Guy, O., & Figarella, C. (1981) *Scand. J. Gastroenterol. 16* (Suppl. 67), 59-61.
Han, J. H., Stratowa, C., & Rutter, W. J. (1987) *Biochemistry 26*, 1617-1625.
Hardman, N. (1986) *Biochem. J. 234*, 1-11.
Huang, Y., & Hui, D. Y. (1991) *J. Biol. Chem. 266*, 6720-6725.
Hui, D. Y., & Kissel, J. A. (1990) *FEBS Lett. 276*, 131-134.
Kesaniemi, Y. A., & Miettinen, T. A. (1987) *Eur. J. Clin. Invest. 17*, 391-395.
Kirgessner, T. G., Chuat, J. C., Heinzmann, C., et al. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9647-9651.
Kissel, J. A., Fontaine, R. N., Turck, C., et al. (1989) *Biochim. Biophys. Acta 1006*, 227-236.
Kyger, E. M., Wiegand, R. C., & Lange, L. C. (1989) *Biochem. Biophys. Res. Commun. 164*, 1302-1309.
Lindstrom, M. B., Sternby, B., & Borgstrom, B. (1988) *Biochim. Biophys. Acta 959*, 178-184.
Lone, Y. C., Simon, M. P., Kahn, A., et al. (1986) *J. Biol. Chem. 261*, 1499-1502.
Maulet, Y., Camp, S., Gibney, G., et al. (1990) *Neuron 4*, 289-301.
Mickel, F. S., Weidenbach, F., Swarovsky, B., et al. (1989) *J. Biol. Chem. 264*, 12895-12901.
Mount, S. M. (1982) *Nucleic Acids Res. 10*, 459-472.
Rudd, E. A., & Brockman, H. L. (1984) in *Lipases* (Borgstrom, B., & Brockman, H. L., Eds.) pp 185-204, Elsevier Science Publishers, Amsterdam.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5463-5467.
Sarkar, S. (1984) *Prog. Nucleic Acid Res. Mol. Biol. 31*, 267-293.
Sudhof, T. C., Goldstein, J. L., Brown, M. S., et al. (1985) *Science 228*, 815-822.
Williams, R. J., McCarthy, A. D., & Sutherland, C. D. (1989) *Biochim. Biophys. Acta 1003*, 213-216.

Molecular Cloning, Sequence Analyses, and Expression of Complementary DNA Encoding Murine Progesterone Receptor[†,‡]

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ABSTRACT: Progesterone receptors exist in two molecular forms commonly designated as "A" and "B" forms, the relative proportion of which can vary among species. In murine tissues, progesterone receptor exists predominantly as the "A" form which, in mammary glands, is also under developmental regulation [Shyamala et al. (1990) *Endocrinology 126*, 2882-2889]. Therefore, toward resolving the molecular mechanisms responsible for the predominance of the "A" form of progesterone receptor in murine tissues and its developmental regulation, we have isolated, sequenced, and expressed the complementary DNA corresponding to the mouse progesterone receptor. Nucleotide sequence analysis revealed two in-frame ATG codons, such that the largest open reading frame beginning with the first codon could encode a polypeptide with an estimated molecular weight of 99 089, while the shorter open reading frame beginning with the second codon could produce a polypeptide with a calculated molecular weight of 81 829. The murine progesterone receptor had complete identity for the DNA binding domain of human and rabbit progesterone receptors and 99% homology with the chicken progesterone receptor; for the steroid binding domain, it had 96% homology with human and rabbit progesterone receptors and 86% homology with chicken progesterone receptors. Expression of the complete complementary DNA in Chinese hamster ovary cells yielded a protein which bound the synthetic progestin promegestone with an equilibrium dissociation constant of approximately 1 nM, and in Western blot analyses revealed both "A" and "B" forms of immunoreactive receptor.

The progesterone receptor (PR) belongs to the superfamily of nuclear steroid receptors which regulate steroid-dependent gene expression by interacting with discrete cis-acting DNA elements present in the 5'-flanking region of target genes [for reviews, see Yamamoto (1985), Evans (1988), Green and Chambon (1988), Beato (1989), and Carson-Jurica et al. (1990)]. Among the various steroid receptors, PR is somewhat unique in that it exists as two forms: a larger molecular form of approximately 108 000-120 000 daltons and a smaller one of approximately 80 000-94 000 daltons (designated as B and A, respectively), the relative proportion of which varies among species [for reviews, see Schrader et al. (1981) and Wei and Horwitz (1986)]. The "A" forms of the receptor repesent an N-terminally truncated naturally occurring variant of the "B" form arising from alternate codon utilization (Conneely et al., 1987a,b; Gronemeyer et al, 1987; Kastner et al., 1990). There

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is also evidence that the synthesis of the "A" and "B" forms of human PR may be regulated by two distinct promoters (Kastner et al., 1990) and that the two forms are encoded by distinct mRNAs (Jeltsch et al., 1990; Kastner et al., 1990; Wei et al., 1990).

Although the rodent systems were some of the earliest experimental systems used to examine the regulation of PR (Feil et al., 1972), as yet, the cloning and sequencing of complementary DNA (cDNA) encoding rodent PR have not been accomplished. Thus, information concerning the primary structure of rodent PR mRNA and its corresponding amino acid sequence, similar to that reported for chicken (Conneely et al., 1987b; Jeltsch et al., 1986), rabbit (Loosfelt et al., 1986), and human PR (Misrahi et al., 1987), is unavailable. Our laboratory is involved in studying the steroid hormonal and developmental regulation of mammary PR using the mouse as the experimental animal (Shyamala, 1985), and we have shown that in contrast to PR from the other two mammalian species studied so far (rabbit and human), murine PR exists predominantly as the A form (Shyamala et al., 1990; Schneider et al., 1991), suggesting that in this species both the mechanisms responsible for regulating the synthesis of PR and hence its functionality may differ. Since our initial attempts to identify murine PR mRNA transcripts corresponding to the two forms of PR using heterologous PR cDNA probes had encountered difficulties, to facilitate our own studies and the overall understanding of the mechanisms responsible for the species-specific differences in the A and B forms of PR, we have cloned, sequenced, and expressed the cDNA encoding murine PR.

