Expression of Two Forms of Prolactin Receptor in Rat Ovary and Liver

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The screening of a size-selected cDNA library from the ovary revealed the existence of a second form of PRL receptor in the rat. The polypeptide sequence deduced from cDNAs has a much longer cytoplasmic domain (357 amino acids) than the form previously identified in the liver (57 amino acids). Nucleotide sequence analysis and comparison with rabbit, mouse, and human PRL receptor cDNAs suggests that the two forms of rat PRL receptor result from alternative splicing of a primary transcript. Complementary DNAs encoding the long form of the receptor were also found in a library prepared from estradiol-treated rat liver, although they represent a minor fraction of total PRL receptor cDNAs obtained from this tissue. DNA polymerase chain reaction amplification of cDNA confirmed the presence of the two receptor forms in both the ovary and liver. Northern analysis, using probes that specifically hybridize with either form of mRNA, indicates a major transcript of 1.8 kilobases (kb) in estradiol-treated liver, which encodes the receptor with a short cytoplasmic domain, while the long form of the receptor is encoded by mRNAs of 2.5 and 3 kb. In the ovary, a complex pattern of hybridization to multiple mRNAs (1.8–5.5 kb) is obtained with the probe specific to the long form, and essentially only a 5.5-kb mRNA is obtained with the probe specific to the short form. The predicted size of the mature form of the long PRL receptor (PRL-R2) is 591 amino acid residues. The cytoplasmic domain of this receptor shows strong overall sequence similarity with the corresponding residues of the rabbit (67%) and human (66%) PRL receptors and four regions of localized identity with the rabbit and human GH receptor. (Molecular Endocrinology 4: 1136–1143, 1990)

0888-8809/90/1136–1143$02.00/0
Molecular Endocrinology

INTRODUCTION

PRL receptors are widely distributed in various tissues, and PRL exerts numerous actions in its target cells (1). Biochemical studies, including cross-linking experiments, and the heterogeneity of PRL receptor mRNAs suggest the existence of different M_r forms of the PRL receptor in the rat (2–6). Recently, we have characterized a cDNA from estradiol (E_2)-treated rat liver (F3) (2) which encodes a mature receptor protein of 291 amino acid residues. The size of the protein deduced from this cDNA is smaller than that of the rabbit (7) and human (8) PRL receptors. To further identify forms of PRL receptor proteins in the rat, we have screened cDNA libraries from the ovary, E_2-treated liver, and kidney, using probes derived from the original liver cDNA F3.

RESULTS

Ovarian cDNA

A size-selected cDNA library from rat ovary (8×10^4 recombinants) was screened using a RNA probe derived from the rat PRL receptor cDNA (F3) (2), yielding two positive clones. The inserts were subcloned in the Bluescript vector (Stratagene, La Jolla, CA) and analyzed by restriction enzyme digestion and nucleotide sequence determination.

The longest cDNA clone obtained from our size-selected ovarian cDNA library, O1, contains 201 base-pairs (bp) identical to the nucleotide sequence of F3, encoding amino acids (aa) 196–261 (Fig. 1). From that position, corresponding to nucleotide 842 of the liver cDNA F3, the sequences differ completely, resulting in a much longer open reading frame in the ovarian cDNA. Using this first ovarian sequence (O1) as a probe, a second rat ovary cDNA library (obtained from Stratagene) was screened, yielding several clones, one of which, O2, contains the full coding sequence as well as some 5′ and 3′ noncoding sequences. The entire nucleotide sequence of the cloned DNA is presented in Fig. 2.

In addition to containing a sequence encoding a new C-terminal portion of the PRL receptor protein, this cDNA differs from the liver cDNA (F3) in a part of its 5′ noncoding sequence. Thus, the first 118 nucleotides of O2 are different from the corresponding bases of F3 cDNA.

Rat Liver cDNA

An E_2-treated rat liver cDNA library (7.3×10^5 recombinants) was screened using synthetic oligonucleotide probes RPR-28 and RPR-31, derived from the sequence of the ovarian cDNA 01 (see Fig. 1). Several cDNAs were isolated, the longest of which, G8 (1938 bp), starts at nucleotide 77 of F3 (2) and is identical until nucleotide 841, after which the sequence is different from that of F3 but identical to that of the ovarian clones O1 and O2. As for the ovarian clones (O1 and O2), the sequence of this liver cDNA (G8) does not seem to contain the entire 3′ noncoding region of the message, since no AATAAA polyadenylation signal is present in the cloned DNA.

