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Molecular cloning of a novel luteinizing-hormone/human-chorionic-gonadotropin-receptor cDNA

Identification of a long 3′ untranslated region and cDNA sequence of the major transcript in rat ovary

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The biological action of luteinizing hormone/human chorionic gonadotropin (lutropin/choriogonadotropin) in the ovary is mediated by interaction with its specific receptor. Lutropin/choriogonadotropin-receptor hnRNA is processed into multiple mRNAs. However, nucleotide sequences for many of the transcripts, including the major form (6.7 kb), have yet to be determined. In an attempt to identify a cDNA encoding the major transcript, we have isolated a 3.5-kb cDNA clone from a rat ovary cDNA library. The 3.5-kb cDNA recognized only two (6.7 kb, 4.4 kb) of the three (6.7, 4.4, 2.6 kb) ovarian lutropin/choriogonadotropin-receptor transcripts when used as a probe. The first 732 nucleotides of the newly identified 3.5-kb cDNA showed 98% identity to the 3′ untranslated region (3′ UTR) of the previously cloned cDNA corresponding to the 4.4-kb transcript. Southern blot analysis indicated that the 3.5-kb cDNA and the C-terminal domain of the lutropin/choriogonadotropin-receptor originate from the same gene. Oligonucleotide-directed cleavage of the 6.7-kb lutropin/choriogonadotropin-receptor mRNA by RNase H revealed that the newly identified 3.5-kb cDNA is a 3′ extension of the 4.4-kb transcript. We propose that the nucleotide sequence of the 6.7-kb lutropin/choriogonadotropin-receptor transcript, the major form found in rat ovary, contains a long 3′ UTR, which has not been previously identified.

The luteinizing hormone/human chorionic gonadotropin (lutropin/choriogonadotropin) receptor is a glycoprotein expressed in rat ovary. The interaction of lutropin/choriogonadotropin with cell-surface receptors leads to activation of adenylate cyclase, which in turn, stimulates a number of intracellular functions, including steroidogenesis [1–3]. Rat, porcine, human, and murine lutropin/choriogonadotropin-receptor cDNAs have been cloned [4–7]. The cloned receptors show characteristics typical of stimulatory guanine-nucleotide-binding-regulatory(G)-protein-coupled receptors [8, 9], except that on the basis of hydropathy plot, the lutropin/choriogonadotropin receptor exhibits a large extracellular N-terminal domain and a smaller intracellular C-terminal [4].

The lutropin/choriogonadotropin receptor is encoded by a single-copy gene spanning 75–96 kb and consisting of 11 exons [10, 11]. However, multiple transcripts of the lutropin/choriogonadotropin receptor are present in all target tissues [12–16]. Wang et al. showed that the sizes and abundance of lutropin/choriogonadotropin-receptor transcripts varied in different gonad tissues and cell types [12]. Sokka et al. have reported that in the neonatal period, rat ovary expresses various transcript sizes during different stages of development [13]. They suggested that alternative splicing may play a major role in generating the multiple transcripts during this period. In rat ovary, one major 6.7-kb transcript, and two minor transcripts corresponding to 4.4 kb and 2.6 kb, and a less abundant 1.8-kb transcript have been reported [12, 14, 15]. It is thought that the 3.0-kb cDNA cloned by Mac Farland et al. corresponds to the 4.4-kb transcript in rat ovary [4]. Although the major transcript in the ovary is 6.7 kb, its nucleotide sequence has not been established. One objective of this study was to determine the identity of the 6.7-kb transcript.

A second objective was to determine whether the lutropin/choriogonadotropin-receptor transcript contains a previously unidentified 3′ untranslated region (3′ UTR), since this region has been implicated as a determinant of mRNA stability in other systems [17]. We have previously shown that the receptor mRNA is post-transcriptionally regulated during ligand-induced down-regulation [14]. Since specific sequences in the 3′ UTR can modulate message stability [17], we hypothesized that the major transcript may contain additional regulatory sequences, and thus a longer 3′ UTR than formerly recognized. Our results show that we have cloned a lutropin/choriogonadotropin-receptor cDNA that encodes a novel 3′ UTR that may serve as a focal point for determining mRNA stability.

MATERIALS AND METHODS

Construction of the C-terminal lutropin/choriogonadotropin-receptor probe

A 750-nucleotide probe was constructed by reverse transcription, followed by PCR as described by Kawasaki et al.

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Abbreviations. 3′ UTR, 3′ untranslated region; RNase H, ribonuclease H.

[18] using ovarian RNA as a template. The probe corresponded to nucleotides 1951−2697 of the sense strand of the receptor cDNA cloned by MacFarland et al. [4]. The primer sequences to generate the cDNA were 5′ TGACAAGCTTCGGGCGGAGCTTTACAG 3′ and 5′ GCTCAGATGGGCTCCTGGCC 3′. The probe was cloned into a Bluescript SK^+ vector (Stratagene) between the *Hin*dIII and *Sma*I restriction sites.