Materials and Methods

Description of Probes. The probe used for screening the cDNA library, hPR54, was a gift from Dr. B. W. O'Malley and contained a 2.6-kb fragment of the human PR cDNA consisting of the entire coding region except for 431 base pairs from the 5′ end (Wei et al., 1988). The insert was cut from the pGEM3 vector by appropriate restriction enzymes, gel-purified, and used either as is or after additional digestion with *Hin*dIII to yield a 2.2-kb 5′ fragment (5′hPR) and 0.4-kb 3′ fragment (3′hPR), which were used as probes for determining 5′ versus 3′ localization of newly isolated clones. The probes were labeled with [α-^{32}P]dCTP and [α-^{32}P]dATP (3000 Ci/mmol) using a multiprime labeling system. The specific activity of the probe was typically 2×10^9 cpm/μg of DNA.

cDNA Library Construction and Screening. Total RNA was isolated from intact mouse uteri using guanidine thiocyanate (Chomczynski & Sacchi, 1987) and fractionated on oligo(dT)–cellulose columns to obtain poly(A+) RNA. Oligo(dT)-primed cDNA was synthesized from 15 mg of poly(A+) RNA and used in the construction of a λgt11 cDNA library (Clonetech, Palo Alto, CA). The library contained 2.0×10^6 independent recombinants of which 85% contained inserts. This library was amplified to yield a titer of 1.7×10^{10}/mL and screened by using standard procedures (Sambrook et al., 1989). Positive clones which were detected on two replica filters were replated, rescreened, and clonally purified.

Subcloning and Sequencing. DNA prepared from positive clones was analyzed for insert size by digestions with *Eco*RI and visualization on a 1% agarose, TBE (89 mM Tris–borate, pH 8, and 1 mM EDTA) gel with appropriate molecular weight markers. The insert DNA of interest was isolated by running similar digests on 1% low melting agarose–TBE gels and subcloned into Bluescript vector (Stratagene, Inc., San Diego, CA) according to standard methods. Sequential deletions were generated in the plasmid DNA by using an exonuclease III–mungbean nuclease kit (Stratagene), and the resulting subclones were sequenced by dideoxy chain termination (Sanger et al., 1977) using Sequenase (U.S. Biochemical Corp., Cleveland, OH) following the supplier's protocol. In instances where "chewing back" by exonuclease III resulted in gaps in the sequence, these were covered by using specific sequencing primers synthesized on the basis of sequences already obtained from the exonuclease III clones. Regions of compression were resolved with inosine triphosphate. Sequences were analyzed by using the EuGene (Baylor College of Medicine) sequence analysis program.

Northern Blot Analysis. Northern blot analyses were performed with mouse uterine and vaginal poly(A+) RNA using standard procedures. The blots were hybridized at 42 °C with random primer labeled cDNA inserts [$(2–4) \times 10^9$ cpm/μg of DNA], sequentially washed with 0.1 × SSC/0.1% SDS at room temperature and 0.1 × SSC/0.1% SDS at 60 °C, and autoradiographed.

Stable Expression of the Full-Length cDNA in Chinese Hamster Ovary Cells. The murine PR sequences from mRP11 and mPR2 (Figure 1) were ligated to produce a 3.0-kb sequence containing the entire coding region, which was then inserted into the *Eco*RI site of the eukaryotic expression vector, pSV2NeoCMV, provided by Dr. Paul Yaswen, at Lawrence Berkeley Laboratory, University of California. This vector, derived from pSV2Neo (Southern & Berg, 1982), carries the murine cytomegalo virus promoter (Dorsch-Hasler et al., 1985) upstream from the inserted sequence.

Chinese hamster ovary (CHO) cells (1.5×10^5/100-mm plate) were transfected with mPR expression plasmid DNA (10 mg/plate) together with denaturated salmon sperm DNA (20 mg/plate) as carrier, using the calcium phosphate technique (Sambrook et al., 1989). After 2 h, the DNA–media mixture was replaced with 10 mL of fresh media, and the cells were grown for 3 days to attain confluency. At day 3, the media was removed and replaced with 800 mg/mL G418 to select for stable transfectants containing the mPR expression vector.

Steroid Binding Assays. Cells were homogenized in a buffer which contained 10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 20 mM sodium molybdate, and 10% (v/v) glycerol, pH 7.4 (TEDG), to which leupeptin (48 μg/mL), bacitracin (100 μg/mL), aprotinin (77 μg/mL), and pepstatin (1 μg/mL) had been added. The homogenate was centrifuged for 1 h at 105000*g* at 4 °C to obtain cytosol, aliquots of which were then labeled with varying concentrations of [^{3}H]promegestone[1] (R5020) (New England Nuclear, Boston, MA) either alone (to determine total binding) or with a 100-fold excess of unlabeled R5020 (to determine nonspecific binding). All incubations contained a 100-fold excess of unlabeled dexamethasone to block glucocorticoid binding sites. Free steroid was separated from bound steroid by fractionating the incubated cytosols on minicolumns of Sephadex LH-20.

Western Blot Analyses. For detection of immunoreactive PR in CHO cells, cytosolic extracts were subjected to Western blot analyses using mouse monoclonal anti-PR antibodies hPRa 4, 5, and 7, raised against human PR (Clarke et al., 1987), and αPR6, a mouse monoclonal antibody raised against avian PR (Sullivan et al., 1986). The antigen–antibody complexes were identified by reacting the blots initially with rabbit anti-mouse immunloglobulin G antibodies followed by incubation with ^{125}I-labeled protein A (0.1 μCi/mL) and

[1] Promegestone (R5020) = 17α,21-dimethyl-19-norpregna-4,9-diene-3,20-dione.

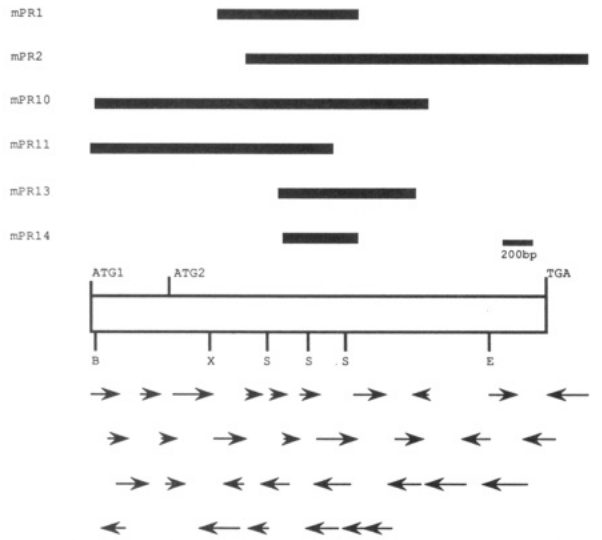

FIGURE 1: Restriction map and sequencing strategy for murine PR cDNA. The relative positions of the λgt11 clones used in obtaining the cDNA sequence are indicated in the upper part of the figure. Some restriction sites used in the initial characterization of these clones are shown below the open box which indicates the coding region (B, *Bam*HI; E, *Eco*RI; S, *Sac*II; X, *Xho*I). The positions of the first and second ATG codons and the first TGA stop codon are also indicated. *The arrows below indicate the direction and extent of each sequence* derived from the plasmid subclones as determined by the dideoxy chain termination procedure. The inset scale shows the length in base pairs.

