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Nucleotide sequence of the cDNA encoding the precursor of the β subunit of rat lutropin

[lutropin (luteinizing hormone)/glycoprotein hormones/mRNA/recombinant DNA]

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ABSTRACT **We have determined the nucleotide sequences of cDNAs encoding the precursor of the β subunit of rat lutropin, a polypeptide hormone that regulates gonadal function, including the development of gametes and the production of steroid sex hormones. The cDNAs were prepared from poly(A)$^+$ RNA derived from the pituitary glands of rats 4 weeks after ovariectomy and were cloned in bacterial plasmids. Bacterial colonies containing transfected plasmids were screened by hybridization with a ^{32}P-labeled cDNA encoding the β subunit of human chorionic gonadotropin, a protein that is related in structure to lutropin. Several recombinant plasmids were detected that by nucleotide sequence analyses contained coding sequences for the precursor of the β subunit of lutropin. Complete determination of the nucleotide sequences of these cDNAs, as well as of cDNA reverse-transcribed from pituitary poly(A)$^+$ RNA by using a synthetic pentadecanucleotide as a primer of RNA, provided the entire 141-codon sequence of the precursor of the β subunit of rat lutropin. The precursor consists of a 20 amino acid leader (signal) peptide and an apoprotein of 121 amino acids. The amino acid sequence of the rat lutropin β subunit shows similarity to the β subunits of the ovine/bovine, porcine, and human lutropins (81, 86, and 74% of amino acids identical, respectively). Blot hybridization of pituitary RNAs separated by electrophoresis on agarose gels showed that the mRNA encoding the lutropin β subunit consists of approximately 700 bases. The availability of cDNAs for both the α and β subunits of lutropin will facilitate studies of the regulation of lutropin expression.**

Lutropin (LH; luteinizing hormone) is a glycoprotein hormone synthesized and secreted by gonadotropes of the anterior pituitary gland in mammals and lower vertebrates and is essential for gametogenesis and the production of gonadal steroids. Lutropin is a member of a family of structurally related polypeptide hormones that also includes pituitary thyrotropin and follitropin and placental chorionic gonadotropin (1–3). Each hormone consists of two nonidentical, noncovalently associated subunits, α and β. The primary structures of the α subunits are nearly identical among hormones within a species, whereas the primary structures of the β subunits differ greatly and confer the specific biological activities of the hormones (4). However, the β subunits share enough similarity in their primary structures to suggest that they arose by duplications of a single ancestral gene (4–6). For example, 82 of the 112 amino acids in the sequence of the β subunit of human pituitary lutropin are identical to those comparable residues in the sequence of the β subunit of human chorionic gonadotropin (4). In addition, individual subunits have no known biologic activities; formation of the heterodimer is necessary for activity (4). The regulation of the production of lutropin is under complex control by factors such as the hypothalamic gonadotropin-releasing hormone, which is a potent stimulator of lutropin release, and gonadal steroid hormones, which exert complex negative and positive feedback effects on lutropin release or synthesis.

Recently, it has been shown that the α and β subunits of the glycoprotein hormones are encoded by separate genes (7–11). The α subunit in most species is synthesized initially in the form of a nascent precursor of M_r 14,000 (7–15), whereas the lutropin β subunit is synthesized initially as a nascent precursor of M_r 15,000–17,000 (13–17). These precursors are both processed cotranslationally by the enzymic removal of the NH_2-terminal leader peptides and also by the addition of carbohydrates. The further glycosylation of the subunits, the formation of intramolecular disulfide linkages, and the association of the subunits lead to the production of the bioactive α/β dimer (18–20). Although considerable information is available about the post-translational processes involved in the biosynthesis of lutropin and other glycoprotein hormones, very little is known about the cellular mechanisms involved in the regulation of the expression of the α and β subunit genes.

As an initial step to determine the cellular mechanisms by which the α and β subunit genes of lutropin are expressed, we have cloned recombinant cDNAs encoding both of the subunits. We previously reported the nucleotide sequences of the cDNAs encoding the precursor for the α subunit of the rat and mouse glycoprotein hormones (21, 22). In this communication, we report the complete amino acid sequence of the precursor of the β subunit of rat lutropin (pre-LHβ) derived from the nucleotide sequences of cDNAs encoding the subunit precursor.

