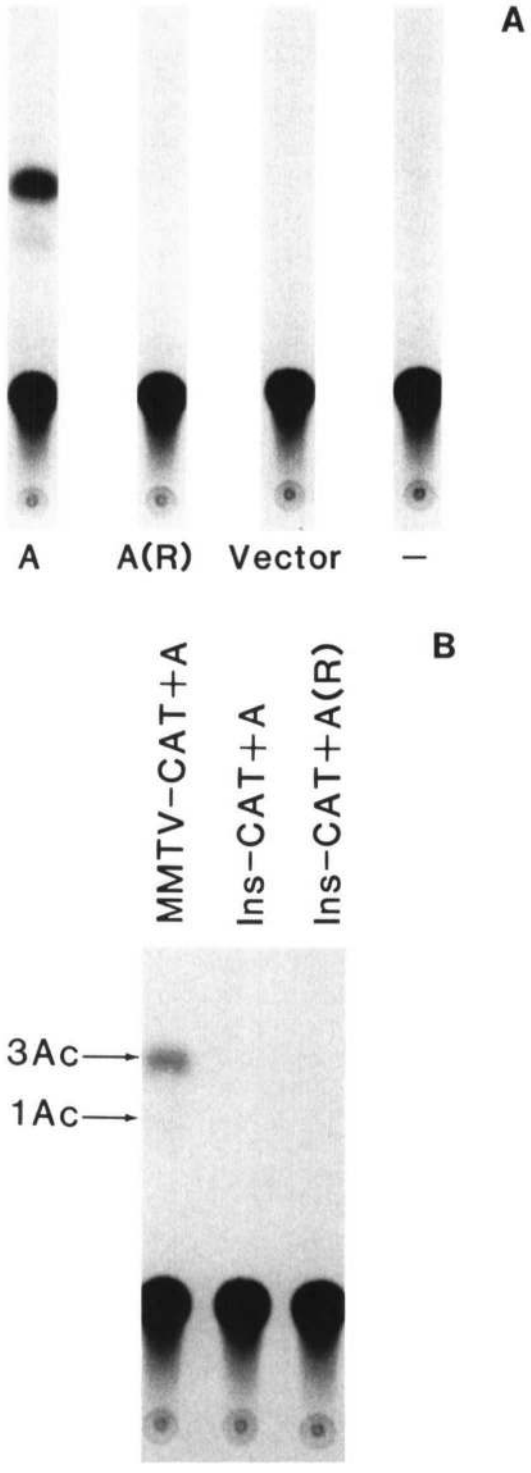


**Fig. 6.** Transient Cotransfections of Receptor cDNA and Target Gene Plasmids into CV-1 Cells

*Panel A*, CAT activity expressed in CV-1 cells after cotransfection of pAHCAT with: 1) 5 $\mu$g plasmid p91023(B)$cPR_A$ in the sense orientation (A); 2) p91023(B)$cPR_A$ in the antisense orientation (A(R)); 3) p910239(B) without cDNA; and 4) pAHCAT alone without receptor plasmid. *Panel B*, Target gene specificity of gene activation by receptor: 1) 5 $\mu$g (A) p91023(B)$cPR_A$ with 5 $\mu$g pAH CAT; 2) 5 $\mu$g p91023 (B) $cPR_A$ with 5 $\mu$g Ins-CAT; and 3) 5 $\mu$g receptor plasmid p91023(B)$cPR_A$ in the antisense orientation with 5 $\mu$g Ins-CAT.

functional PR which was capable of inducing the MMTV promoter to express CAT activity. Cotransfection of the pAHCAT construct with p91023(B) alone or with the same receptor cDNA inserted into p91023(B) in the reverse orientation showed no CAT activity and demonstrates that the CAT activity observed with the p91023(B) $cPR_A$ construct is dependent on receptor expression.