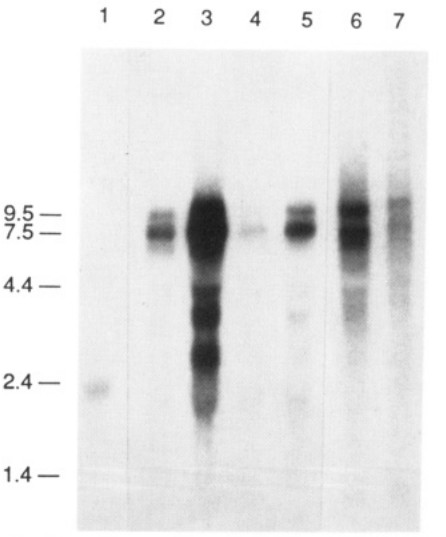

FIGURE 2: Northern blot analyses for rodent PR mRNA. Poly(A+) RNAs from mouse liver (lane 1), uteri (lanes 2 and 3), vagina (lanes 4 and 5), and rat uterus (lane 6) and vagina (lane 7) were electrophoretically fractionated, blotted, and hybridized to ^{32}P-labeled mPR1 as described in text. Samples in lanes 1, 3, 5, 6, and 7 correspond to tissues obtained from nonovariectomized animals while those in lanes 2 and 4 correspond to tissues from ovariectomized animals. The *position of the molecular weight standards (kb) is indicated on the* left.

autoradiography. Detailed procedures for Western blot analyses of mouse PR using these antibodies have been described by us previously (Schneider et al., 1991).

Results and Discussion

Cloning of the Murine Progesterone Receptor cDNA. Initial screening of the cDNA library with hPR54 yielded 2 stable cDNA clones from a total of 5×10^5 recombinants. These clones, designated as mPR1 and mPR2, contained inserts of approximately 0.9 and 2.1 kb, respectively. Rescreening the cDNA library with mPR1 resulted in the isolation of four additional stable cDNA clones. A schematic representation of the various cDNA clones along with a partial restriction map is shown in Figure 1. All six clones hybridized to hPR54 and also to 5′hPR, while mPR2 also hybridized to 3′hPR, suggesting that these clones contained inserts homologous to the human PR. These clones also hybridized to mouse uterine and vaginal poly(A+) RNA in Northern blot analyses, and an example of such an experiment using mPR1 is shown in Figure 2. As may be seen, multiple species of mRNA were detected in the uterus and vagina among which transcripts corresponding to approximately 6.9 and 8.7 kb were the most abundant and whose cellular concentration was also clearly ovarian dependent (compare lanes 2 and 3 and also lanes 4 and 5). In all species examined so far, Northern blot analyses for PR using cDNA-derived probes have also yielded multiple transcripts (Conneely et al., 1987b; Wei et al., 1988; Read et al., 1988; Misrahi et al., 1988). mPR1 did not hybridize with mouse liver poly(A+) RNA (lane 1), and also, as expected, in both mouse and the rat, the mRNA transcripts were more abundant in uterus as compared to the levels present in vagina (compare lanes 3 and 5 and also lanes 6 and 7). Regardless of the origin of the probe, i.e., whether it was derived from the 5′ end or 3′ end, the same species of mRNA (as shown for mPR1) were detected (data not shown). These results are also similar to those previously reported for Northern blot analyses for PR mRNA using large fragments of cDNA-derived probes in other species.

Initial analysis by Southern blots revealed that mPR2 and mPR11 could cross-hybridize (data not shown), indicating that these two clones contained overlapping sequences. Since the total length of these two overlapping clones was approximately 3.0 kb (Figure 1), sufficient to encode the larger "B" form of murine PR of 115 kDa (Schneider et al., 1988, 1991), these clones were chosed for sequencing in entirety to deduce the primary structure of murine PR mRNA. The strategy adopted for sequencing is shown in Figure 1.

Sequence Analyses of the Mouse Progesterone Receptor. The combined sequence of mPR11 and mPR2 consisted of 3014 nucleotides with the longest open reading frame starting with an ATG at position 1 (Figure 3) and ending with 2 consecutive TGA stop codons at position 2770. As found with other PR cDNAs (Conneely et al., 1987b; Gronemeyer et al., 1987; Misrahi et al., 1987; Loosfelt et al., 1987), there were two in-frame ATG codons present in the cDNA-deduced amino acid sequence at positions 1 and 166 (Figure 3). A comparison of the mouse PR with human PR revealed an insertion of 3 amino acids and a deletion of 13 amino acids in the murine receptor, and all of these deletions were downstream from the second methionine codon (Figure 4). Despite this, in this region, several of the putative sites proposed for posttranslational modification of human PR (Misrahi et al., 1987), were conserved in the mouse which included the consensus site for tyrosine phosphorylation at amino acid 329, two consensus sites for phosphorylation by casein kinases (amino acids 231 and 542), and a slightly modified nuclear transfer signal (with a substitution of lysine for an arginine) at positions 184–188. In addition, the two putative glycosylation sites (amino acids 654–666), one consensus site for phosphorylation by tyrosine kinase (amino acid 591), and one consensus site for phosphorylation by casein kinase (amino acid 783) found in human PR (Misrahi et al., 1987) were also conserved in the mouse PR (Figure 3). A comparison of murine PR with the three other PRs cloned so far revealed that, at the level of amino acid sequence, the murine PR had an overall homology with human, rabbit, and chicken PR of 81%, 82%, and 50%, respectively, which at the level of nucleotide sequence

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                                                  -7: GGTCGTC
                                                      .......

   1: ATG ACT GAG CTG CAG GCA AAG GAT CCG CAG GTT CTC CAC ACG TCT
      met thr glu leu gln ala lys asp pro gln val leu his thr ser    :15

  46: GGC GCT TCG CCC TCC CCC CCA CAC ATC GGG TCC CCC TTG CTT GCA
      gly ala ser pro ser pro pro his ile gly ser pro leu leu ala    :30

  91: CGC TTG GAC TCA GGT CCC TTC CAA GGG AGC CAG CAC TCG GAC GTG
      arg leu asp ser gly pro phe gln gly ser gln his ser asp val    :45

 136: TCG TCT GTA GTC TCG CCT ATA CCG ATC TCC CTG GAC GGG CTG CTT
      ser ser val val ser pro ile pro ile ser leu asp gly leu leu    :60

 181: TTT CCT CGG TCC TGC CGG GGT CCC GAG CTC CCA GAC GGA AAG ACA
      phe pro arg ser cys arg gly pro glu leu pro asp gly lys thr    :75

 226: GGG GAC CAG CAG TCG CTG TCC GAC GTG GAG GGC GCT TTC TCT GGG
      gly asp gln gln ser leu ser asp val glu gly ala phe ser gly    :90

 271: GTA GAA GCC ACT CAT AGG GAG GGA GGC AGA AAT TCC AGA CCC CCG
      val glu ala thr his arg glu gly gly arg asn ser arg pro pro    :105

 316: GAG AAG GAC AGC AGA CTC TTA GAC AGT GTC TTA GAC TCG TTG TTG
      glu lys asp ser arg leu leu asp ser val leu asp ser leu leu    :120