MATERIALS AND METHODS

Enzymes. Restriction endonucleases and polynucleotide kinase were obtained from Bethesda Research Laboratories and New England BioLabs. Reverse transcriptase (RNA-dependent DNA nucleotidyltransferase) (avian myeloblastosis virus) was obtained from J. W. Beard (Life Sciences, St. Petersburg, FL).

Identification of Clones Containing cDNAs Encoding Rat Pre-LHβ. cDNAs encoding rat pre-LHβ were selected from cloned recombinant cDNAs prepared from poly(A)$^+$ RNA derived from the anterior pituitary gland of ovariectomized rats (22). Bacterial clones containing recombinant plasmids (pBR322) bearing lutropin-related sequences were selected by colony hybridization (22–23) with a 460-base pair DNA fragment (*Hin*dIII/*Ava* I) of a cDNA encoding the precursor of the β subunit of human chorionic gonadotropin (24). The cDNA of the human

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Abbreviations: pre-LHβ, precursor of lutropin (luteinizing hormone) β subunit; NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate.

chorionic gonadotropin β subunit was labeled by nick-translation with [α-^{32}P]dCTP (New England Nuclear; 3,000 Ci/mol; 1 Ci = 3.7 × 10^{10} Bq) to a specific activity of 3–4 × 10^8 cpm/μg (25). Hybridization was performed at 46°C for 48 hr with the ^{32}P-labeled cDNA of the human chorionic gonadotropin β subunit in the presence of 6× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 10× Denhardt's solution (1× Denhardt's solution = 0.02% Ficoll-400/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone-40), 0.5% sodium dodecyl sulfate, and sonicated denatured salmon sperm DNA (100 μg/ml). The filters were subsequently washed twice at 41°C for 30 min in 250 ml of 5× NaCl/Cit/1× Denhardt's solution/0.5% sodium dodecyl sulfate, rinsed twice at room temperature in 250 ml of 0.03× NaCl/Cit, and dried prior to exposure to Kodak XAR x-ray film; exposure was with an intensifying screen at −80°C for 1–7 hr.

Nucleotide Sequence Analyses. The nucleotide sequences of the cDNA inserts of recombinant plasmids containing coding information for pre-LHβ were determined on both strands by using the strategy depicted in Fig. 1. Specifically, the cDNA inserts contained nucleotides corresponding to the amino acids −4 to +40 (pLHβ-1), +4 to +108 (pLHβ-2), +40 to the poly(A) tract (pLHβ-3), and +96 to the poly(A) tract (pLHβ-4). In composite, these cDNAs represent the entire coding region of the apoprotein of rat lutropin β as well as the entire 3′ untranslated region for its mRNA. The sequence of the 5′ region of the mRNA was determined by chemical analysis of cDNAs synthesized using total RNA from the pituitary glands of ovariectomized rats as template and a synthetic pentadecanucleotide as a primer (27). This approach provided the nucleotide sequence of the 5′ coding region of the mRNA for the β subunit precursor of rat lutropin (Fig. 1). The pentadecanucleotide primer used had the sequence 5′-A-C-A-G-G-C-C-G-G-C-A-C-A-G-T-3′ which is complementary to codons +8 to +12 of the apoprotein of rat lutropin β. The oligonucleotide was synthesized by the phosphotriester approach using protected dinucleotides and polystyrene support (Bachem Fine Chemicals, Torrance, CA) (28). After deprotection of reactive side groups, the pentadecanucleotide was separated from smaller oligonucleotides by high-pressure liquid chromatography using anion-exchange (Partisil 10 SAX; Whatman) and reverse-phase (C_{18}; Waters Associates) columns. The pentadecanucleotide was 5′-end-labeled with [γ-^{32}P]ATP (6,000 Ci/mmol; New England Nuclear) and phage T4 polynucleotide kinase to a specific activity of 5 × 10^6 cpm/pmol. The correct nucleotide sequence was confirmed by chemical analysis (26). In a typical primed synthesis of cDNA, we utilized 20 μg of total pituitary RNA, 50 pmol of 5′-end-labeled pentadecanucleotide, and 100 units of reverse transcriptase in a total volume of 100 μl.