To determine whether the enhancer activation seen with the expressed receptor is specific for the target gene, we used a construct containing the insulin promoter and enhancer regions fused to CAT (Ins-CAT) in a second cotransfection assay. The insulin gene is not normally expressed in CV-1 cells. As shown in Fig. 6B, no CAT activity was observed from this construct when cotransfected with the p91023(B) $cPR_A$ construct in the sense (A) or antisense (A(R)) orientation. A positive control demonstrating CAT activity of the pAHCAT construct with p91023(B) $cPR_A$ is shown in lane 1. A similar cotransfection was carried out using pSV2CAT. This construct expresses CAT activity in CV-1 cells using the SV40 promoter. Transfection of receptor sequences with this construct did not increase the basal expression of CAT activity (data not shown). The results indicate that the MMTV activation seen with the expressed cDNA is specific for the target gene enhancer sequences.

The hormone dependency of target gene activation was measured by culturing CV-1 cells which had been cotransfected with both p91023(B) $cPR_A$ and pAHCAT constructs in charcoal-stripped serum in the absence of progesterone and with increasing concentrations of progesterone added to the medium. The CAT activity observed at each concentration of progesterone is shown in Fig. 7, A and B. The data show that the induction of MMTV is progesterone dependent. Analysis of the relative CAT activity at the various progesterone concentrations allowed an estimation of the equilibrium constant ($K_d$) for progesterone activation of receptor at approximately $3.3 \times 10^{-10}$ M. This value coincides closely with the equilibrium constant PR binding observed with the native PR in chick oviduct (18).

## DISCUSSION

The data presented in this paper demonstrates that the cDNA clones we have isolated and sequenced encode a functional cPR. The conclusions are based on three criteria: 1) the polypeptide product expressed from the cloned receptor cDNA is capable of binding progesterone; 2) the PR complex is immunoreactive with antiprogesterone receptor monoclonal antibodies; and 3) finally, the cloned receptor is capable of inducing transcription of a target gene. The induction is both target gene specific and progesterone dependent.

The complete open reading frame in the cPR cDNA encodes a protein of 787 amino acids in length with a calculated mol wt for the B form of the receptor of 85.9 K. This value is approximately 20 K lower than the estimated mol wt of the receptor B protein using denaturing gel analysis (2). A possible explanation for this discrepancy may be provided by the appearance of a long interrupted glutamic acid track consisting of 25 amino acid residues which may result in retarded migration of the protein in sodium dodecyl sulfate acrylamide gels. This glutamic acid track is not contained in the rabbit or hPR sequences (13, 14). The cDNA sequence reported here corresponds in length to a 4.5 kb mRNA detected in chick oviduct. Three additional mRNAs are detected also with sizes of 8.0, 3.6, and 1.8 kb. There is no evidence to date to suggest that the three larger mRNAs encode functionally distinct proteins. We have isolated a variant cDNA which may encode the 1.8 Kb mRNA. This cDNA encodes a polypeptide which contains the entire immunoreactive domain of the receptor but lacks the hormone binding domain and half of the DNA binding domain.

Comparison of the amino acid sequence of cPR with the steroid and thyroid hormone receptors demonstrates two regions of striking amino acid homology which are positionally conserved among the receptors. They comprise the DNA-binding domain (C1) and a hydrophobic region (C2) within the hormone-binding domain of the receptors. The homology between the different steroid hormone receptors is highest between the PR and GR where the amino acid conservation extends throughout the carboxy terminal half of these receptors. Furthermore, analysis of hydrophobic plots of the carboxy terminal half of these proteins demonstrates that many of the amino acid changes observed in the homologous regions arise from conservative amino acid substitutions and result in a remarkably similar hydrophobic structure in the DNA- and hormone-binding domains of the two steroid receptors. It has been shown that both the GR and PR bind to the same enhancer DNA sequences on MMTV DNA (23). In addition the steroid ligands for these two receptors share certain structural features including requirement for a $\Delta^4$-3-Keto function and the 20-Keto group (26). In light of these observations, the high structural homology observed in the major functional domains of these receptors is not surprising.