 361: ACT CCC TCC GGA CCG GAA CAG AGT CAC GCC AGC CCT CCA GCC TGC
      thr pro ser gly pro glu gln ser his ala ser pro pro ala cys    :135

 406: GAG GCC ATC ACT TCC TGG TGT CTC TTT GGG CCA GAG CTT CCA GAA
      glu ala ile thr ser trp cys leu phe gly pro glu leu pro glu    :150

 451: GAC CCC CGC AGT GTC CCT GCT ACC AAA GGG TTG TTG TCC CCG CTC
      asp pro arg ser val pro ala thr lys gly leu leu ser pro leu    :165

 496: ATG AGT CGG CCA GAG ATC AAG GTC GGC GAC CAG TCC GGG ACA GGA
      met ser arg pro glu ile lys val gly asp gln ser gly thr gly    :180

 541: CGA GGA CAG AAG GTG CTG CCC AAA GGA CTG TCA CCA CCC AGG CAG
      arg gly gln lys val leu pro lys gly leu ser pro pro arg gln    :195

 586: CTG TTG CTC CCT ACC TCG GGG AGT GCT CAC TGG CCC GGG GCA GGG
      leu leu leu pro thr ser gly ser ala his trp pro gly ala gly    :210

 631: GTG AAG CCG TCC CCG CAG CCA GCT GCA GGG GAG GTG GAA GAG GAC
      val lys pro ser pro gln pro ala ala gly glu val glu glu asp    :225

 676: AGT GGC CTG GAG ACC GAG GGC TCT GCC AGT CCG CTT CTA AAG AGC
      ser gly leu glu thr glu gly ser ala ser pro leu leu lys ser    :240

 721: AAA CCT CGA GCA CTG GAA GGC ACC GGC CAG GGA GGA GGA GTC GCA
      lys pro arg ala leu glu gly thr gly gln gly gly gly val ala    :255

 766: GCC AAC GCG CCG TCA GCG GCC CCA GGC GGT GTC ACT CTG GTC CCA
      ala asn ala pro ser ala ala pro gly gly val thr leu val pro    :270

 811: AAG GAA GAT TCA CGG TTT TCT GCT CCT AGG GTC TCC TTG GAG CAA
      lys glu asp ser arg phe ser ala pro arg val ser leu glu gln    :285

 856: GAC TCT CCC ATT GCC CCC GGG CGC TCC CCA CTG GCC ACC ACA GTG
      asp ser pro ile ala pro gly arg ser pro leu ala thr thr val    :300

 901: GTG GAT TTC ATC CAT GTG CCC ATC CTG CCT CTG AAC CAC GCA CTC
      val asp phe ile his val pro ile leu pro leu asn his ala leu    :315

 946: CTG GCC GCC CGC ACC CGG CAG CTG CTG GAG GGG GAG AGC TAC GAC
      leu ala ala arg thr arg gln leu leu glu gly glu ser tyr asp    :330

 991: GGC GGG GCC ACA GCA GGG CCC TTT TGC CCG CCT AGG TCG CCC TCC
      gly gly ala thr ala gly pro phe cys pro pro arg ser pro ser    :345

1036: GCG CCA TCC ACC CCG GTG CCC CGC GGT GAC TTC CCA GAC TGC ACC
      ala pro ser thr pro val pro arg gly asp phe pro asp cys thr    :360

1081: TAC CCT CTG GAA GGC GAC CCC AAA GAG GAC GTG TTC CCT CTT TAC
      tyr pro leu glu gly asp pro lys glu asp val phe pro leu tyr    :375

1126: GGC GAC TTC CAG ACG CCT GGC TTG AAG ATC AAG GAG GAG GAG GAA
      gly asp phe gln thr pro gly leu lys ile lys glu glu glu glu    :390

1171: GGC GCG GAT GCT GCT GTG CGC TCG CCG CGC CCC TAC CTG TCG GCT
      gly ala asp ala ala val arg ser pro arg pro tyr leu ser ala    :405

1216: GGA GCC AGC TCC TCC ACC TTC CCA GAC TTC CCG CTG GCA CCC GCG
      gly ala ser ser ser thr phe pro asp phe pro leu ala pro ala    :420

1261: CCG CAG GCA GCG CCA TCC TCC AGG CCC GGA GAA GCG GCG GTG GCC
      pro gln ala ala pro ser ser arg pro gly glu ala ala val ala    :435

1306: GGC GGA CCC AGC AGC GCC GCG GTG TCG CCA GCG TCC TCC TCC GGC
      gly gly pro ser ser ala ala val ser pro ala ser ser ser gly    :450

1351: TCC GCG CTG GAG TGC ATC CTG TAC AAA GCG GAA GCG CCG CCC ACG
      ser ala leu glu cys ile leu tyr lys ala glu ala pro pro thr    :465

1396: CAG GGT TCG TTC GCG CCA CTG CCG TGC AAG CCC CCA GCC GCC GCG
      gln gly ser phe ala pro leu pro cys lys pro pro ala ala ala    :480

1441: TCC TGC CTA CTA CCC CGG GAC AGC CTG CCG GCC GCC CCC GGC ACC
      ser cys leu leu pro arg asp ser leu pro ala ala pro gly thr    :495

1486: GCC GCA GCA CCC GCC ATC TAC CAG CCG CTC GGC CTC AAT GGG CTC
      ala ala ala pro ala ile tyr gln pro leu gly leu asn gly leu    :510

1531: CCG CAG CTG GGC TAC CAG GCC GCG GTG CTC AAG GAC AGC CTG CCC
      pro gln leu gly tyr gln ala ala val leu lys asp ser leu pro    :525

1576: CAG GTC TAC CCG CCA TAC CTC AAC TAC CTG AGG CCA GAT TCA GAA
      gln val tyr pro pro tyr leu asn tyr leu arg pro asp ser glu    :540

1621: GCC AGC CAG AGC CCA CAG TAT GGC TTT GAT TCC TTA CCT CAG AAG
      ala ser gln ser pro gln tyr gly phe asp ser leu pro gln lys    :555

1666: ATC TGC TTA ATC TGC GGG GAT GAA GCA TCT GGC TGT CAC TAT GGC
      ile cys leu ile cys gly asp glu ala ser gly cys his tyr gly    :570

1711: GTG CTT ACC TGT GGG AGC TGC AAG GTC TTC TTT AAG AGG GCA ATG
      val leu thr cys gly ser cys lys val phe phe lys arg ala met    :585

1756: GAA GGG CAG CAT AAC TAT TTA TGT GCT GGA AGA AAT GAC TGC ATT
      glu gly gln his asn tyr leu cys ala gly arg asn asp cys ile    :600

1801: GTT GAT AAA ATT CGC AGA AAA AAC TGC CCA GCA TGT CGT CTG AGA
      val asp lys ile arg arg lys asn cys pro ala cys arg leu arg    :615