Dot Matrix Computer Analysis. The sequence of the mRNAs encoding the β subunit precursors of rat lutropin and human chorionic gonadotropin were compared by using a dot matrix computer program designed by J. Novotny (29).

Size Estimation of the mRNA Encoding Pre-LHβ. Total RNA from the pituitary glands of ovariectomized rats was prepared by extraction in guanidinium thiocyanate (30), denatured by glyoxal (31), separated by electrophoresis on 1.4% agarose gels, and transferred to a nitrocellulose filter by the method of Thomas (32). The filters were dried and incubated with pLHβ-2, labeled by nick translation with [^{32}P]dCTP to a specific activity of 3–4 × 10^8 cpm/μg (21–22). The filters were washed and exposed to Kodak XAR x-ray film, using an intensifying screen at −80°C for 48 hr.

RESULTS AND DISCUSSION

Identification of cDNAs Encoding the Precursor of the β Subunit of Lutropin. Eight of approximately 1,400 bacterial clones containing recombinant cDNAs were detected with the ^{32}P-labeled cDNA of the β subunit of human chorionic gonadotropin. The nucleotide sequences of the four largest cDNAs were determined in their entirety on both strands by the method of Maxam and Gilbert (26) (Fig. 1). The portion of the coding

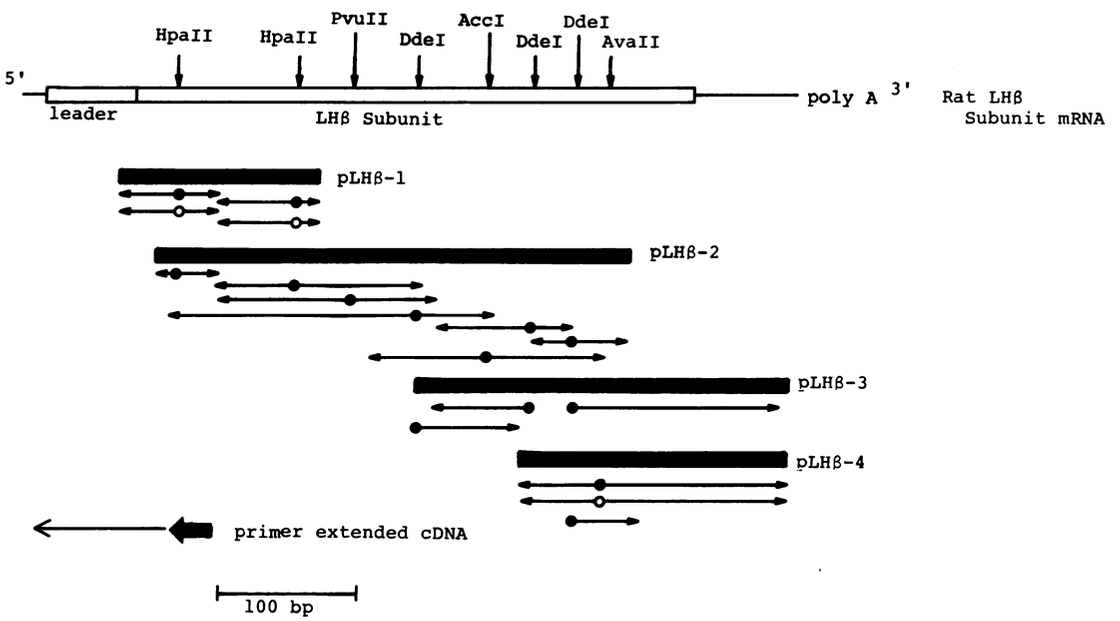

FIG. 1. DNA sequencing strategy for the cDNAs of the precursor of the β subunit of rat lutropin. DNA fragments of the four rat pre-LHβ cDNA (pLHβ-1–4) inserts were produced by the indicated restriction endonucleases, were labeled at either the 5′ (●) or the 3′ (○) end, and were prepared for nucleotide sequence determination by the method of Maxam and Gilbert (26). DNA fragments labeled at a single end were generated by cleavage of end-labeled fragment with a second restriction endonuclease. Arrows indicate regions of cDNAs whose sequences were determined from a given cleavage site. Heavy arrow denotes region of cDNA for which a synthetic pentadecanucleotide was used as a primer to synthesize 5′-directed cDNA (light arrow). bp, Base pairs; leader, signal peptide region of the precursor; and LHβ subunit, apoprotein region of the precursor.