Several more cDNAs corresponding to the short form of the PRL receptor were also isolated from this library and were shown to contain a slightly longer 3′ noncoding region than that of our initial clone F3, as exemplified by clone L1, which contains 21 extra nucleotides followed by several A residues, presumably from the poly(A) tail.

Kidney cDNAs

Among 18 cDNAs isolated from a rat kidney library (4×10^5 recombinants), four were fully characterized. All four correspond to the short form of the receptor. The longest cDNA, K4 (1475 bp), starts at nucleotide −41 of F3 and contains six additional residues at the 3′ end, followed by a run of 11 A residues.

Northern Blot Analysis

To determine the abundance of mRNAs encoding the two forms of the PRL receptor in liver and ovary, probes were prepared that corresponded to regions unique to each cDNA. Thus, a *Pst*I-*Eco*RV fragment of F3 (F3/P-V; 243 bp), corresponding to part of the 3′ noncoding sequence of F3, and a *Hin*dIII-*Xho*I fragment of the ovarian clone (O1/H-X; 269 bp), corresponding to the middle of the coding region of the long cytoplasmic domain, were subcloned in the plasmid Bluescript. ^{32}P-Labeled cRNAs were prepared and used for Northern blot analysis of polyadenylated RNA from E_2-treated rat liver and rat ovary (Fig. 3). When E_2-treated rat liver RNA was hybridized with probe F3/P-V, a strong signal at 1.8 kilobases (kb) and a very weak band at 3.0 and 5.5 kb were seen. After hybridization with probe O1/H-X, signals were also observed at 2.5, 3.0, and 5.5 kb. The relative intensities of these signals, as determined by densitometry of the x-ray films between the 1.8-, 3.0-, and 5.5-kb bands are 33.5, 2.0, and 1 using probe F3/P-V; for the 2.5-, 3.0-, and 5.5-kb bands, the relative intensities are 4.3, 7.0, and 1 using the probe specific to the long form (O1/H-X). In the rat ovary, the O1/H-X probe recognizes several mRNAs, ranging from 1.8–5.5 kb. With a shorter film exposure, definite bands of 2.3–2.8, 3.2–4.0, and 5–5.5 kb are distinguishable, but the major signal is at 5.5 kb. These bands did not disappear, even under stringent washing conditions [75 C; 0.1 × SSC, (15 mM NaCl, 1.5 mM Na citrate) -0.1% sodium dodecyl sulfate]. The F3/P-V probe detects essentially only a 5.5-kb mRNA.

Polymerase Chain Reaction (PCR)

To confirm the presence of messages encoding the short and long cytoplasmic domains within the same tissue and to look for potential additional forms, cDNAs were prepared from total RNA from rat ovary and poly(A)$^+$ RNA from E_2-treated rat liver and amplified by the DNA PCR. Several synthetic oligonucleotides from the coding regions shared by the twc forms were used as forward primers and one from a sequence corresponding to a portion unique to each type of receptor were used as reverse primers. The position and directions of three of the primers used are shown in Fig. 1. The DNAs were amplified through 30 cycles, then analyzed on an agarose gel. To confirm that the fragments obtained were indeed derived from PRL receptor cDNA, the material was transferred to a nylon filter and hybridized with a probe that recognized both forms. Using the particular set of primers depicted in Fig. 1, when the short form is present, the fragment amplified is of 330 bp, and when the long form is present, the DNA fragment is 420 bp long (Fig. 4). This experiment demonstrates that within the regions amplified, the short and long forms of the PRL receptor in the ovary and liver are the same, since the sizes of the PCR products are identical in both tissues. The same overall result was observed with other forward primers, the most 5′ of which started at bp 113 of the cDNA sequence shown in Fig. 2 (results not shown).