1846: AAG TGT TGT CAG GCT GGC ATG GTC CTT GGA GGT CGT AAG TTT AAG
      lys cys cys gln ala gly met val leu gly gly arg lys phe lys    :630

1891: AAG TTT AAT AAA GTC CGA GTT ATG AGA ACC CTT GAC GGT GTT GCT
      lys phe asn lys val arg val met arg thr leu asp gly val ala    :645

1936: CTC CCC CAG TCG GTG GGC CTT CCT AAC GAG AGC CAG GCC CTG AGC
      leu pro gln ser val gly leu pro asn glu ser gln ala leu ser    :660

1981: CAG AGA ATC ACC TTT TCA CCA AAT CAA GAA ATT CAA CTG GTC CCG
      gln arg ile thr phe ser pro asn gln glu ile gln leu val pro    :675

2026: CCA CTC ATC AAC CTG CTC ATG AGC ATT GAG CCT GAT GTG ATC TAT
      pro leu ile asn leu leu met ser ile glu pro asp val ile tyr    :690

2071: GCA GGG CAT GAC AAC ACA AAG CCT GAC ACT TCC AGC TCT TTG CTG
      ala gly his asp asn thr lys pro asp thr ser ser ser leu leu    :705

2116: ACC AGT CTC AAC CAA CTA GGC GAG AGA CAA CTG CTT TCA GTA GTC
      thr ser leu asn gln leu gly glu arg gln leu leu ser val val    :720

2161: AAA TGG TCT AAA TCT CTG CCA GGT TTC CGG AAC TTA CAC ATT GAT
      lys trp ser lys ser leu pro gly phe arg asn leu his ile asp    :735

2206: GAC CAG ATA ACC CTG ATT CAG TAC TCC TGG ATG AGC CTG ATG GTG
      asp gln ile thr leu ile gln tyr ser trp met ser leu met val    :750

2251: TTT GGC CTG GGG TGG AGG TCG TAC AAG CAT GTC AGT GGA CAG ATG
      phe gly leu gly trp arg ser tyr lys his val ser gly gln met    :765

2296: CTA TAT TTT GCA CCT GAT CTA ATC CTA AAT GAG CAG AGG ATG AAG
      leu tyr phe ala pro asp leu ile leu asn glu gln arg met lys    :780

2341: GAG CTG TCA TTC TAC TCG CTG TGC CTT ACC ATG TGG CAA ATC CCA
      glu leu ser phe tyr ser leu cys leu thr met trp gln ile pro    :795

2386: CAG GAG TTT GTC AAA CTC CAG GTG ACC CAT GAG GAA TTC CTC TGT
      gln glu phe val lys leu gln val thr his glu glu phe leu cys    :810

2431: ATG AAA GTC TTA CTA CTT CTT AAC ACA ATT CCT TTG GAA GGA CTG
      met lys val leu leu leu leu asn thr ile pro leu glu gly leu    :825

2476: AGG AGT CAA AGC CAG TTT GAA GAG ATG AGA TCA AGC TAT ATC CGC
      arg ser gln ser gln phe glu glu met arg ser ser tyr ile arg    :840

2521: GAA TTG ATC AAG GCA ATT GGT TTA AGA CAG AAA GGG GTT GTC CCC
      glu leu ile lys ala ile gly leu arg gln lys gly val val pro    :855

2566: ACG TCA CAG CGC TTC TAC CAA CTC ACA AAA CTT CTC GAC AGC TTG
      thr ser gln arg phe tyr gln leu thr lys leu leu asp ser leu    :870

2611: CAT GAT CTT GTG AAA CAG CTC CAC CTG TAC TGC TTG AAT ACA TTC
      his asp leu val lys gln leu his leu tyr cys leu asn thr phe    :885

2656: ATC CAA TCC CGG ACA CTG GCT GTG GAA TTT CCG GAA ATG ATG TCT
      ile gln ser arg thr leu ala val glu phe pro glu met met ser    :900

2701: GAA GTT ATT GCT GCC CAG TTG CCC AAG ATC CTG GCG GGC ATG GTG
      glu val ile ala ala gln leu pro lys ile leu ala gly met val    :915

2746: AAG CCG CTC CTC TTT CAC AAA AAG TGA TGACTTTT CTTTGTT
      lys pro leu leu phe his lys lys *** ........ .......
```

FIGURE 3: Nucleotide sequence analyses and the deduced amino acid sequence of the cDNA encoding murine PR. The nucleotides are numbered on the left-hand side of the sequence starting with +1 for the first ATG. The first stop codon is indicated by asterisks. The numbers in the right-hand column refer to the amino acid sequence of the longest open reading frame.

corresponded to 79%, 79%, and 59%. As observed previously with other members of the streoid receptor superfamily, among the three mammalian PRs, the DNA and steroid binding domains were highly conserved such that, at the level of amino acids, murine PR had complete identity with the human and rabbit PR in the DNA binding domain and 96% identity in the steroid binding domain (Table I). Among the sequences of the various PR cDNAs cloned so far, the N-terminal (A/B) and the hinge (D) regions are known to be the most divergent (Misrahi et al., 1987), and a comparison of these other known sequences with murine PR confirmed this (Table I). The principal difference between the mouse and the other two mammalian species appeared to be the divergence in codon usage such that at the nucleotide level, in both the steroid and DNA binding domains, even in regions which were identical between the human and rabbit PR cDNAs, there was a reasonable divergence in the mouse. For example, a 37-nucleotide region in the DNA binding domain corresponding to nucleotides 1707–1743 and a 44-nucleotide region in the steroid binding domain corresponding to nucleotides 2416–2459 of the human cDNA (Misrahi et al., 1987) show a complete identity with the rabbit PR cDNA (Loosfelt et al., 1986) but reveal a 14% mismatch in the former and a 18% mismatch for the latter region in the murine PR cDNA (Figure 3). Synthetic oligonucleotides corresponding to these two regions had failed to detect murine PR mRNA in our earlier studies.