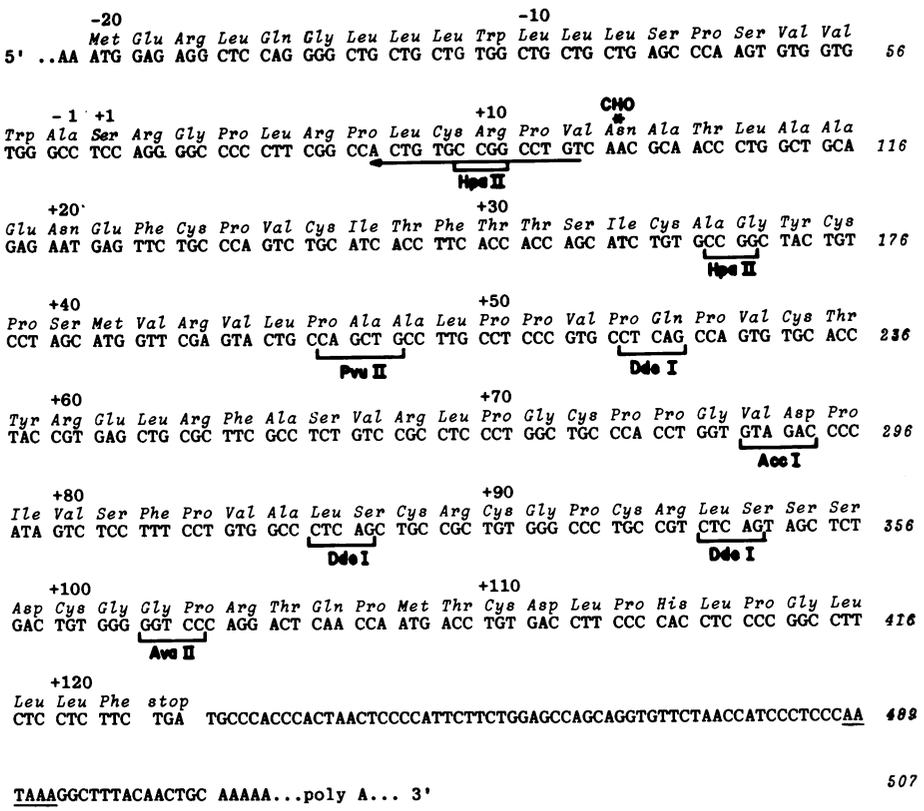

FIG. 2. The composite nucleotide and corresponding amino acid sequences of the sense strands of cDNAs representing the coding and 3'-untranslated regions of pre-LHβ mRNA. The DNA sequence was determined as described in the text. The leader sequence includes amino acid residues -20 to -1, and the apoprotein includes amino acid residues $+1$ to $+121$. The arrow underlining codons $+8$ to $+12$ indicates the region in the sequence complementary to the synthetic pentadecanucleotide used to prime the synthesis of 5'-directed cDNAs. Brackets denote sites of cleavages by restriction endonucleases used in determining the sequences of the cDNAs. CHO* at Asn-13 (codon $+13$) indicates the predicted site of N-glycosylation analogous to that found in other β subunits of glycoprotein hormones (1).

	1									10									20					
rat	Ser	Arg	Gly	Pro	Leu	Arg	Pro	Leu	Cys	Arg	Pro	Val	Asn	Ala	Thr	Leu	Ala	Ala	Glu	Asn	Glu	Phe	Cys	Pro	Val
bovine/ovine	-	-	-	-	-	-	-	-	-	Gln	-	Ile	-	-	-	-	-	-	-	Lys	-	Ala	-	-	-
porcine	-	-	-	-	-	-	-	-	-	-	-	Ile	-	-	-	-	-	-	-	Asp	-	Ala	-	-	-
human	-	-	Glu	-	-	-	-	Trp	-	His	-	Ile	-	-	Ile	-	-	Val	-	Lys	-	Gly	-	-	-
hamster	-	-	-	-	-	-	-	-	-	-	-	Ile	-	-											
	○	○		○	○	○	○		○		○		○	○		○	○		○		○		○	○	○