DISCUSSION

We have isolated several cDNAs from rat ovary and liver which differ from the previously reported PRL

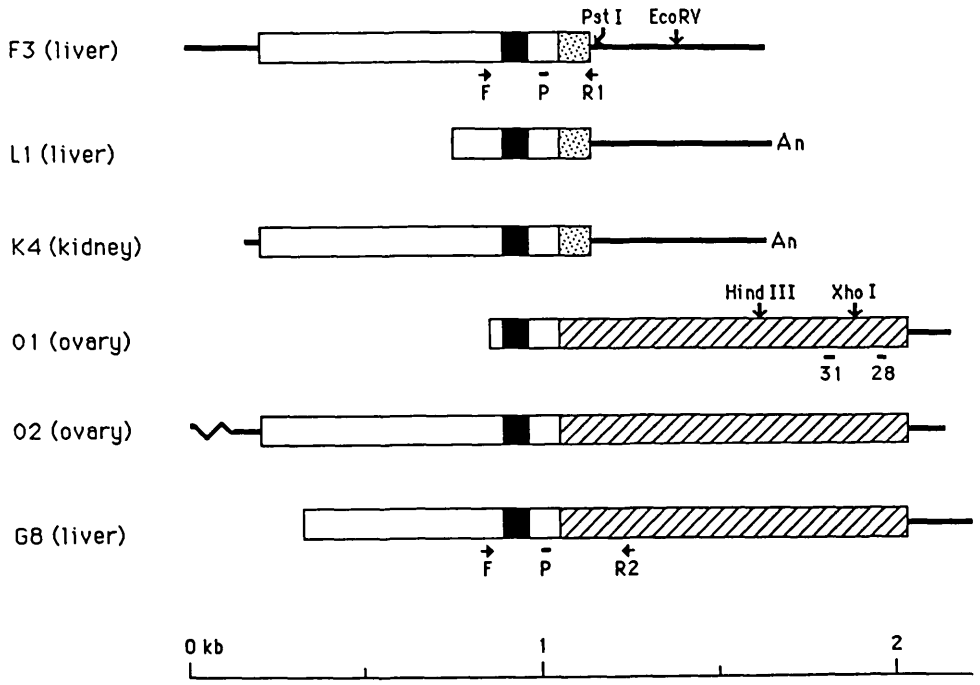

Fig. 1. Schematic Representation of Various Rat PRL Receptor cDNAs

The coding sequence contained in these clones is represented by *boxes*. The *black area* is the region encoding the transmembrane portion of the receptor. The *dotted area* is the region unique to the short form of the receptor, and the *hatched area* represents the long cytoplasmic domain. The *thick lines* at the beginning and end of the coding regions represent the noncoding sequences contained in the cDNAs. The *zig-zag line* in O2 corresponds to a different 5′ noncoding region in that cDNA. The location of the restriction sites used to generate fragments specific to each type of cDNA is shown. The numbers 28 and 31 refer to oligonucleotides (20-mers) used as probes (see text). The location of the oligonucleotide primers used in the PCR experiment is also shown. The F (forward) and R (reverse) 1 and 2 oligos were 30-mers, while P (probe) was a 20-mer.

receptor cDNA sequence (F3) in the rat, in that they encode a predicted protein with a much longer cytoplasmic domain. Nucleotide sequence analyses show that the 5′ coding portion of these clones is identical to that of the previously identified liver cDNA clone (F3) until nucleotide 842. The position at which the change in sequence of the two forms of PRL receptor occurs in the rat corresponds to an exon-intron junction in the closely related GH receptor gene (9).

Recently, three different cDNAs sequences of PRL receptor were reported to be present in the mouse (10). Two of these encode receptor proteins with a short cytoplasmic domain (39 and 50 *vs.* 57 aa in the rat), while the third is thought not to be translated into a protein because of a frame shift at codon 78. No such heterogeneity in the C-terminal portion of the short form of the PRL receptor was observed in the rat, as deduced from the nucleotide sequence of more than 12 cDNAs. However, the position at which the three mouse sequences diverge is identical to that where the short and long forms of rat receptors differ, thus supporting the hypothesis that the different mRNAs result from alternative splicing of a single gene.

Northern blot analysis of E_2-treated liver RNA (Fig. 3) using probes derived from regions unique to each type of cDNA (O1 or F3) shows a major transcript of 1.8 kb which hybridizes to the probe F3/P-V but not to the probe derived from the long form (O1/H-X probe). In the liver, the O1/H-X probe detects RNA fragments of 2.5, 3.0, and 5.5 kb. As judged by the intensity of the signals obtained with this probe on Northern blot analysis and the relative low abundancy of cDNAs corresponding to this form of receptor in the liver cDNA library, it is concluded that the long form is a minor component of the PRL receptor population in this tissue.

