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A Novel Progesterone-Induced Messenger RNA in Rabbit and Human Endometria. Cloning and Sequence Analysis of the Complementary DNA†

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ABSTRACT: Complementary DNAs (cDNAs) prepared from messenger RNAs (mRNAs) isolated from endometria of 5 day pregnant rabbits were inserted into the plasmid pBR322. A library of 2400 recombinant plasmid clones was prepared and screened by differential in situ hybridization with cDNAs prepared from mRNAs of rabbits either injected with progesterone or untreated by the hormone. Clones encoding uteroglobin were identified and discarded. Several progesterone-induced and progesterone-repressed clones were identified. One of them corresponded to a relatively frequent mRNA (0.2% of clones in the library) of 2300 nucleotides. The induction of this messenger RNA by progesterone was totally suppressed by the antagonist RU486. This compound displayed a limited agonistic activity when administered alone. A very small increase in mRNA concentration was observed after estradiol administration. The messenger RNA was also found in the liver (where it was constitutively expressed), the ovaries, and the Fallopian tubes of rabbits. A cross-hybridizing messenger RNA was detected in human endometrium during the luteal phase. Sequence analysis showed that the messenger RNA encoded a protein of 370 amino acids with a calculated molecular weight of 40 800. A search in Genbank and National Biomedical Research Foundation data banks showed no identity or marked similarity with previously published DNA or protein sequences.

Physiological and pharmacological studies of progesterone and progestin have, in many cases, used as a model system the important histological changes provoked by these hormones in the rabbit endometrium. These hormonal effects form the basis of the classical tests of Clauberg (Clauberg, 1933) and McGinty (McGinty et al., 1939).

Consequently, it appeared important to try to understand the molecular mechanisms underlying the action of progestins in rabbit endometrium. Initially this was undertaken by analyzing the regulation of uteroglobin synthesis and secretion. Uteroglobin (Beier, 1968) or blastokinin (Krishnan & Daniel, 1967) is the major secretory protein of the progesterone-stimulated endometrium. Its gene has been isolated (Atger et al., 1981; Snead et al., 1981; Suske et al., 1983), and many features of its hormonal regulation have been unraveled

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(Torkkeli et al., 1977; Kopu et al., 1979; Savouret et al., 1980; Loosfelt et al., 1981; Heins & Beato, 1981; Kumar et al., 1982; Bailly et al., 1983).

However, progesterone provokes a wide range of modifications in the endometrium, many of which are necessary for implantation of the blastocyst. Several modifications of secreted or membrane-attached proteins have been described in the rabbit (Dunbar & Daniel, 1979; Kirchner, 1980; Beier, 1982; Saxena & Sahib, 1983; Ricketts et al., 1984; Lampelo et al., 1985) and in the human endometrium (Iacobelli et al., 1981; MacLaughlin et al., 1982; Strinden & Shapiro, 1983; Bell et al., 1986). Moreover, it appeared interesting to study the regulation by progesterone of several different genes in order to assess the generality of the phenomena that were observed. We thus used the "differential hybridization" method to clone a cDNA corresponding to a novel progesterone-regulated mRNA in the rabbit endometrium. Nucleotide sequence studies showed that the protein that was encoded by this cDNA had not been described previously. A homologous mRNA was detected in the human endometrium by cross-hybridization experiments, and preliminary studies indicated that it was also progesterone regulated.

Materials and Methods

Animals. Endometria were isolated from either 5 day pregnant or prepubertal (1 kg) New Zealand female rabbits.

Hormonal Treatments. For a 6-day period, groups of 10–20 prepubertal rabbits received daily injections of 1 mL of sesame oil containing progesterone (0.1, 0.2, or 5 mg), estradiol (5 μg), tamoxifen [2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine] (75 or 500 μg), and RU38486 (17β-hydroxy-11β-[4-(dimethylamino)phenyl]-17α-(1-propynyl)-estra-4,9-dien-3-one) (10 mg). Control animals received the vehicle alone.