cDNA library screening

A rat ovary cDNA library (day-15-pregnant rat corpora lutea) was purchased from Stratagene and the procedure for plaque hybridization was obtained from the enclosed Lambda ZAP II/*Eco*RI cloning kit and Berger et al. [19]. The cDNA library, already cloned into the Lambda ZAP II vector, was plated on XL-1 Blue *Escherichia coli* cells and transferred to nitrocellulose. After the blots were denatured and neutralized, they were heated at 80°C under vacuum for 2 h. Blots were prehybridized in 2×Pipes (0.8 M NaCl, 0.02 M Pipes), pH 6.5, 50% formamide, 0.5% SDS, and 100 µg/ml salmon sperm DNA at 42°C for 2 h. The C-terminal probe was radiolabeled using [α-^{32}P]dCTP (ICN) and the Klenow fragment of DNA polymerase [20] and hybridized to blots overnight at 42°C in fresh buffer at 10000000 cpm/filter. Hybridized blots were washed with 0.1×NaCl/Cit (NaCl/Cit is 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS at 65°C for 15 min. The washed blots were exposed overnight at −70°C to Kodak XAR film in a cassette containing intensifying screens. Positive clones were isolated from the original agar plates and put into SM buffer (0.1 M NaCl, 0.01 M $MgSO_4$, 0.05 M Tris, pH 7.5, 0.004% gelatin). The pBluescript plasmid was excised from the Lambda ZAP II vector with R408 helper phage (Stratagene).

Animals and tissues

Pseudopregnancy was induced using the procedure described by Parlow [21]. 21-day-old female Sprague Dawley rats were injected with 50 U PMSG (pregnant mare serum gonadotropin; Calbiochem), followed by 25 U choriogonadotropin (Sigma) 56 h later. When indicated, down-regulation was induced in pseudopregnant rats by injecting 50 U choriogonadotropin on day 5 of pseudopregnancy; control rats received saline. Day-15-pregnant Sprague Dawley rats were used when indicated. Ovaries were collected and either processed immediately or stored in liquid nitrogen until needed.

RNA extraction

RNA was extracted from tissues using the procedure of Chomczynski and Sacchi [22]. Tissues were homogenized in a solution of guanidine isothiocyanate, 2 M sodium acetate, pH 4.0, and extracted with water-saturated phenol and chloroform/isoamyl alcohol (49:1, by vol.). RNA remained in the aqueous phase and was precipitated overnight at −20°C using 3 volumes of ethanol. RNA was spectrophotometrically quantified, and the purity was determined by the ratio of A_{260}/A_{280}.

cDNA probes used in Northern and Southern analysis

The 750-nucleotide C-terminal (CT) probe used to screen the cDNA library to isolate the 3.5-kb cDNA clone was also used in Northern and Southern analyses. The plasmid containing the 3.5-kb insert isolated from the cDNA library was linearized using *Bam*HI when used as a full-length (FL) probe. The 3.5-kb cDNA clone was cleaved to isolate smaller cDNA probes. Four fragments from *Sac*I/*Hin*dIII digestion of the 3.5-kb cDNA (0.9, 0.3, 0.8 and 1.2 kb) were designated as A, B, C, and D, respectively. Probe E (650 nucleotides) was isolated following *Bam*HI/*Kpn*I digestion of the plasmid containing the 3.5-kb insert. The N-terminal (NT) probe corresponding to nucleotides 9−535 of a lutropin/choriogonadotropin-receptor cDNA, was obtained from Dr William Moyle at the Robert Wood Johnson (Rutgers) Medical School [23].

Northern blot hybridization

Procedures were essentially the same as those described by Davis et al. [24]. Total RNA was separated by electrophoresis in a 1.2% agarose gel containing 3% formaldehyde. RNA was blotted to nitrocellulose using 10× NaCl/Cit. The blot was heated at 80°C under vacuum for 2 h then hybridized. Hybridization was basically the same as that described by Sambrook et al. [25]. Blots were prehybridized at 42°C for 2 h in a solution containing 0.75 M NaCl, 0.05 M Tes, 0.05 M EDTA, pH 7.1, 1× Denhardt's solution, 50% deionized formamide and 100 µg/ml salmon sperm DNA. The probes were radiolabeled using [α-^{32}P]dCTP (ICN) and the Klenow fragment of DNA polymerase [20] and hybridized to blots overnight at 42°C in fresh buffer at 20000000 cpm/230 cm^2 of nitrocellulose. Hybridized blots were washed twice with 2× NaCl/Cit, 0.1% SDS for 20 min at room temperature and once at 60°C for 30 min. The washed blots were exposed overnight at −70°C to Kodak XAR film in a cassette containing intensifying screens.

DNA sequencing

DNA denaturation and primer annealing were performed as described by Hsiao [26]. Sequence was obtained from both strands by chain-termination DNA sequencing [27] using the Sequenase Version 2.0 kit (USB) and [α-^{35}S]dATP[S] (NEN) incorporation or fluorescein-based automated sequencing (ABI). Sequence analysis was carried out by means of the MacVector 3.5 program (IBI).

Southern blot hybridization

DNA was isolated from day-6-pseudopregnant rat spleen using Stratagene's DNA Extraction kit according to the protocol of Miller et al. [28]. Procedure for Southern blotting and hybridization was obtained from Sambrook et al. [25]. 10 µg DNA was digested with 300 U *Bam*HI, separated on a 0.7% agarose gel, denatured (1.5 M NaCl, 0.5 M NaOH), neutralized (1 M Tris, pH 7.4, 1.5 M NaCl), and blotted to nitrocellulose with 10× NaCl/Cit. The blot was heated at 80°C under vacuum for 2 h. Blots were prehybridized at 42°C for 2 h in a solution containing 6× NaCl/Cit, 5× Denhardt's reagent, 0.5% SDS, 50% formamide, and 100 µg/ml salmon sperm DNA. The probes were radiolabeled using [α-^{32}P]dCTP (ICN) and the Klenow fragment of DNA polymerase [20] and were hybridized to blots overnight at 42°C at 10000000 cpm. Hybridized blots were washed with 2× NaCl/Cit, 0.1% SDS for 15 min at room temperature, 0.1× NaCl/Cit, 0.5% SDS for 30 min at 37°C, and 0.1× NaCl/Cit, 0.5% SDS for 30 min at 68°C. The washed blots were