Northern blot analysis of ovarian RNA (Fig. 3) shows a complex pattern of mRNAs, the most apparent of which ranges from 2.3–5.5 kb using the probe specific for the long form, whereas essentially only a 5.5-kb form was seen with the probe specific for the short form. Despite stringent washes of the membranes (75 C; 0.1 × SSC), none of the bands disappeared (data not shown), suggesting that the ovary possesses a variety of specific mRNAs for the PRL receptor. These different mRNAs may correspond to additional alternative splicing of the PRL receptor gene. In the ovary, PRL receptors have been found not only in corpora lutea, but also in granulosa cells of medium- and large-sized follicles (11–13). It is conceivable that the different messages encode receptors performing different functions in the ovary.

PCR amplification of cDNAs prepared with RNA from ovary and E_2-treated liver and Southern blot analysis (Fig. 4) clearly demonstrates that the mRNAs encoding the two forms of PRL receptor are present in the ovary

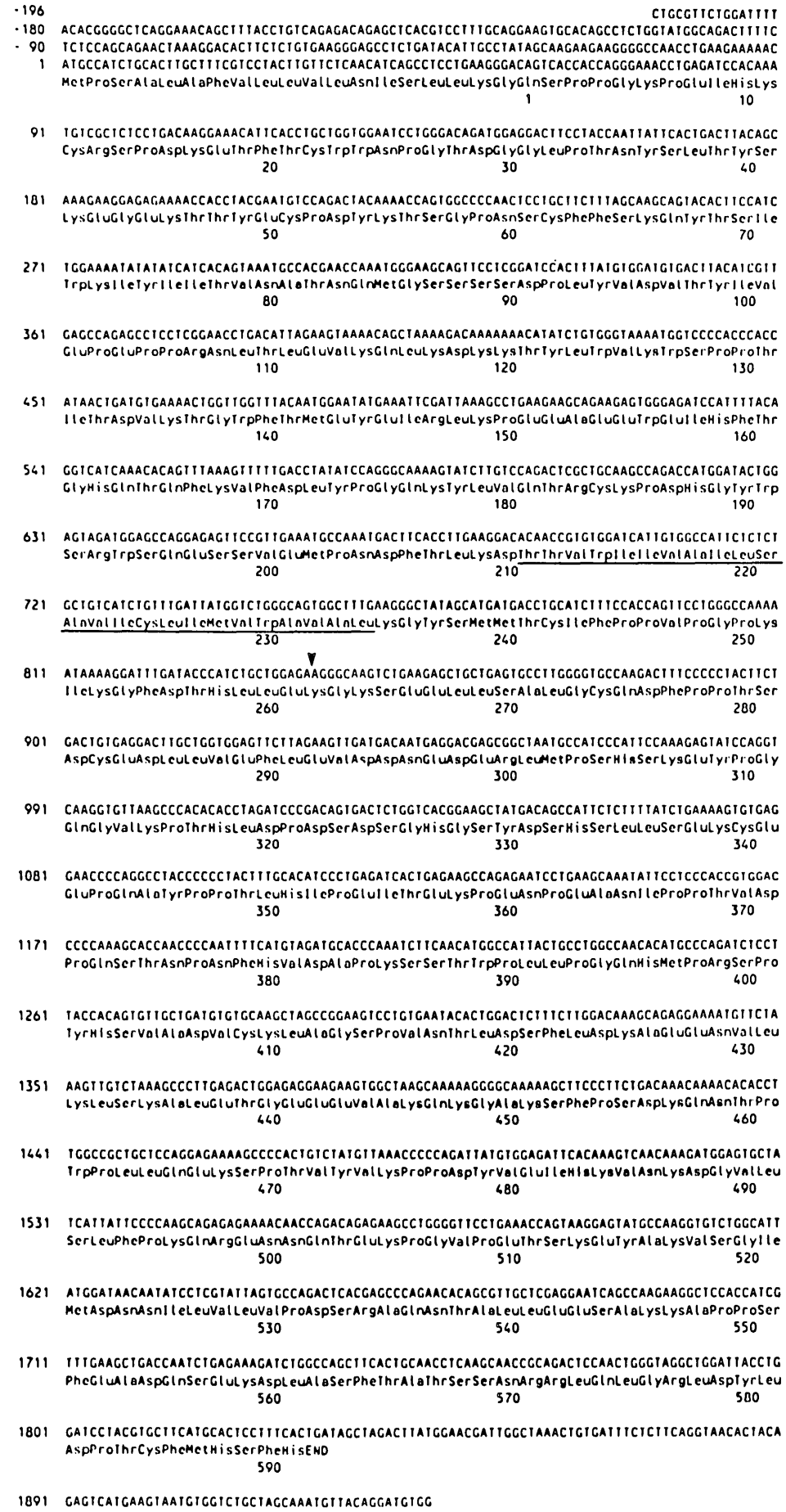

Fig. 2. Nucleotide Sequence and Deduced Amino Acid Sequence of Rat Ovary cDNA (O2)

Nucleotides are numbered on the *left*, based on the numbering of F3 (2), and aa of the mature protein are numbered *below* the sequence. The transmembrane region is *underlined*. The *arrowhead* indicates the position in the coding sequence at which this cDNA differs from the liver cDNA F3 (2).

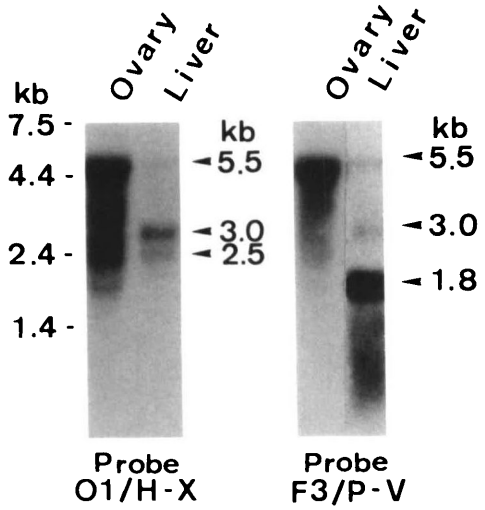