```
     ↓
 hPR MTELKAKGPRAPHVAGGPPSP*EVGSPLLCRPAAGPFPGSQTSDTLPEVSAIPISLDGLLFPRPCQGQDPSDEKTQDQQSLSDVEGAYSRAEATRGA*** 96
 mPR     Q  D QVL TS AS   PHI     A LDS   Q   H  VSSV  P            S R PELP G  G           F GV  HRE*** 97
rbPR        E         A   T    Q  G  DP   Q     EASSV             G      N R G    PP        FPGV  PE  *** 97
 cPR    V S ET   SS RDGAVL*LQAPPSRGEAEGIDVA*****************     Y  SSDEEEEEEEEPQQREEEEEEEEEEEEEEEEEDRDCP 82

                                                                               ↓
 hPR *****GGSSSSPPEKDSGLLDSVLDTLLAPSGPGQSQPSPPACEVTSSWCLFGPELPEDPPAAPATQRVLSPLMSRSGC*KVGDSSGTAAAHKVLPRGLS 190
 mPR *****  RN R      R       S  T    E  HA      AIT             RSV   KGL       PEI*    Q   GRGQ    K    191
rbPR ***** D   R                         HA  AT  AI P      D     R     KG  A     PED* A                   191
 cPR SYRPG   LS****  C**       F   AAHAAP************ S     V  V V   *********   GPEQ AV AGPGAPGPSQ*  PGA 154

 hPR PARQLLLPASESPHWSGAP*****VKPSPQAAAVEVEEEDGSESEESAGPLLKGKPRALGGAAAGGGAAAVPPGAAAGGVALVPKEDSRFSAPRVALVEQ 285
 mPR  P      T G A  P  G*****      P  G    DS L T G  S    S     E TGQ   V  NA S  P   T              S *   285
rbPR  S      S G    PA*******      P   Q D   S    GTV      Q              P AS                      S A   284
 cPR ************ L P  DSLNVA  AR GP*******  A  NRAPGLPGAEERGF****************************************P R 195

 hPR DAPMAPGRSPLATTVM************DFIHVPILPLNHALLAARTRQLLEDES*YDGGAGAASAFAPPRSSPCASSTPVAVGDFPDCAYPP*DAEPKD 371
 mPR  S I           V************                        G  *     T GPFCP  R** S P    PR      T  L*EGD  E 369
rbPR    V         S V*************            F  T       G  *     A   P V Q G  S       G      T   *       370
 cPR   G**  EGG  PAAAASPAAVEPGAGQ YL        S F  S      DV AA   *****   G*     SVPAADLAEYG*****   P GKEGP 282

 hPR DAYPLYSDFQPPALKIKEEEEGAEASARSPRSYLVAGANPAAFPDFPLGPPPP**LPPRATPSRPGEAAVTAAPASASVSSASSSGSTLECILYKAEGAP 469
 mPR  VF   G   T G          D AV    P  S   SSST      A A QAA*****PS        AGG S  A  P      A     •       463
rbPR   F   G              A   A  A  T              Q AA   PS    VPS        A S G      S                   470
 cPR FA*** GE  S*       GV L****************   P P    AK********************    DFAQPPRAGQEPS   V     **  340