Comparison of the amino acid sequences of cPR, rbPR, and hPR showed that the primary structure of the receptor is highly conserved between species from the DNA-binding domain in the central portion of cPR to the carboxy terminal end. Although the $NH^2$-terminal region of the protein is also highly conserved between the rbPR and hPR (13, 14) in terms of both domain length and amino acid sequence, this region shows low overall amino acid homology with cPR where the domain is shorter by approximately 140 amino acids. The functional significance of this domain diversity in the cPR is unclear at the present time.

The data obtained from transient transfection experiments demonstrates that the cloned receptor cDNA construct encodes fully functional PR. The cDNA construct used in these experiments used an internal methionine codon to initiate translation of transcribed mRNAs. Use of this internal methionine to express a

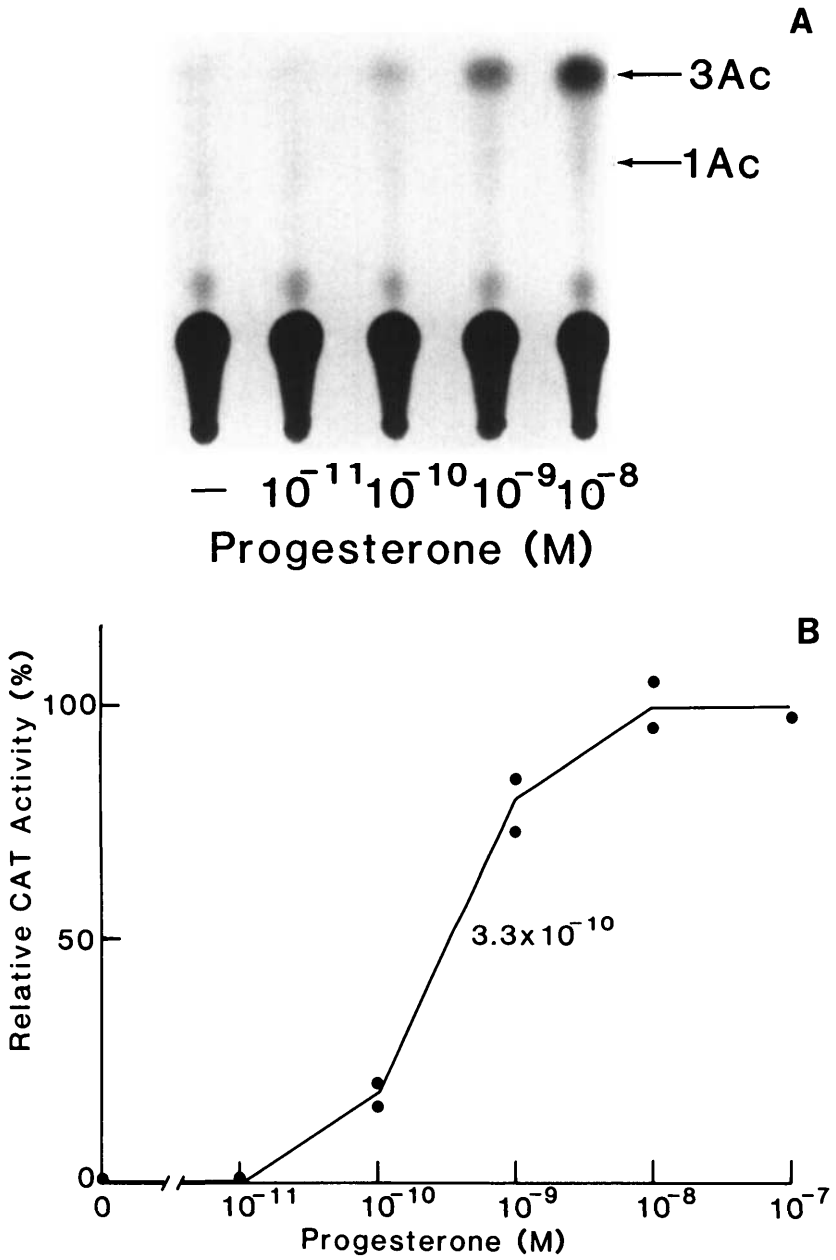


**Fig. 7.** Progesterone Dependency of MMTV Activation by the Cloned Receptor

A, Autoradiogram of thin layer chromatographic assay for CAT activity. Cells were cultured in charcoal-stripped serum without progesterone or with added progesterone at concentrations shown. Five micrograms of receptor and pAHCAT reporter plasmids were used for transfections. B, Relative CAT activity as a function of progesterone concentration. The relative CAT activity was determined by counting the radioactive products obtained at each progesterone concentration. 3AC, 3-Acetylchloramphenicol; 1AC, 1-acetylchloramphenicol.

receptor protein should yield a polypeptide of 72 kilodaltons in size and 659 amino acids in length. This cloned receptor induces transcription of MMTV in a progesterone-dependent manner and is thus a functional receptor molecule. In recent studies we have used the complete cPR cDNA including 5′-untranslated sequences to express PR A and B proteins both *in vivo* and *in vitro*. These data indicate that the cDNA sequence described in Fig. 2 codes for both the A and B forms of the PR. The first ATG in reading frame represents the initiation codon for the larger B form. The second ATG represents an internal codon used for synthesis of the A form. Thus the two forms of the receptor are generated by alternate initiation of translation from two in phase AUG codons in a single mRNA (unpublished observations).