Fig. 3. Northern Blot Analysis of PRL Receptor mRNA Expression in Rat Ovary and Liver

Twenty micrograms of poly(A)$^+$ RNA (ovary) or 10 μg poly(A)$^+$ RNA (liver) were used for each lane. Hybridization was performed with cRNA probe specific to the long form (O1/H-X) and the short form (F3/P-V). The x-ray film was exposed for 16 h with an intensifying screen at −70 C.

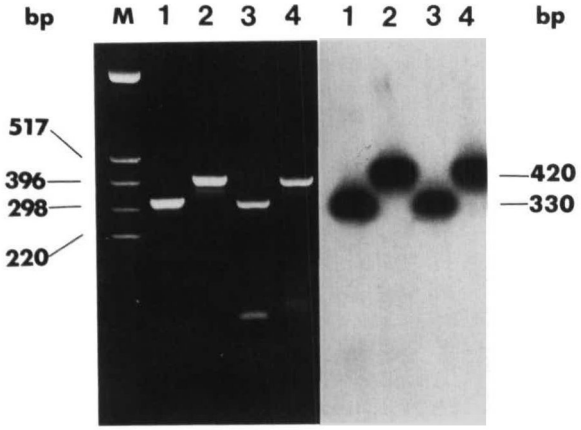

Fig. 4. Agarose Gel and Southern Blot Analysis

One tenth of the amplified cDNA reaction mixture was applied to each lane of a 2% agarose gel. Lanes 1 and 2 are amplified DNA from rat ovary, and lanes 3 and 4 are DNA from rat liver. The DNAs were transferred to a nylon membrane and hybridized with a synthetic oligonucleotide probe (P, Fig. 1) common to the long and short forms of the receptor. The *left panel* is the ethidium bromide-stained gel, and the *right panel* is the Southern hybridization.

and liver in the rat and that no additional sequences (or deletions) are present between the primers used.

Recently, the heterogeneity of ovarian PRL receptors was again demonstrated. Three different M_r forms (80,000, 40,000, and 34,000) were identified by HPLC and immunoblot analysis (14). However, the 11 amino acid sequence (MKVFEPIPLVI) presented as a part of the 80-kDa putative receptor is not present in the long form of the PRL receptor that we have described here. It would, therefore, represent a third type of PRL receptor protein in the rat.