					30										40									50
rat	Cys	Ile	Thr	Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Ser	Met	Val	Arg	Val	Leu	Pro	Ala	Ala	Leu	Pro
bovine/ovine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Lys	-	-	-	-	Val	Ile	-	-
porcine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Arg	-	-	-	-	-	-	-	-
human	-	-	-	Val	Asn	-	Thr	-	-	-	-	-	-	-	Thr	-		-	-	-	Gln	-	Val	-	-
	○	○	○			○		○	○	○	○	○	○	○		○		○	○	○				○	○

										60									70					
rat	Pro	Val	Pro	Gln	Pro	Val	Cys	Thr	Tyr	Arg	Glu	Leu	Arg	Phe	Ala	Ser	Val	Arg	Leu	Pro	Gly	Cys	Pro	Pro	Gly
bovine/ovine	-	Met	-	-	Arg	-	-	-	-	His	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
porcine	-	-	-	-	-	-	-	-	-	-	-	-	Ile	-	-	-	Ser	-	-	-	-	-	-	-	-
human	-	Leu	-	-		-	-	-	-	-	Asp	Val	-	-	Glu	-	Ile	-	-	-	-	-	-	Arg	-
			○	○		○	○	○	○					○		○		○	○	○	○	○	○		○

					80										90									100
rat	Val	Asp	Pro	Ile	Val	Ser	Phe	Pro	Val	Ala	Leu	Ser	Cys	Arg	Cys	Gly	Pro	Cys	Arg	Leu	Ser	Ser	Ser	Asp	Cys
bovine/ovine	-	-	-	Met	-	-	-	-	-	-	-	-	-	His	-	-	-	-	-	-	-	-	Thr	-	-
porcine	-	-	-	Thr	-	-	-	-	-	-	-	-	-	His	-	-	-	-	-	-	-	-	-	-	-
human	-	-	-	Val	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Arg	-	Thr	-	-	-
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									110				114					119		121	
rat	Gly	Gly	Pro	Arg	Thr	Gln	Pro	Met	Thr	Cys	Asp	Leu	Pro	His	Leu	Pro	Gly	Leu	Leu	Phe	
bovine/ovine	-	Pro	Gly	-	-	Glx	-	Leu	Ala	-	Asx	His	-	Pro	-	-	Asp	Ile	-		
porcine	-	Pro	Gly	-	Ala	Glx	-	Leu	Ala	-	Asx	Arg	-	Pro	-	-	-	-	-		
human	-	-	-	Lys	Asp	His	-	Leu	-	-	-	His	-	Gln							
							○			○			○								

FIG. 3. Primary structures of the lutropin β subunits in mammals. The known amino acid sequences of bovine/ovine (33–35), porcine (35, 36), human (37, 38), and hamster (39) lutropin β subunits are compared with the sequence deduced from the nucleotide sequence of DNA encoding rat lutropin β (Fig. 1). A - indicates identity; a ○ indicates invariant positions. The primary structures of bovine and ovine lutropin β are identical. The hamster lutropin β sequence is a partial NH_2-terminal sequence (39) and contains residues 1 to 14. Rat lutropin β consists of 121 amino acid residues; bovine/ovine, 119; porcine, 119; and human, 112.

sequence of the mRNA not included in the cloned cDNAs was completed by chemical analysis of ^{32}P-labeled cDNA synthesized by using RNA from the pituitary glands of ovariectomized animals as template and a synthetic pentadecanucleotide as a primer. The composite nucleotide sequences of these cDNAs were unambiguous and the amino acid sequence deduced therefrom established that the cDNAs encoded a polypeptide of 141 amino acids (Fig. 2) with a leader (signal) sequence of 20 amino acids and an apoprotein of 121 amino acids. The M_r calculated from the sequence is 15,200. A comparison of the amino acid sequence of the polypeptide deduced from the cDNA sequences with the corresponding amino acid sequences of the lutropin β subunits of other species is shown in Fig. 3. The relatively high level of amino acid identity between this polypeptide and ovine/bovine, porcine, and human lutropin β subunit (81, 86, and 74%, respectively) indicates that it is the precursor of the β subunit of rat lutropin. Further comparisons of the β subunits of lutropins indicate that the NH_2-terminal regions of the apoproteins are more alike than the corresponding COOH-terminal regions. In addition to sequence heterogeneity of the COOH terminus there are also differences in the lengths of the β subunits. This finding is consistent with evidence that the COOH-terminal region of lutropin is not required for biologic action (1). Finally, the level of amino acid identity between rat lutropin and human chorionic gonadotropin β subunits is 60%, if one discounts the COOH-terminal extension of human chorionic gonadotropin β subunit. We have assigned the site of cleavage of the leader sequence to the Ala-Ser at positions -1 and $+1$ by analogy to the sequence of the chorionic gonadotropin β subunit (4) as well as by comparison of the NH_2-terminal serine of the rat lutropin β to the NH_2-termini of the sequences of the known lutropin β subunits (4) (see Fig. 3).