 hPR PQQGPFAPPPCKAPGASGCLLPRDGLPSTSASAAAAGAAPALYPALGLNGLPQ*LGYQAAVLKEGLPQVYPPYLNYLRPDSEASQSPQYSFESLPQKI   566
 mPR  T  S   L   P A AS      S  AAPGT   ****  I QP        •         DS                        G D           556
rbPR         L   P   GA              G           T        •                T                              567
 cPR  LLPGAYG  A*  ********* S      ****** P G  SP     HH A  FP          LC     G V   T T   S              422
```

FIGURE 4: Alignment of the A/B region of PR corresponding to human (hPR), mouse (mPR), rabbit (rbPR), and chicken (cPR). The human PR sequence (Misrahi et al., 1987) is given in its entirety, and for others, only the residues that differ from human PR are shown. Gaps introduced to maximize the alignment of four proteins are indicated by asterisks. The proposed translational start sites for the hPR and cPR for forms of B and A (also conserved in the mouse) are indicated by arrows.

Table I: Comparison of Mouse PR Nucleic Acid and Amino Acid Sequences with PR from Other Species[a]

domains:	N-terminal A/B	DNA binding C	hinge D	steroid binding E
amino acids:	1–556	557–634	635–670	671–923
nucleic acids:	1–1668	1669–1920	1903–2010	2011–2769
nucleotide sequence				
human PR	75	92	81	85
rabbit PR	76	91	70	84
chicken PR	47	85	61	77
amino acid sequence				
human PR	72	100	78	96
rabbit PR	74	100	75	96
chicken PR	26	99	58	86

[a]The percentage homology of various domains of mouse PR cDNA to corresponding domains of cDNA for human PR (Misrahi et al., 1987), rabbit PR (Loosfelt et al., 1986), and chicken PR (Gronemeyer et al., 1987; Conneely et al., 1987) is shown.

In retrospect, we believe that it was this difference in codon usage which probably contributed to our difficulties in our initial attempts to detect murine mRNA with heterologous human and avian PR cDNA probes using standard hybridization techniques. Since murine PR cDNA also readily detects the rat PR mRNA species (Figure 2), it suggests that the nucleotide sequence of rat PR cDNA (as yet unknown) may more closely resemble that of the mouse.

Expression of Murine PR cDNA in CHO Cells. Six neomycin-resistant colonies were obtained from CHO cells transfected with mPR cDNA which were analyzed for the presence of PR by steroid binding assays using the synthetic progestin promogestone (R5020) and also by Western blot analyses using anti-PR antibodies. CHO cells transfected with the expression vector containing an irrelevant insert did not have any significant specific binding of [H^3]R5020, and neither did three of the six neomycin-resistant colonies (data not shown). The other three neomycin-resistant colonies exhibited significant binding of R5020, and there were no appreciable differences between these colonies with regard to their ability to bind R5020 (data not shown). The binding data corre-

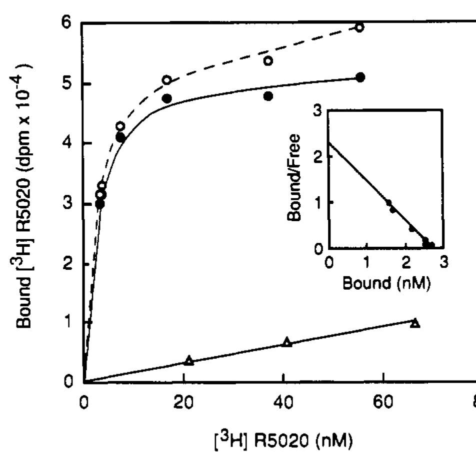

FIGURE 5: Saturation analysis of [^{3}H]R5020 binding by the cytoplasmic extracts of Chinese hamster ovary cells transfected with murine PR cDNA. Aliquots of cytosol were incubated with [^{3}H]R5020 either alone (○) or in the presence of a 100-fold excess of unlabeled R5020 (△) before filtration on Sephadex LH-20 columns; (●) indicates specific binding. The insert shows the Scatchard plot of the specific binding.

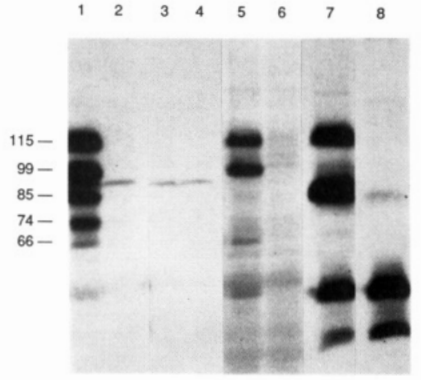

FIGURE 6: Immunoblot analyses for PR in cytosolic extracts of CHO cells tranfected with murine PR cDNA. Aliquots of cytosol from CHO cells tranfected with murine PR cDNA, either positive (lanes 1, 2, 5, and 6) or negative (lanes 3 and 4) for PR as measured by steroid binding assays and murine uterus (lanes 7 and 8), were processed for Western blot analyses as described in the text. Subsequent to electrophoretic fractionations, samples were either left as is (lanes 2, 4, 6, and 8) or incubated with hPRa 4, 5, and 7 (lanes 1, 3, and 7) or αPR6 (lane 5) prior to reacting with rabbit anti-mouse immunoglobulin G antibodies and ^{125}I-labeled protein A. The positions of the various immunoreactive forms of PR and their estimated molecular weights ($\times 10^{-3}$) are indicated on the left.

sponding to one of these colonies are shown in Figure 5. As may be seen, the cytoplasmic extract of these CHO cells bound R5020 with a high affinity (K_D = 1.16 nM), and it was estimated that these cells contained approximately 280 000 sites of PR per cell.

Analyses of the cytoplasmic extract for immunoreactive PR revealed two major and one minor antibody-specific bands when probed with αPR6 (Figure 6, compare lanes 5 and 6) and five bands when probed with hPRa 4, 5, and 7 (Figure 6, compare lanes 1 and 2). All these bands were absent in CHO cells which had been transfected with the murine PR cDNA but lacked steroid binding activity (Figure 6, lanes 3 and 4). The calculated molecular weights of the two major bands and one minor band detected with αPR6 were 115 000, 99 000, and 66 000, respectively, while those detected with hPRa 4, 5, and 7 were 115 000, 99 000, 85 000, 74 000, and 66 000. As observed by us previously (Schneider et al., 1991), murine uterine cytosol (run as control) revealed two antibody-specific bands corresponding to proteins of 115 and 83–85 kDa (Figure 6, lanes 7 and 8). Since the epitope detected by αPR6 is located upstream from the second methionine codon (Conneely et al., 1987a) and thus reacts only with the "B" form of PR from several species (Sullivan et al., 1986) including the mouse (Schneider et al., 1988), it follows that the bands in the CHO cell extract detected with αPR6, i.e., the 115 000-, 99 000-, and 66 000-Da proteins, all correspond to variants of the "B" form of murine PR while the 85 000- and 74 000-Da protein correspond to "A" forms. Transient expression of both human and chicken PR cDNAs and their derivatives has shown that the "A" form of the receptor represents the product of translational initiation at the second methionine codon and as such represents an N-terminally truncated natural variant of the "B" form (Conneely et al., 1987a; Gronemeyer et al., 1987; Krett et al., 1988; Kastner et al., 1990). As such, if in the mouse both the first and second methionine codons were to serve as translation initiation sites, from the cDNA-deduced amino acid sequence (Figure 3) they might be expected to produce two proteins with estimated molecular weights of 99 000 and 81 829 corresponding to the "B" and "A" forms of the receptor, respectively. Therefore, among the different forms of "B" receptor detected in CHO cell extracts, we believe that the protein with an apparent molecular weight of 99 000 represents the native murine PR while that with an apparent molecular weight of 115 000 represents its counterpart which has been covalently modified. And since it is well documented that rabbit, chicken, and human PR can all be naturally phosphorylated in intact cells even in the absence of any treatment with steroid (Logeat et al., 1985; Denner et al., 1987; Wei et al., 1987; Sullivan et al., 1988), we believe that the protein with the apparent molecular weight of 115 000 may most likely represent the phosphorylated counterpart of the protein with the apparent molecular weight of 99 000. Indeed, in all murine target tissues tested so for, the apparent molecular weight of the "B" form of PR is 115 000 (Schneider et al., 1991). The likelihood that the protein with the apparent molecular weight of 115 000 may represent the phosphorylated state of a native 99-kDa protein is also reinforced by the fact that, similar to the mouse, the predicted molecular weight of the "B" form of PR encoded by the human PR cDNA is also approximately 99 000 (Misrahi et al., 1987) while human "B" receptors in target cells consist of triplets with approximate molecular weights of 114 000, 117 000, and 120 000 (Sheridan et al., 1989). Similarly, the phosphorylated state of the "B" form of chicken PR also differs in molecular weight by approximately 20 000 as compared to the predicted molecular weight based on the deduced amino acid sequence of cloned cDNA (Conneely et al., 1987b). Since similar to the "B" form, the "A" form of PR can also be phosphorylated (Sullivan et al., 1988; Sheridan et al., 1989) and in all murine tissues tested so far the "A" form of the receptor has an approximate molecular weight of 83 000–85 000 (Schneider et al., 1991), the protein with an apparent molecular weight of 85 000, detected in CHO cell extracts, can conceivably represent the phosphorylated state of the 81 829-dalton protein. If indeed the 115- and 85-kDa proteins expressed in CHO cells correspond to the "A" and "B" proteins found in murine target tissues, it will support the argument that the presence of 5′UTR is not essential to generate the "A" form of PR (Kastner et al., 1990) since the murine PR cDNA used in our transfected studies contained only seven nucleotides upstream from the coding region (Figure 3) and thus essentially did not contain any 5′UTR. On the other hand, it is to be noted that the relative ratio of "A" and "B" forms of PR in transfected cells favors the "B" form (Figure 6) while that found in vivo in murine tissues favors the "A" form (Schneider et al., 1991). This could be either because (a) as found with human and chicken PR (Jeltsch et al., 1990; Kastner et al., 1990; Wei et al., 1990), murine "A" and "B" forms are also encoded by distinct mRNAs and in transfected cells transcripts corresponding to the "A" form are not generated as efficiently as compared to the transcripts encoding the "B" form, or (b) while 5′UTR is not essential for the generation of "A" form, it is important for translational efficiency as previously proposed (Conneely et al., 1987a). It is also conceivable that cell-specific factors play an important role at all levels of PR expression and this, at least in part, may be responsible for the observed differences in the ratio of "A" and "B" forms found between transfected CHO cells and murine target tissues in vivo.

Finally, it is to be noted that in addition to the readily identifiable "B" and "A" forms, other forms of PR are also present in transfected cells, i.e., the protein with an apparent molecular weight of 74 000 lacking immunoreactivity with αPR6 and the protein with an apparent molecular weight of 66 000 exhibiting immunoreactivity. These forms of PR have never been detected by us previously in any of the murine target tissues either by photoaffinity labeling or by immunoblot experiments (Schneider et al., 1991). These receptor forms

may, therefore, represent products either of premature translational arrest in CHO cells or of site-specific proteolysis. Alternatively, they may also correspond to as yet unidentified murine PR forms analogous to the new form of PR (C-receptor) discovered in human cells (Wei et al., 1990). To define the precise mechanisms underlying the generation of the various forms of murine PR and the relative abundance of "A" form in target tissues from these species, it will be necessary to determine the entire structure of murine PR gene and characterize all the relevant mRNA species and their end products. As such, the availability of murine PR cDNA should facilitate studies designed to examine in detail the molecular basis for the species-specific regulation of PR gene expression, and herein lies the overall importance of our present studies. In addition, the availability of murine PR gene will also facilitate the construction of the "gene trap" vector, for introduction into mouse embryonic stem cells by homologous recombination, in future studies designed to analyze the importance of PR in regulating the development of its target tissues such as the mammary gland.

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REFERENCES

Beato, M. (1989) *Cell 56*, 335–344.

Benton, W. D., & Davis, R. W. (1977) *Science 196*, 180–182.

Carson-Jurica, M. A., Schrader, W. T., & O'Malley, B. W. (1990) *Endocr. Rev. 11*, 210–220.

Chomczynski, P., & Sacchi, N. (1987) *Anal. Biochem. 162*, 156–159.

Clarke, C. L., Zaino, R. J., Feil, P. D., Miller, J. V., Steck, M. E., Ohlsson-Wilhem, B. M., & Satyswaroop, P. G. (1987) *Endocrinology 121*, 1123–1132.

Conneely, O. M., Maxwell, B. L., Toft, D. O., Schrader, W. T., & O'Malley, B. W. (1987a) *Biochem. Biophys. Res. Commun. 149*, 493–501.

Conneely, O. M., Dobson, A. D. W., Tsai, M.-J., Beattie, W. G., Toft, D. O., Haetraby, C. S., Zaruchi, T., Schrader, W. T., & O'Malley, B. W. (1987b) *Mol. Endocrinol. 1*, 517–525.

Denner, L. A., Bingman, W. E., III, Greene, G. L., & Weigel, N. L. (1987) *J. Steroid Biochem. 27*, 235–243.

Dorsch-Hasler, K., Keil, G. M., Weber, F., Jasin, M., Schaffner, W., & Koszinowski, U. H. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8325–8329.

Evans, R. M. (1989) *Science 240*, 889–895.

Feil, P. D., Glasser, S. R., Toft, D. O., & O'Malley, B. W. (1972) *Endocrinology 91*, 738–746.

Green, S., & Chambon, P. (1988) *Trends Genet. 4*, 309–314.

Gronemeyer, H., Turcotte, B., Quirin-Stricker, C., Boucquel, M. T., Meyer, M. E., Krozowski, Z., Jeltsch, J. M., Lerouge, T., Garnier, J. M., & Chambon, P. (1987) *EMBO J. 6*, 3985–3994.

Jeltsch, J. M,. Krozowski, Z., Quirin-Stricker, C., Gronemeyer, H., Simpson, R. J., Garnier, J. M., Krust, A., Jacob, F., & Chambon, P. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5424–5428.

Jeltsch, J. M., Turcotte, B., Garnier, J. M., Lerouge, T., Krozowski, Z., Gronemeyer, H., & Chambon, P. (1990) *J. Biol. Chem. 265*, 3967–3974.

Kastner, P., Krust, A., Turcotte, B., Stropp, U., Tora, L., Gronemeyer, H., & Chambon, P. (1990) *EMBO J. 9*, 1603–1614.

Krett, N. L., Wei, L. L., Francis, M. D., Nordeen, N. K., Gordon, D. F., Wood, W. M., & Horwitz, K. B. (1988) *Biochem. Biophys. Res. Commun. 157*, 278–285.

Logeat, F., Le Cung, M., Pamphile, R., & Milgrom, E., (1985) *Biochem. Biophys. Res. Commun. 131*, 421–427.

Loosfelt, H., Atger, M., Misrahi, M., Guiochon-Mantel, A., Meriel, C., Logeat, F., Benarous, R., & Milgrom, E. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 9045–9049.

Misrahi, M., Atger, M,. D'Auriol, L., Loosfelt, H., Meriel, C., Fridlansky, F., Guiochon-Mantel, A., Galibert, F., & Milgrom, E. (1987) *Biochem. Biophys. Res. Commun. 143*, 740–748.

Misrahi, M., Loosfelt, H., Atger, M., Meriel, C., Zerah, V., Dessen, P. A., & Milgrom, E. (1988) *Nucleic Acids Res. 16*, 5459–5472.

Read, L. D., Snider, C. E., Miller, J. S., Greene, G. L., & Katzenellenbogen, B. S. (1988) *Mol. Endocrinol. 21*, 263–271.

Sambrook, J., Fritsch, E. F., & Maniatis, T., Eds. (1989) *Molecular Cloning—A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sanger, F., Nicklen, S., & Coulson, A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Schneider, W., Toft, D. O., Sullivan, W. P., & Shyamala, G. (1988) *J. Steroid Biochem. 29*, 297–306.

Schneider, W., Ramachandran, P. G., Satyaswaroop, P. G., & Shyamala, G. (1991) *J. Steroid Biochem. Mol. Biol. 38*, 285–291.

Schrader, W. T., Birnbaumer, M. E., Hughes, M. R., Weigel, N. L., Grody, W. W., & O'Malley, B. W. (1981) *Recent Prog. Horm. Res. 37*, 583–632.

Sheridan, P. L., Francis, M. D., & Horwitz, K. B. (1989) *J. Biol. Chem. 264*, 7054–7058.

Shyamala, G. (1985) in *Molecular Mechanism of Steroid Hormone Action* (Moudgil, V. K., Ed.) pp 423–435, Walter de Gruyter & Co., New York.

Shyamala, G., Schneider, W., & Schott, D. (1990) *Endocrinology 126*, 2882–2889.

Southern, P. J., & Berg, P. (1982) *J. Mol. Appl. Genet. 1*, 327–341.

Sullivan, W. P., Beito, T. G., Proper, J., Krco, C. J., & Toft, D. O. (1986) *Endocrinology 119*, 1549–1557.

Sullivan, W. P., Smith, D. F., Beito, T. G., Kroco, C. J., & Toft, D. O. (1988) *J. Cell Biochem. 36*, 103–119.

Wei, L. L., & Horwitz, K. B. (1986) *Steroids 46*, 677–695.

Wei, L. L., Sheridan, P. L., Krett, N. L., Francis, M. D., Toft, D. O., Edwards, D. P., & Horwitz, K. B. (1987) *Biochemistry 26*, 6262–6272.

Wei, L. L., Krett, N. L., Francis, M. D., Gordon, D. F., Wood, W. M., O'Malley, B. W., & Horwitz, K. B. (1988) *Mol. Endocrinol. 2*, 62–72.

Wei, L. L., Gonzalez-Aller, C., Wood, W. M., Miller, L. A., & Horwitz, K. B. (1990) *Mol. Endocrinol. 4*, 1833–1840.

Yamamoto, K., (1985) *Annu. Rev. Genet. 19*, 209–252.