The expression of a functional PR protein in these studies has now facilitated examination of the structure-function relationships of the protein by our ability to assay the functional activities of altered receptors generated by a site-directed mutagenesis of cPR cDNAs. Moreover, expression of the A and B forms of the

receptor in heterologous cells will allow us to examine the relative functional significance of these two receptor proteins.

## MATERIALS AND METHODS

### Isolation of cDNA Clones

Total RNA was isolated from estrogen-stimulated chickens using guanidine thiocyanate and purified by oligo(dT) cellulose chromatography. (Collaborative Research, Inc., Lexington, MA). Twenty five micrograms of poly $(A)^+$ RNA was used to prepare oligo(dT)-primed cDNA and random oligonucleotide-primed cDNA by the method of Gubler and Hoffman (27). The cDNA was modified for insertion into λgt11 as previously described (28). Two independent λgt11 cDNA libraries were generated: a random primed λgt11 cDNA library containing $8 \times 10^6$ primary recombinants and an oligo(dT)-primed cDNA library containing $6 \times 10^6$ primary cDNA recombinants. An additional chick oviduct cDNA library generated in pCD by the method of Okayama and Berg (34) was kindly provided by Jeffrey Northrop and Gordon Ringold (Stanford University, Stanford, CA). The libraries were plated at a density of 5,000–10,000 recombinants per 150 mm Petri dish. Nitrocellulose filters were hybridized with nick-translated cPR2 DNA overnight at 68 C in 6× SSC (0.9 M NaCl, 0.09 M NaCitrate) and 1.0% dried milk. The specific activity of the probe was approximately $3 \times 10^8$ cpm/μg. The filters were washed three times for 30 min at 68 C with 1× SSC. The filters were then autoradiographed overnight at −70 C. Sequence analysis was carried out on positive clones on both strands by the methods of Maxam and Gilbert (21) and/or the dideoxy chain termination method of Sanger *et al.* (20).

### Northern Hybridization Analysis

Total cellular RNA (10μg) from oviducts of estrogen-stimulated chickens was electrophoresed in a 1.5% Agarose gel in the presence of 2.2 M formaldehyde (29). The RNA was transferred to nitrocellulose and hybridized with $1 \times 10^7$ cpm $^{32}$P-labeled antisense RNA probe (30) obtained from the cPR2 cDNA (11). Molecular sizes were determined using RNA size standards (BRL).