Analyses by filter hybridization of the total mRNA from the pituitary glands of ovariectomized rats, using the ^{32}P-labeled lutropin β cDNA rLHβ-2, reveal that the mRNA encoding the precursor of rat β lutropin consists of about 700 bases (Fig. 4). On the basis of our findings that the coding region and the 3′ untranslated region of the mRNA are 423 and 79 bases, respectively, and assuming that the poly(A) tract of pre-mRNA is 100–150 bases in length, we estimate that the 5′ untranslated region, not determined in its entirety in our studies, is 50–100 bases in length.

Computer Analyses by Dot Matrix of Rat Lutropin β and Human Chorionic Gonadotropin β cDNAs. The determination of the sequence of the coding and 3′ untranslated regions of the rat lutropin β mRNA also allows us to compare the nucleotide sequence with that of the mRNA encoding the β subunit of human chorionic gonadotropin, the only other β subunit mRNA whose sequence has been determined to date.

To characterize further the similarities and differences between the cDNA sequences of the rat lutropin β and the human chorionic gonadotropin β, we used a dot matrix computer program (29), which compares each base of one sequence with all the bases of the other sequence and places a dot where they match and a blank space where they do not. The dot matrix (Fig. 5) is filtered so that a dot appears only when three consecutive bases in the sequences match. Although there are similarities between these two sequences, the longest homolo-

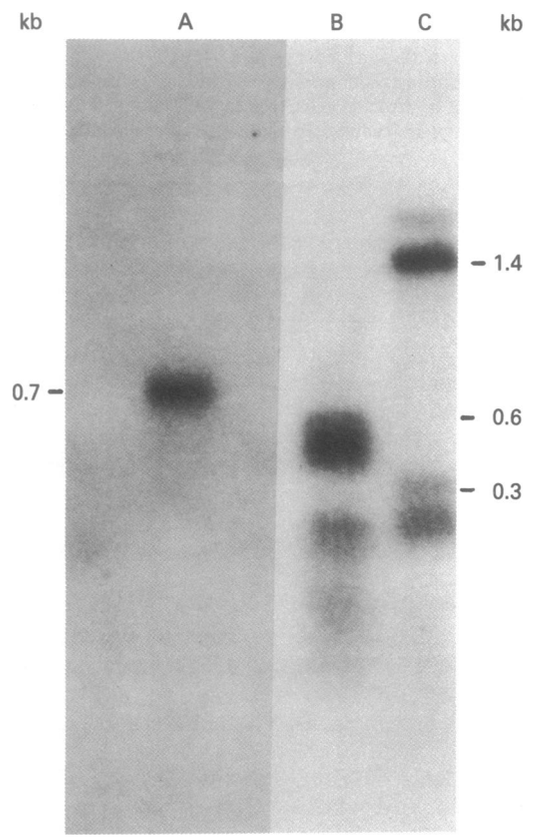

FIG. 4. Determination of the size of mRNA encoding the precursor of the rat lutropin β subunit. Total RNA (5 μg) prepared from the pituitary glands of ovariectomized rats was denatured and subjected to electrophoresis on a 1.4% agarose slab gel, transferred to nitrocellulose filter paper, and hybridized with ^{32}P-labeled rat lutropin β cDNA (pLHβ-2). The autoradiogram of the washed and dried filter paper is shown. Lane A, total RNA of the pituitary gland of the ovariectomized rat; lanes B and C, DNA molecular size markers, *Hpa* II digest of pBR322 and *Ava* II digest of pBR322, respectively. kb, Kilobases.