Fallopian tubes and ovaries were taken from untreated adult female rabbits.

Human endometrium was obtained from patients undergoing gynecological surgery. Clinical records, endometrial histology, and estradiol and progesterone assays in blood were used to determine the day of the cycle. In some cases endometrial samples were also obtained from patients undergoing abortion. Informed consent was obtained from all patients.

Isolation of cDNAs Corresponding to Progesterone-Regulated Messenger RNAs. RNAs extracted (Cathala et al., 1983) from endometria of rabbits in the fifth day of pregnancy were chromatographed on oligo(dT)–cellulose. Double-stranded cDNAs were prepared from 1 μg of mRNA, digested with S1 nuclease, and inserted into the *Pst*I site of pBR322 by the dC-dG tailing procedure (Atger et al., 1980). A library of 2400 clones was established in the RR1 strain *Escherichia coli*.

Seven hundred colonies were grown on triplicate filters. Two filters were used for differential in situ hybridization (Grunstein & Hogness, 1975). One filter was hybridized with cDNA probes prepared from mRNAs of progesterone-treated rabbits and the other filter with cDNAs prepared from mRNAs of control rabbits injected with the vehicle alone (specific activity of probes was (1–2) × 10^5 cpm/ng; 3 × 10^6 cpm were used per 8.5-cm filter).

The third filter was used to identify uteroglobin cDNA clones: it was hybridized with nick-translated uteroglobin cDNA probe (Atger et al., 1980) (specific activity (1–1.5) × 10^5 cpm/ng).

Progesterone-induced clones, other than those encoding uteroglobin, were identified and submitted to a second differential hybridization. Clone $rEPIP_1$ (rabbit endometrial progesterone induced protein) was selected for further experiments.

Preparation of a Library Containing Clones Corresponding to the Full Length of rEPIP mRNA. Poly(A+) RNAs from 5 day pregnant rabbits were centrifuged on a sucrose gradient. Northern blots with a nick-translated radioactive $rEPIP_1$ DNA allowed fractions containing the highest concentration of rEPIP messenger RNA to be identified.

Single-stranded cDNAs were prepared in the presence of random primers (Maniatis et al., 1982) and 2000 units/mL reverse transcriptase (PH Stehelin, Basel). The second strand was synthesized by the RNase H method (Gubler & Hoffman, 1983). After dG elongation, 600 ng of the cDNAs were chromatographed on a Bio-Gel A50 column (Bio-Rad, Richmond, CA). Four nanograms of the largest fractions were used to prepare a library of 3600 recombinant pBR322 clones in the RR1 strain of *E. coli*. The cDNAs were inserted into the *Sph*I site of dC-tailed pBR322.

DNA Sequence Determination. Clone $rEPIP_1$ was sequenced according to the procedure of Maxam and Gilbert (1980). Restriction fragments were end-labeled by using T4 polynucleotide kinase (Boehringer, Mannheim) and [γ-^{32}P]-ATP (Amersham) after dephosphorylation with calf intestine alkaline phosphatase (Boehringer, Mannheim). Fragments labeled at one end were generated by redigestion with an appropriate restriction enzyme.

Clones $rEPIP_2$ and $rEPIP_3$ were sequenced by the dideoxynucleotide chain termination procedure (Sanger et al., 1977). After initial shotgun cloning in M13, sequencing was completed by oriented cloning of restriction fragments (Messing & Vieira, 1982). Regions rich in G-C were sequenced in the presence of inosine.

In all cases both strands were analyzed and all restriction sites were overlapped.

To search for homologous sequences we screened the National Biomedical Research Foundation Protein Sequence Database (1986) and the National Institutes of Health Genetic Sequence Databank (1986).