The nucleotide sequence of the ovarian cDNA O1 and O2 encodes a cytoplasmic domain of 357 aa (Fig. 2) instead of the 57 aa encoded by F3 [rat (r) PRL-R2 and rPRL-R1 of Fig. 5]. The predicted size of the mature protein (rPRL-R2) is, therefore, 591 aa. This size is almost identical to that of the PRL receptor in the rabbit (592 aa) (7) and the human (598 aa) (8). The predicted M_r of the mature nonglycosylated protein is 66,676. Since the extracellular region contains the same glycosylation sites as F3 (2), the M_r of the glycosylated receptor should be 75,000–80,000. Western blot analysis of rat ovary shows bands of 84,000, 51,000, and 42,000 M_r using a monoclonal antibody specific to the extracellular domain (5). The 51,000 M_r band seen on Western blot may correspond to the long form of the PRL receptor which has been degraded during membrane preparation or electrophoresis, in spite of the fact that numerous protease inhibitors were used. The 42,000 and 84,000 M_r forms are probably a monomer and dimer of the short form of the receptor encoded by the 1.8-kb mRNA representing clone F3 (5, 6).

Figure 5 shows a comparison between the sequences of the cytoplasmic domains of the long form of the rat PRL receptor (rPRL-R2) and the short form (rPRL-R1), the rabbit (rbPRL-R) and human (hPRL-R) PRL receptors, as well as rabbit (rbGH-R) and human (hGH-R) GH receptors (15). Although the long form of the rat PRL receptor has virtually no similarity to the last 30 aa of the short form, it shows strong similarity with the entire sequence of the long form of the rabbit (67%) and human (66%) receptors. When conservative aa residue substitutions are considered, these values increase to 70% and 77%. The overall sequence similarities with the cytoplasmic domains of rabbit and human GH receptors are 19% and 18%, respectively. However, four areas of significantly higher sequence identity between the long form of PRL receptors and GH receptors are clearly seen. The first of these regions of 25 residues in length is located close to the transmembrane domain (aa 243–267) and is 68% identical. Three additional regions (residues 285–295, 319–329, and 474–483) of lesser (>40%) but significant identity are also found. These conserved regions possibly play a role in the process of signal transduction, which remains a mystery for both PRL and GH.

The identification of two different forms of the PRL receptor within the same tissue (liver and ovary) suggests that these forms may perform different functions. Since the two probes used in the Northern blot (Fig. 3) were of comparable size, and the film exposure time was identical, it is possible to directly compare the intensities of the bands and arrive at an approximate expression level of the two forms in the ovary and liver. Thus, there is a preponderance of the short form in liver, whereas the ovary has more of the long form. In the kidney because only four cDNAs were fully analyzed, a definitive statement cannot be made; however,