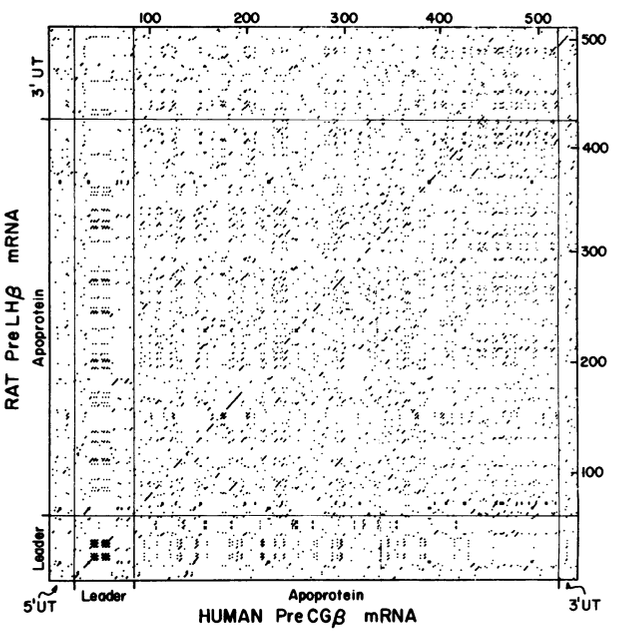

FIG. 5. Dot matrix computer program comparison of the nucleotide sequences representing the mRNAs encoding the precursors of rat lutropin β subunit (pre-LHβ) and human chorionic gonadotropin β subunit (pre-CGβ). Each dot in the matrix denotes bases in the corresponding sequences that begin triplet nucleotide stretches of identity between the two sequences. Regions designated in the dot matrix are apoprotein, apoprotein portion of the β subunit precursor; leader, the NH_2-terminal precursor extension of the apoprotein; and 5′ UT and 3′ UT, 5′ and 3′ untranslated regions of the mRNA, respectively. The apoprotein of human chorionic gonadotropin β subunit is larger than that of the rat lutropin β subunit because of a COOH-terminal extension peptide. The scales indicate the number of bases.

gous sequence consists of 18 bases and corresponds to codons +34 to +38, the conserved sequence Cys-Ala-Gly-Tyr-Cys (CAGYC in the single-letter code), which is present in all the β subunits of the glycoprotein hormones as well as in the serine proteases (4). It is likely that this region of homology in the nucleotide sequences was a major determinant in providing the hybridization signal resulting in the detection of the cloned rat lutropin β cDNAs by using the human chorionic gonadotropin β DNA as a labeled hybridization probe.

Of particular interest has been the finding that the additional 30 amino acids at the COOH terminus of chorionic gonadotropin β (human lutropin β = 112 amino acids, human chorionic gonadotropin β = 145 amino acids) appear to have arisen at some point in evolution by way of a mutation in a codon that signals the end of translation. This mutation results in a read-through translation for an additional 30 codons before termination occurs at a sequence A-A-T-A-A-A that also presumably serves as the signal for addition of poly(A) to the 3′ end of the mRNA (24). A comparison of the nucleotide sequences of the human chorionic gonadotropin and rat lutropin β cDNAs by using the dot matrix program reveals that the similarities in the nucleotide sequences end abruptly at the end of the coding region of the rat lutropin β; the nucleotide sequence of the 3′ untranslated region of the rat lutropin β diverges markedly from that of the corresponding sequence of human chorionic gonadotropin β that codes for the COOH-terminal 30 amino acids of the subunit.

Somewhat surprising was the finding of a much greater conservation between the base sequences encoding the rat and human gonadotropin (73%) than between the corresponding amino acid sequences (60%), indicating the occurrence during evolution of a greater number of base substitutions that result in a change in amino acid assignment (replacement substitution) than base substitutions that retain the amino acid coding assignment (silent substitution). A similar disparity in base and amino acid homologies has been observed in the conservation of the corresponding nucleotide and encoded amino acid sequences of rat and human growth hormone and prolactin genes (40).

The availability of cloned cDNAs encoding both the α and β subunits of rat lutropin will facilitate investigation of the structure and organization of the gonadotropin genes. The cDNAs will also provide hybridization probes for the study of the regulation of the expression of the lutropin subunit genes.

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