### Recombinant Plasmids

A 3.1 Kb receptor cDNA fragment encoding sequence from nucleotide 501 to the *Eco*RI site at nucleotide 3575 in the 3′-untranslated region of the cDNA was used for receptor expression studies. The 5′-end of the cDNA corresponded to the end of a λgt11 cDNA clone which had been repaired using the Klenow fragment of DNA polymerase I and *Eco*RI linkers added. The cDNA was inserted into the *Eco*RI site of p91023(B) downstream of the adenovirus major late promoter. The p90123(B) expression vector was a gift from R. J. Kaufman (Genetics Institute, Cambridge, MA). THe pAHCAT construct used in cotransfection assays was provided by Dr. Steven Nordeen (University of Colorado Health Science Center, Fort Colleus, CO). This construct contained MMTV DNA sequences from −1160 to +106 fused to the bacterial CAT gene and the (SV40) polyadenylation signal in plasmid pxf3. The Ins-CAT construct contained enhancer-promoter sequences from −461 to +118 of the rat insulin II gene fused to CAT-SV40 construct.

### Cell Transfections

COS M-6 and CV-1 cells were grown at 37 C in Dulbecco's Modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum, 100 μg/ml penicillin and 100 μg/ml streptomycin. CV-1 cells used for hormone dependency studies were cultured in charcoal-stripped serum in the absence of progesterone or with added progesterone at the concentrations specified in Figure 6. The 5–10 μg of plasmid constructs were introduced into the cells by calcium phosphate precipitation (31). For hormone-binding analysis, 40 μg receptor plasmid were used to transfect COS M-6 cells. For the induction studies, 5 μg receptor and reporter plasmids were cotransfected into CV-1 cells. Progesterone was added to the culture medium 24 h after transfection. At 48 h post transfection the cells extracts were assayed for CAT activity as previously described (32).

### Acknowledgments

Received May 11, 1987. Accepted May 26, 1987.

Address correspondence and requests for reprints to: Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, 1 Baylor Plaza, Houston, Texas 77030.

This work was supported by NIH Grants HD-07857-16 and HD-08188.

* Supported by NIH Postdoctoral Fellowship GM-10332.

## REFERENCES

1. Ericksson H, Gustafsson JA (eds) 1983 Steroid Hormone Receptors: Structure and Function. Nobel Symposium 57. Elsevier, Amsterdam
2. Schrader WT, Birnbaumer ME, Hughes MR, Weigel NL, Grody WW O'Malley BW 1981 Studies on the structure and function of the chicken progesterone receptor. Recent Prog Horm Res 37:583–633
3. Yamamoto KR 1985 Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 19:209–252
4. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM 1985 Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 318:635–641
5. Miesfeld R, Rusconi S, Godowski PJ, Maler BA, Okret S, Wikström A-C, Gustafsson J-A, Yamamoto KR 1986 Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. Cell 46:389–399
6. Green S, Walter P, Kumar V, Krust A, Bornert J-M, Argos P, Chambon P 1986 Human oestrogen receptor cDNA sequence, expression and homology to v-erb A. Nature 320:134–139
7. Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J 1986 Sequence and expression of human estrogen receptor complimentary DNA. Science 231:1150–1154
8. Krust A, Green S, Argos P, Kumar V, Walter P, Bornert J-M, Chambon P 1986 The chicken estrogen receptor sequence: homology with v-erb A and the human oestrogen and glucocorticoid receptors. EMBO J 5:891–897
9. Weinberger C, Thompson CC, Ong ES, Lebo R, Groul DJ, Evans RM 1986 The c-erb-A encodes a thyroid hormone receptor. Nature 324:641–646
10. Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H, Vennström B 1986 The c-erb-A protein is a high affinity receptor for thyroid hormone. Nature 324:635–640
11. Conneely PM, Sullivan WP, Toft DO, Birnbaumer M, Cook RG, Maxwell BL, Zarucki-Schulz T, Greene GL, Schrader WT, O'Malley BW 1986 Molecular cloning of the chicken progesterone receptor. Science 233:767–770
12. Jeltsch JM, Krozowski Z, Quirin-Stricker C, Gronemyer H, Simpson RJ, Garnier JM, Krust A, Jacob F, Chambon P 1986 Cloning of the chicken progesterone receptor. Proc Natl Acad Sci USA 83:5424–5428