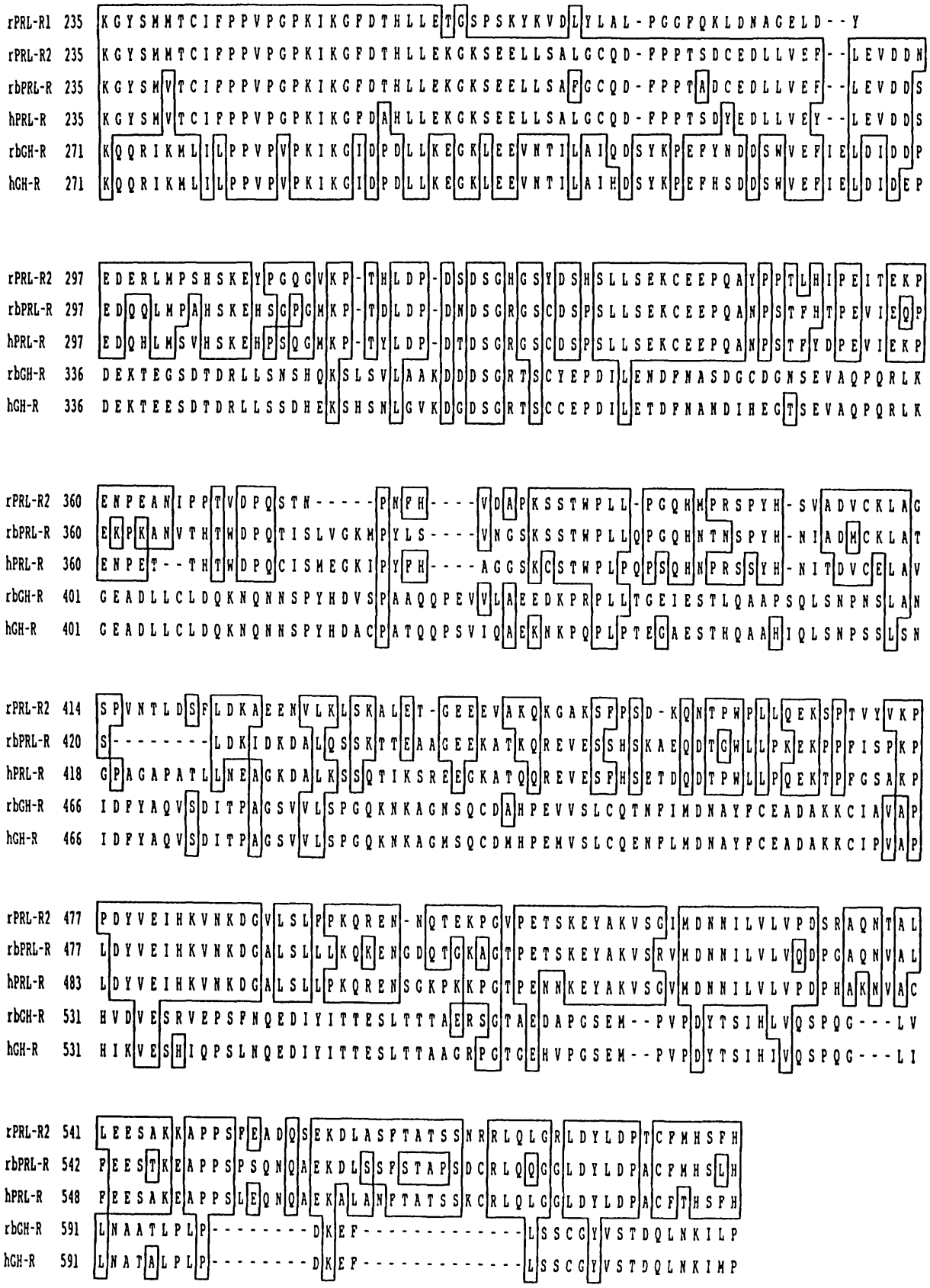

Fig. 5. Structural Comparison of the Cytoplasmic Domains of PRL and GH Receptors

The long form of the rat PRL receptor (rPRL-R2) is compared with the rabbit PRL receptor (rbPRL-R), human PRL receptor (hPRL-R), rabbit GH receptor (rbGH-R), and human GH receptor (hGH-R). The short form of the rat PRL receptor (rPRL-R1) is also included. Numbers indicate the residue of the mature receptor. Amino acids identical to those of rPRL-R2 are *boxed*.

the fact that all four were of the short form suggests that this form predominates.

Interestingly, a portion of the 5′ noncoding sequence of the ovarian cDNA O2 is different from the corresponding liver cDNA sequence F3. The change occurs at position −81. The fact that this sequence is identical in 71% of the corresponding bases in a human PRL receptor cDNA (8) argues against it being artefactual. We also have preliminary evidence of heterogeneity of the 5′ region of the cDNA encoding the short form of the receptor (Banville, D. and P. A. Kelly, unpublished observations). The heterogeneity of the 5′ noncoding sequence of rat PRL receptor mRNAs is analogous to the situation described for the related GH receptor mRNAs (15) and may represent the use of different promoter elements. This may also provide an explanation for the observation that the short form of the PRL receptor is encoded by a mRNA of 1.8 kb in the liver and 5.5 kb in the ovary.

MATERIALS AND METHODS

Complementary DNA Libraries

In addition to the size-selected rat liver λgt11 library previously described (2), a second library was prepared from rat liver as well as one from rat ovary. Size-selected cDNA libraries were constructed as follows. Complementary DNA was synthesized from poly(A)$^+$ RNA from E_2-treated rat liver or adult rat ovaries. After the addition of *Eco*RI linkers to the oligo(dT)-primed double stranded cDNA, fragments larger than 1 kb were selected on a Bio-Gel column (16) and were inserted into the phage expression vector λgt10 (Stratagene). A size-selected kidney library in λgt11 was kindly provided by Dr. J. C. Edman in Dr. W. J. Rutter's laboratory (San Francisco, CA). Finally, a λZap library from rat ovary was obtained from Stratagene.