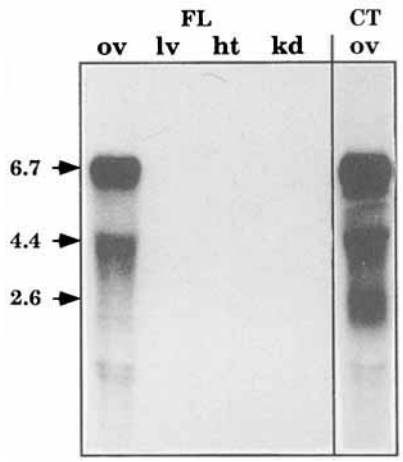

Fig. 1. Hybridization of full-length (FL) 3.5-kb cDNA probe to RNA from different tissues and C-terminal (CT) cDNA probe to ovarian RNA. Each lane contained 25 μg total RNA extracted from day-6 pseudopregnant rats. Samples shown are ovary (ov), liver (lv), heart (ht), and kidney (kd). The 6.7-, 4.4-, and 2.6-kb bands are indicated.

exposed overnight at −70°C to Kodak XAR film in a cassette containing intensifying screens.

Oligonucleotide-directed cleavage with RNase H

Procedures for DNA/RNA annealing and hybridization with RNase H were modified from Goodwin et al. [29], Smith et al. [30], and Hake et al. [31]. 15 μg DNA oligomers corresponding to different regions of the 3.5-kb cDNA sequence and the lutropin/choriogonadotropin-receptor cDNA were annealed to 40 μg total RNA isolated from day-6 pseudopregnant rat ovaries for 1 h at 37°C in 40 mM Hepes, pH 7.9, 10 mM $MgCl_2$, 60 mM KCl, and 1 mM dithiothreitol. To the DNA/RNA mixture, 2 U RNase H (BMB) was added and incubation continued for 30 min at 37°C. The reaction was terminated by extraction with water-saturated phenol and chloroform/isoamyl alcohol (49:1, by vol.). The RNA was concentrated by ethanol precipitation and subjected to Northern analysis. The probes used for hybridization are described above.

RESULTS

Isolation and characterization of the 3.5-kb cDNA clone

A 15-day-pregnant-rat corpus luteum library was screened using a cDNA probe (CT) that corresponded to the C-terminal domain extending to the 3′ UTR region of the previously cloned lutropin/choriogonadotropin-receptor, and a 3.5-kb cDNA clone was isolated. In Fig. 1, the 3.5-kb cDNA was labeled and used as a full-length (FL) probe in Northern analysis of RNA from different tissues. The 3.5-kb cDNA (FL) hybridized to ovarian RNA, and not to RNA isolated from non-target tissues, including liver, heart and kidney. Moreover, the 3.5-kb cDNA (FL) recognized the 6.7-kb and 4.4-kb transcripts and did not hybridize to the 2.6-kb transcript that is recognized by the C-terminal (CT) probe.

For initial characterization, the 3.5-kb cDNA clone was restriction digested with *Sac*I and *Hin*dIII to release the cDNA fragments seen in Fig. 2A. All four fragments recognized the 6.7-kb and 4.4-kb transcripts in pseudopregnant rat ovary but not the RNA from ovaries that were down-regulated for 24 h, as shown in Fig. 2B. Previous studies in our laboratory [14, 16] have shown that lutropin/choriogonadotropin-receptor cDNA probes did not hybridize to ovarian RNA isolated from pseudopregnant rats 24 h after down-regulation with choriogonadotropin. Fig. 2C shows that three of the four fragments (A, C, D), plus the 3.5-kb cDNA clone (FL), hybridized to pregnant rat ovary RNA and recognized the same transcript sizes as in pseudopregnant rats. It appears that there is a 1.8-kb transcript present in pseudopregnant rat ovary RNA, but absent in pregnant rat ovary RNA. However, the 1.8-kb transcript was visualized in pregnant rat ovary RNA after longer exposure of the autoradiogram. Thus, the