13. Loosfelt H, Atger M, Misrahi M, Guiochon-Mantel A, Meriel C, Logeat F, Benarous R, Milgrom E 1986 Cloning and sequence analysis of the rabbit progesterone receptor complimentary DNA. Proc Natl Acad Sci USA 83:9045–9049
14. Misrahi M, Atger M, d'Auriol L, Loosfelt H, Meriel C, Fridlansky F, Guiochon-Mantel A, Galibert F, Milgrom E 1987 Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. Biochem Biophys Res Commun 143:740–748
15. McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR, O'Malley BW 1987 Molecular cloning of complimentary DNA encoding the avian receptor for Vitamin D. Science 235:1214–1217
16. Green S, Chambon P 1986 A superfamily of potentially oncogenic hormone receptors. Nature 324:615–617
17. O'Malley BW, Roop DR, Lai EC, Nordstrom JL, Catterall JF, Swanek GE, Colbert DA, Tsai M-J, Dugaiczyk A, Woo SLC 1979 The ovalbumin gene: organization, structure, transcription and regulation. Recent Prog Horm Res 35:1–42
18. Schrader WT, O'Malley BW 1972 Progesterone-binding components of chick oviduct. Characterization of purified subunits. J Biol Chem 247:51–59
19. Dure L, Schrader WT, O'Malley BW 1980 Covalent attachment of a progestational steroid to chick oviduct progesterone receptor by photoaffinity labelling. Nature 283:784–786
20. Sanger F, Nicklen S, Coulson AR 1977 DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467
21. Maxam AM, Gilbert W 1980 Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol 65:499–560
22. Kozak M 1986 Bifunctional messenger RNAs in eukaryotes. Cell 47:481–483
23. Cato ACB, Miksicek R, Schütz G, Arnemann J, Beato M 1986 The hormone regulatory elements of mouse mammary tumor virus mediates progesterone induction. EMBO J 5:2237–2240
24. Wong GG, Witek JS, Temple PA, Wilkens KM, Leary AC, Luxenberg DP, Jones SS, Brown EL, Kay RM, Orr EC, Shoemaker C, Golde DW, Kaufman RJ, Hewick RM, Wang EA, Clark SC 1985 Human GM-CSF: molecular cloning of the complimentary DNA and purification of the natural and recombinant proteins. Science 228:810–815
25. Sullivan WP, Beito TG, Proper J, Krco CJ, Toft DO 1986 Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 119:1549–1557
26. Ojasoo T, Raynaud J-P 1978 Unique steroid congeners for receptor studies. Cancer Res 38:4186–4198
27. Gubler U, Hoffman B 1983 A simple and very efficient method for generating cDNA libraries. Gene 25:263–269
28. Kulomaa MS, Weigel NL, Kleinsek DA, Beattie WG, Conneely OM, March C, Zarucki-Schulz T, Schrader WT, O'Malley BW 1986 Amino acid sequence of a chicken heat shock protein derived from the complementary DNA nucleotide sequence. Biochemistry 25:6244–6251
29. Lehrach H, Diamond D, Wozney JM, Boedtker H 1977 RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical examination. Biochemistry 16:4743–4751
30. Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR 1984 Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res 12:7035–7056
31. Wigler M, Sweet R, Sim GK, Wold B, Pellicer A, Lacy E, Maniatis T, Silverstein S, Axel R 1979 Transformation of mammalian cells with genes from procaryotes and eucaryotes. Cell 16:777–785
32. Gorman CM, Moffat LF, Howard BH 1982 Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol 2:1044–1051
33. Messing J 1983 New M13 vectors for cloning. Methods Enzymol 101:20–78
34. Okayama H, Berg P 1982 High-efficiency cloning of full-length cDNA. Mol Cell Biol 2:161–170