Nucleic Acid Hybridization and Screening

The cDNA libraries were screened with RNA probes synthesized from cDNA F3, using the method previously described (2), or in the case of the kidney library, with a cDNA probe.

To identify a long form of the PRL receptor in the liver, the size-selected library prepared from E_2-treated rats (2) was screened using ^{32}P-labeled synthetic oligonucleotide probes labeled by 5′-end phosphorylation (17). The location of the robes used (RPR-28 and RPR-31) is shown in Fig. 1. Hybridization was performed as previously described (2), and membranes were washed successively at 4 C, room temperature, and 37 C in 6 × SSC. The nucleotide sequence of cDNAs was determined by the dideoxy chain termination method (18).

Northern Blot Analysis

Poly(A)$^+$ RNA was prepared from adult rat ovaries and E_2-treated rat livers. Poly(A)$^+$ RNA was treated with glyoxal (2), and 20 μg (ovary) or 10 μg (liver) were electrophoresed through 1% agarose in 10 mM NA_2HPO_4. A 0.24- to 9.5-kb RNA ladder (Bethesda Research Laboratories, Gaithersburg, MD) was also included on the gel as M_r markers. Transfer of RNA, hybridization with RNA probes, washing, and autoradiography were performed as previously described (2).

PCR

Complementary DNAs for the PCR reaction were synthesized from 4–5 μg total RNA from ovary or 0.5–1 μg poly(A)$^+$ RNA from E_2-treated rat liver using reverse transcriptase in the BRL cDNA synthesis system (Bethesda Research Laboratories). Complementary DNAs were amplified (30 cycles) with 100 pmol each of two synthetic oligonucleotides. The first RPR-F, a 30-mer corresponding to nucleotides 624–653 (see Fig. 1) is common to both forms; the second, RPR-R1 is a 30-mer complementary to nucleotides 924–953 of F3 and specific to the short form or RPR-R2, a 30-mer complementary to nucleotides 1014–1043 of the long form (Fig. 1). The amplification conditions were: denaturation at 94 C for 1 min, annealing of the primers at 50 C for 2 min, and extension at 72 C for 5 min. After amplification, samples were electrophoresed on a 2% agarose gel, and the DNA was transferred to a nylon membrane (Hybond-N, Amersham, Arlington Heights, IL) for Southern blot analysis. The filters were hybridized to a 5′-end-labeled oligonucleotide probe (P, Fig. 1) derived from a region common to both forms under conditions previously described. To control for potential contamination of the PCR reaction, reactions were performed with RNA that had not been reverse transcribed or with samples lacking RNA in the reverse transcriptase reaction. Each of these control reactions yielded negative results.

Note Added in Proof

A recent report has appeared describing a long ovarian prolactin receptor that contains a consensus sequence for an ATP/GTP type A binding site (Zhang R, Buczko E, Tsai-Morris C-H, Hu Z-Z, Dufau M 1990 Isolation and characterization of two novel rat ovarian lactogen receptor cDNA species. Biochem Biophys Res Commun 168:415–422). Comparison of the 1830 bp of the coding region of the ovarian cDNA described in that paper with the 1830 bp of the long form described in the present manuscript reveals seven single base differences, all of which result in amino acid changes in the predicted protein structure, the three most important of which (nt 1393, 1396, and 1406) cause Lys→Glu, Gln→Glu, and Ala→Gly changes that form part of the putative ATP/GTP binding site reported by Zhang *et al*. In the cDNAs encoding the long form of the prolactin receptor from the rat ovary and liver reported in the present manuscript, which were isolated from three independently prepared cDNA libraries, we found the sequence reported in Fig. 2. Thus, the long form of the PRL receptor that we identified does not contain an ATP/GTP binding site.

Acknowledgments

Received March 6, 1990. Revision received May 11, 1990. Accepted May 21, 1990.

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This work was supported in part by grants from the Medical Research Council of Canada and the NCI of Canada.

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