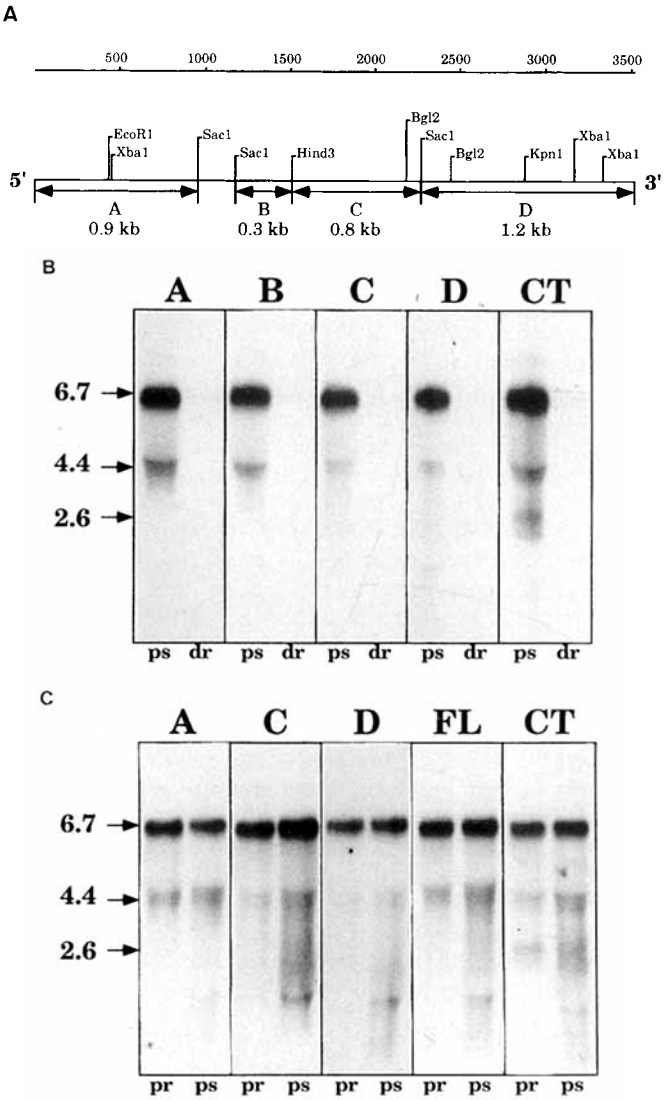

Fig. 2. Hybridization of 3.5-kb cDNA fragments to pseudopregnant, down-regulated, and pregnant rat ovarian RNA. (A) Restriction map of the 3.5-kb cDNA showing the fragments used as probes. (B) Hybridization of probes A, B, C, D, and C-terminal (CT) cDNA to 30 μg total RNA of day-6 pseudopregnant rat ovary (ps) and 24-h down-regulated rat ovary (dr). (C) Hybridization of probes A, C, D, full-length (FL) 3.5-kb cDNA, and C-terminal (CT) cDNA to 25 μg total RNA of day-15-pregnant rat ovary (pr) and day-6-pseudopregnant rat ovary (ps). In both blots, the 6.7-, 4.4-, and 2.6-kb bands are indicated.

3.5-kb cDNA hybridized to specific lutropin/choriogonadotropin-receptor mRNA transcripts in both an expression-specific and tissue-specific manner.

Sequence analysis of the 3.5-kb cDNA clone

The nucleotide sequence of the 3.5-kb cDNA is shown in Fig. 3A. No continuous open reading frames are present in the cDNA clone, suggesting that the sequence corresponds to a non-coding region. Nucleotides 1−732 of the 3.5-kb cDNA clone are 98% identical to the 3′ end of the cDNA cloned by MacFarland et al. [4], indicating that the 3.5-kb cDNA may encode an extension of the lutropin/choriogonadotropin receptor 3′ UTR. A diagram showing where the cDNA cloned by MacFarland et al. [4] and the 3.5-kb cDNA clone overlap is shown in Fig. 3B. The sequence was further examined for the presence of polyadenylation sites and for other characteristic features of the 3′ UTR. The AAUAAA polyadenylation signals [32] and the AUUUA mRNA-destabilization motif [17] are indicated in Fig. 3A. Three polyadenylation sites were found at nucleotides 2198−2203, 3386−3391 and 3479−3484 of the 3.5-kb cDNA. 11 AUUUA mRNA-destabilizing motifs were found in the 3.5-kb cDNA. They appear to cluster in two regions: one within nucleotides 23−267 and another within nucleotides 1498−1925. The presence of these motifs also suggests that the 3.5-kb cDNA encodes a 3′ UTR. Thus, we postulate that the 3.5-kb cDNA plus the coding region of the previously cloned lutropin/choriogonadotropin-receptor cDNA would result in a 5.7-kb cDNA, as shown in Fig. 3B. Since DNA and RNA molecular masses differ, it is possible that the putative full-length 5.7-kb cDNA codes for a lutropin/choriogonadotropin-receptor transcript that is considerably larger than the previously cloned cDNA [4]. Based on this and the following experiments, we propose that the 3.5-kb cDNA encodes the 3′ UTR of the 6.7-kb mRNA transcript.

In an attempt to determine if any of the polyadenylation sites in the 3.5-kb cDNA are utilized, other positive clones isolated during the cDNA library screening were partially sequenced. Since the cDNA library was oligo(dT) primed from pregnant-rat corpus luteum mRNA, the 3′ ends of the cDNA clones should represent the 3′ ends of mRNA transcripts. Partial sequence analysis of the cDNA clones indicated that the 3′ ends of the cDNAs were identical to either the 3′ end of the lutropin/choriogonadotropin-receptor cDNA isolated by MacFarland et al. [4] or the 3′ end of the 3.5-kb cDNA (data not shown). This suggests that the polyadenylation site at nucleotides 2198−2203 does not appear to be functional in the ovary. It is more likely that one of the polyadenylation sites toward the 3′ end of the cDNA is used to generate the 6.7-kb transcript, and that the 4.4-kb transcript is derived from alternative splicing or a non-canonical polyadenylation site.

Localization of the 3.5-kb cDNA within the lutropin/choriogonadotropin-receptor gene

To determine if the 3.5-kb cDNA is encoded by the lutropin/choriogonadotropin-receptor gene, Southern blot analysis was performed using rat spleen DNA. The 5′ end of the 3.5-kb cDNA showed 99% identity to exon 11 of the lutropin/choriogonadotropin-receptor gene isolated by Tsai-Morris et al. and Koo et al. [10, 11]. To establish if the 3′ end of the 3.5-kb cDNA is continuous with its 5′ end, cDNA probes from the middle and the 3′ end of the 3.5-kb cDNA

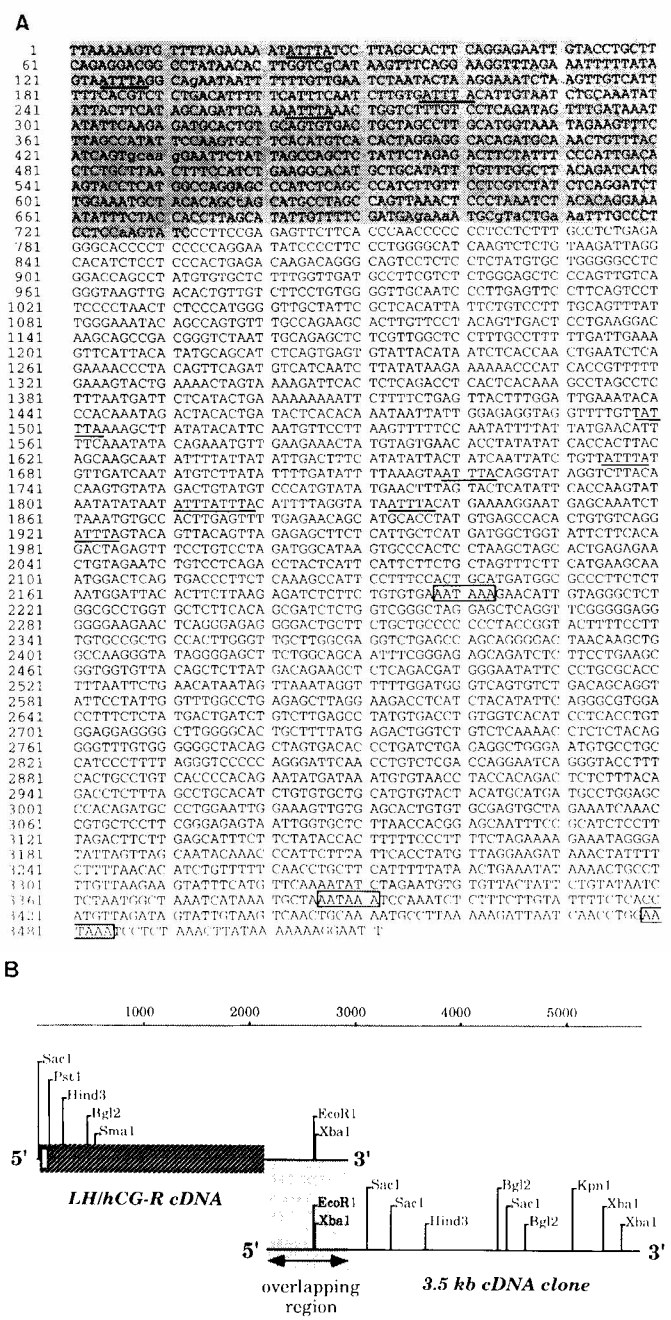

Fig. 3. Nucleotide sequence of the 3.5-kb cDNA isolated from a day-15 pregnant rat corpus luteum cDNA library. (A) The shaded region shows 98% identity to nucleotides 2176−2902 of the lutropin/choriogonadotropin-receptor cDNA cloned by MacFarland et al. [4], with the mismatches shown in lower-case letters. Polyadenylation signals (AAUAAA) are shown in boxes and the AUUUA message-destabilization motifs are underlined. (B) Diagram showing where the 3′ end of the lutropin/choriogonadotropin-receptor cDNA cloned by MacFarland et al. [4] overlaps with the 5′ end of the 3.5-kb cDNA.

(Fig. 4A) were hybridized to *Bam*HI-digested genomic DNA. *Bam*HI was chosen since there are no *Bam*HI recognition sequences in the 3.5-kb cDNA, which should result in a continuous stretch of DNA that could be recognized by all the probes. The results in Fig. 4B show that all three probes recognized the same *Bam*HI-digested 6.5-kb DNA, suggesting

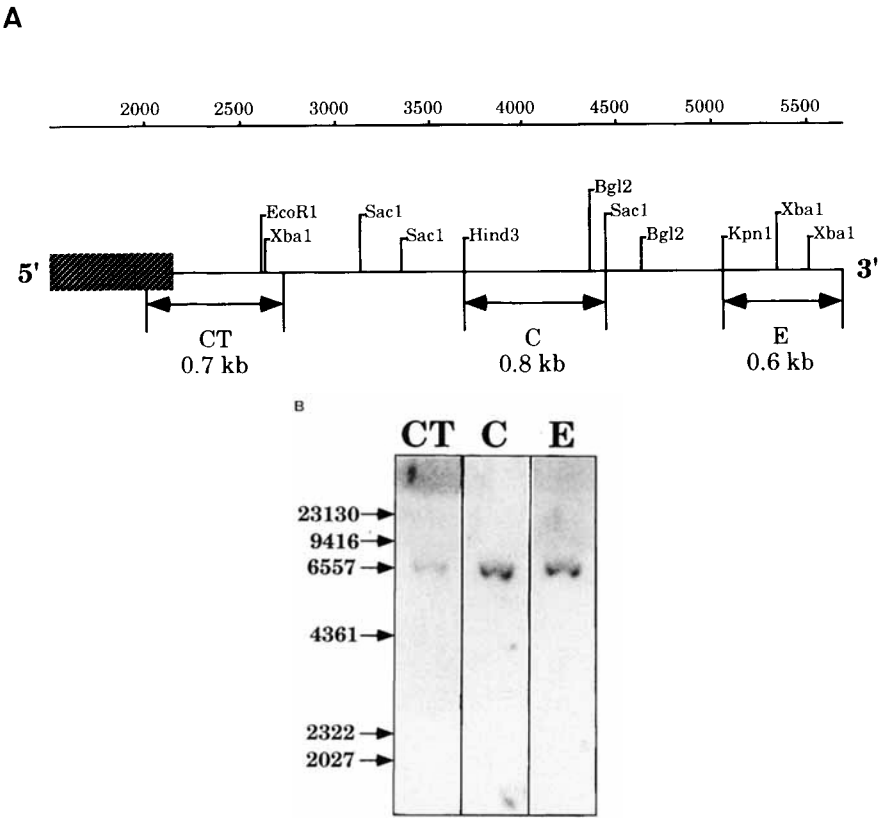

Fig. 4. Hybridization of C-terminal probe and 3.5-kb cDNA probes to rat spleen DNA. (A) Partial restriction map of a putative lutropin/choriogonadotropin-receptor cDNA corresponding to the major transcript in rat ovary showing the C-terminal end (shaded box) of the lutropin/choriogonadotropin-receptor cDNA connected to the 3.5-kb cDNA clone. The location of the fragments used as probes for Southern analysis of day-6 pseudopregnant rat spleen DNA is indicated. (B) Hybridization of C-terminal (CT) cDNA and probes C and E to 10 μg rat spleen DNA digested with *Bam*HI. DNA molecular-mass markers (in bp) are shown on the left.

that the newly identified 3.5-kb cDNA and the exon encoding the C-terminal domain of the lutropin/choriogonadotropin receptor originate from the same gene.

Analysis of lutropin/choriogonadotropin-receptor mRNAs by oligonucleotide-directed cleavage with RNase H

To ascertain whether the 3.5-kb cDNA clone is an extension of the previously cloned lutropin/choriogonadotropin-receptor cDNA clone [4], we utilized RNase H and oligomers from the 3.5-kb cDNA. Since RNase H specifically cleaves RNA in a DNA/RNA hybrid, cleavage of the oligomers annealed to specific sequences of the mRNAs would result in truncated transcripts. The transcripts of interest may be identified by Northern analysis using a specific radiolabeled probe. In Fig. 5A, the location of the oligomers used to hybridize to ovarian RNA prior to RNase H cleavage is indicated. If the oligomers from the 3.5-kb cDNA are an extension of the lutropin/choriogonadotropin-receptor mRNA, then they will direct the truncation of the mRNAs, which can be identified with the N-terminal cDNA probe, as shown in Fig. 5A. The results show that RNase H digestion of the DNA/RNA hybrids resulted in specific cleavages of the lutropin/choriogonadotropin-receptor message to yield fragments of the predicted sizes. Oligomers predicted to be 2.7, 3.0, 3.8, and 4.5 kb away from the 5′ end of the lutropin/choriogonadotropin-receptor message truncated the mRNA at the anticipated sites, as seen in Fig. 5B. Negative controls included oligomers in the sense direction and no oligomer treatment. The RNA treated with sense DNA, which should not hybridize to lutropin/choriogonadotropin-receptor message, and RNA incubated with RNase H and no DNA oligomers did not result in any cleavage of the lutropin/choriogonadotropin-receptor mRNA. It is interesting to note that the N-terminal cDNA probe hybridized to doublets in the samples cleaved with RNase H. It is possible that there are multiple start sites for the lutropin/choriogonadotropin-receptor mRNA, which is consistent with the data of Tsai-Morris et al. [10].

In an attempt to elucidate the identity of the 2.6-kb transcript, experiments were then performed using a C-terminal oligonucleotide (cto) to truncate the lutropin/choriogonadotropin-receptor message at the end of the coding region, as shown in Fig. 6A. Northern blot analysis of lutropin/choriogonadotropin-receptor mRNAs reveals that cDNA probes corresponding to sequences from coding regions predominantly hybridized to the 6.7-, 4.4-, and 2.6-kb transcripts [12, 14, 15], whereas a cDNA probe corresponding only to the 3′ UTR hybridized to the 6.7-kb and 4.4-kb transcripts [16]. Thus, we propose that the 2.6-kb lutropin/choriogonadotropin-receptor transcript might correspond to a message encoding predominantly the open reading frame. As shown in Fig. 6B, the C-terminal oligonucleotide (cto) truncated the lutropin/choriogonadotropin-receptor message to two mRNA transcripts slightly smaller than 2.6 kb. Again, it is possible that the two mRNA transcript sizes may be due to the exis-

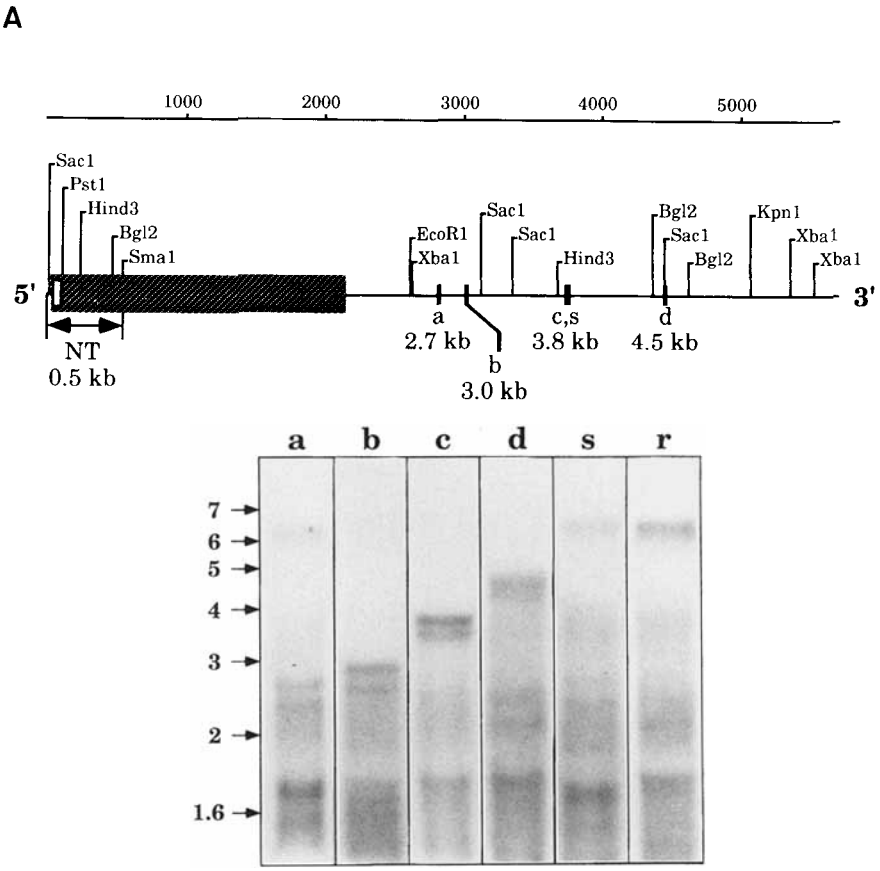

Fig. 5. Oligonucleotide-directed truncations of lutropin/choriogonadotropin-receptor transcript with RNase H. (A) Restriction map of putative lutropin/choriogonadotropin-receptor cDNA (5.7 kb) corresponding to the major transcript in rat ovary. The location of the oligomers used to hybridize to total RNA is indicated as a, b, c, s, and d. Oligomers a, b, c, and d are antisense to RNA and correspond to nucleotides 639–656, 839–855, 1554–1584, 2267–2284, respectively, of the 3.5-kb cDNA. Oligomer s is sense to RNA and corresponds to nucleotides 1578–1597 of the 3.5-kb cDNA. The numbers correspond to the approximate distance of the oligomer from the 5′ end. The location of the N-terminal (NT) cDNA probe is also indicated. (B) Hybridization of N-terminal (NT) cDNA to Northern blot of day-6-pseudopregnant ovarian RNA annealed to oligomers a, b, c, d, and s and digested with RNase H. The RNA-only lane (r) contained no DNA oligomers and was subjected to the same annealing and digestion conditions as the other samples. DNA molecular-size markers (in kb) are shown on the left. Exposure times were varied for optimal visualization.

tence of multiple start sites. In the cto-treated lanes, only the N-terminal and C-terminal cDNA probes hybridized to the transcripts smaller than 2.6 kb, whereas the probes corresponding to the 3′ UTR hybridized only to a mRNA species less than 4.4 kb. Since that same transcript is also recognized by the C-terminal probe (which spans the junction of the cleaved lutropin/choriogonadotropin-receptor mRNA), it is probable that the less than 4.4-kb cleavage fragment corresponds to sequences 3′ of the coding region. In addition, the sum of the two truncated fragments would be approximately equivalent to the 6.7-kb lutropin/choriogonadotropin-receptor transcript. It appears that the 2.6-kb lutropin/choriogonadotropin-receptor mRNA corresponds to a transcript consisting predominantly of the coding region, while the 6.7-kb transcript, which is the major form in rat ovary, contains the coding region plus a large 3′ UTR encoded by the 3.5-kb cDNA clone.

DISCUSSION

We provide strong evidence to support the idea that the 3.5-kb cDNA clone we have isolated encodes a novel 3′ UTR of the lutropin/choriogonadotropin-receptor mRNA. This 3′ UTR appears to be a further extension of the previously identified 4.4-kb lutropin/choriogonadotropin-receptor transcript [4]. Thus, the 4.4-kb transcript with a longer 3′ UTR may represent the major 6.7-kb transcript found in rat ovary. So far, the cDNA corresponding to the 6.7-kb transcript has remained an enigma.

Previous reports [12, 14, 15] have identified the major transcript sizes of lutropin/choriogonadotropin-receptor mRNA as 6.7, 4.4, and 2.6 kb in rat ovary. Upon exposure of the autoradiogram for longer periods, a 1.8-kb transcript was also observed. Smaller lutropin/choriogonadotropin-receptor transcripts have been identified in the pig and rat testis [5, 33] and human and rat ovary [6, 23]. They were postulated to encode truncated forms of the receptor corresponding to the extracellular domain. In every Northern blot presented in this study, all the cDNA probes hybridize to the 1.8-kb fragment after long autoradiographic exposure (data not shown). Since we propose that the 3.5-kb cDNA encodes 3′ UTR of the major 6.7-kb lutropin/choriogonadotropin-receptor transcript, it is puzzling that all the 3.5-kb cDNA probes would hybridize to the 1.8-kb transcript. Three possible explanations may be offered to explain this result. First, alternative splicing may take place to generate the 1.8-kb transcript. Second, there is enough sequence identity between

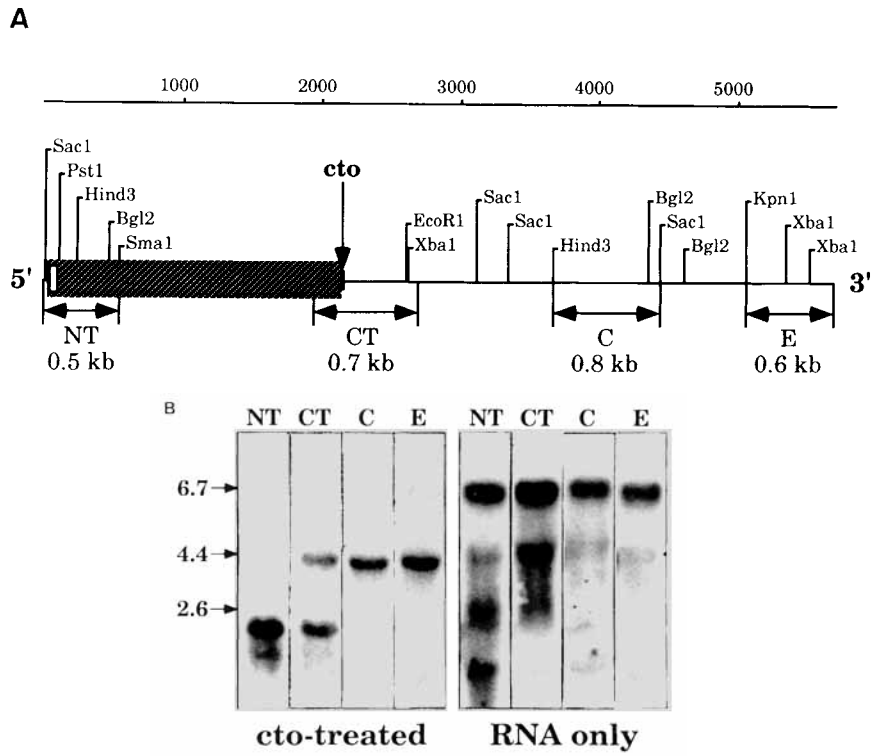

Fig. 6. Oligonucleotide-directed cleavage of lutropin/choriogonadotropin-receptor mRNA after the coding region with RNase H. (A) Restriction map of putative lutropin/choriogonadotropin-receptor cDNA (5.7 kb) corresponding to the major transcript in rat ovary. The location of the C-terminal oligomer (cto) is indicated, as are the N-terminal (NT), C-terminal (CT), C, and E cDNA probes. The C-terminal oligomer (cto) is antisense to nucleotides 2139–2163 of the lutropin/choriogonadotropin-receptor cDNA cloned by MacFarland et al. [4]. (B) Hybridization of cDNA probes to Northern blot of day-6-pseudopregnant ovarian RNA annealed to C-terminal oligomer (cto) and digested with RNase H. The RNA-only lanes contained no DNA oligomers and were subjected to the same annealing and digestion conditions. The major 6.7-, 4.4-, and 2.6-kb transcripts are indicated. Exposure times were varied for optimal visualization.

the 1.8-kb mRNA and the cDNA probes to give a positive hybridization signal. Finally, the 1.8-kb transcript may be a breakdown or degradation product of the larger transcripts.

A related observation was that all cDNA probes derived from the 3.5-kb cDNA were able to hybridize to both the 6.7-kb and 4.4-kb transcripts. If the 4.4-kb transcript was generated by cleavage at an alternative polyadenylation site, it would be expected that a probe corresponding to the 3′ end of the 3.5-kb cDNA (Fig. 6A) would hybridize only to the 6.7-kb transcript. An interesting observation was that the more 3′ probes (Figs 2B and C, and 6B) appear to hybridize with the 4.4-kb transcript with less intensity than the 5′ cDNA probes (Figs 2B and C, and 6B). This suggests that the more 3′ probes share enough sequence identity with the 4.4-kb transcript to result in a positive signal and probably do not represent actual hybridization to that transcript.

The multiple lutropin/choriogonadotropin-receptor mRNAs in rat ovary are regulated in such a manner that during ligand-induced down-regulation, the transcripts decrease coordinately [12, 15, 16]. We have previously shown that the ligand-induced loss of steady-state mRNA is due to increased message degradation and not a result of decreased gene transcription [14]. Thus, it is plausible that the nucleotide sequences regulating message stability are common to all the transcript sizes. Since the 3.5-kb cDNA does not hybridize to the 2.6-kb lutropin/choriogonadotropin-receptor message, it is possible that the primary region regulating mRNA stability is not present in the 3.5-kb cDNA. However, since the 3.5-kb cDNA contains a number of AUUUA destabilizing motifs, it is conceivable that they may play a role in modulating message stability. It has been shown that when the A+U-rich elements of the short lived c-fos, interleukin-2, granulocyte-macrophage colony-stimulating factor, and tissue factor mRNAs are inserted into the 3′ UTR of a normally stable mRNA, they confer destabilization to the chimeric transcript [17, 34–36]. A more recent study of peptidylarginine deiminase [37] has identified a long 3′ UTR that may contribute to rapid mRNA turnover. Thus, the long 3′ UTR we have identified may be involved in the degradation of lutropin/choriogonadotropin-receptor mRNA in response to pharmacological doses of hormone and appears to be consistent with observations in the peptidylarginine deiminase system.

A recent publication by Koo et al. [38] has described the cloning of lutropin/choriogonadotropin-receptor transcripts of varying sizes. However, the 3.5-kb cDNA that we have described is not accounted for in their study. The cloning of an additional 3′ UTR, which is transcribed from a formerly unrecognized extension of exon 11 of the lutropin/choriogonadotropin receptor gene, provides further insight on the generation of multiple transcripts in rat ovary. In addition, the longer 3′ UTR also provides a starting point for the investigation of post-transcriptional regulation of lutropin/choriogonadotropin-receptor mRNAs.

In conclusion, we have presented data supporting the idea that the 6.7-kb transcript, the most abundant lutropin/choriogonadotropin-receptor mRNA in rat ovary, contains a previously unidentified long 3′ UTR. This 3′ UTR may contain specific regulatory elements important for lutropin/choriogonadotropin receptor expression.

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