Primer Extension. A *Nco*I-*Hin*dIII fragment (nucleotides 88–138) from clone $rEPIP_2$, labeled at its 3′ end by filling in the *Nco*I end (0.04 pmol; length 51 nucleotides), was hybridized with 50 μg of poly(A+) RNAs from pregnant rabbits in buffer containing 40 mM 1,4-piperazinediethanesulfonic acid, pH 6.5, 50% formamide, 400 mM NaCl, and 1 mM ethylenediaminetetraacetic acid (10 min at 80 °C, 18 h at 55 °C) (Casey & Davidson, 1977). Primer extension was performed for 1 h at 42 °C in the presence of actinomycin D (25 μg/mL).

After treatment with RNase T1 (Sigma Chemical Co., St. Louis, MO), for 15 min at 37 °C, and ethanol precipitation, the radioactive DNA was analyzed on a 6% polyacrylamide–urea gel.

Results

Cloning of cDNAs Corresponding to Progesterone-Regulated Messengers. Seven hundred cDNA clones were analyzed by differential hybridization (Figure 1). Twenty-one (3%) cross-hybridized with the cloned uteroglobin probe and were discarded. Of the remaining clones, five showed a markedly stronger hybridization with DNAs complementary to mRNAs from progesterone-treated rabbits than from control rabbits. A clear-cut difference, although less striking, was observed for nine other clones. One of the first five clones (clone $rEPIP_1$) was chosen for further experiments.

In addition, eight clones were detected that corresponded to mRNAs repressed by progesterone: two clones displayed

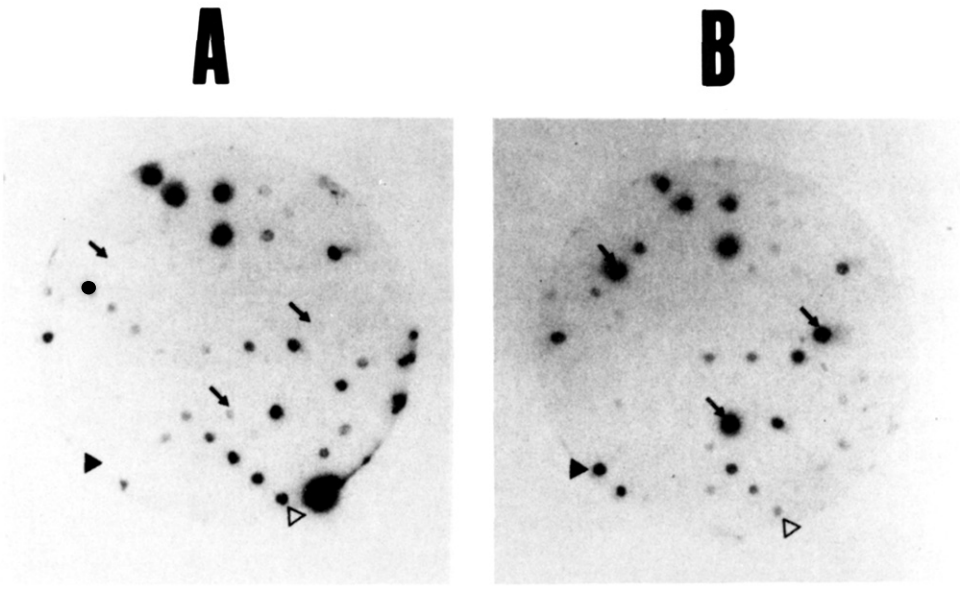

FIGURE 1: Detection of plasmids containing DNAs complementary to progesterone-regulated mRNAs. Bacterial colonies containing recombinant plasmids were grown as replicas on three filters. They were screened in situ with cDNAs prepared from mRNAs of either progesterone-treated rabbits (B) or nontreated rabbits (A), and with a uteroglobin probe (not shown). Symbols: →, clones corresponding to progesterone-induced mRNAs but shown to encode uteroglobin; ▶, clone corresponding to a progesterone-induced mRNA (rEPIP mRNA) shown to be different from uteroglobin mRNA; ▷, clone corresponding to a mRNA whose concentration is decreased by progesterone.

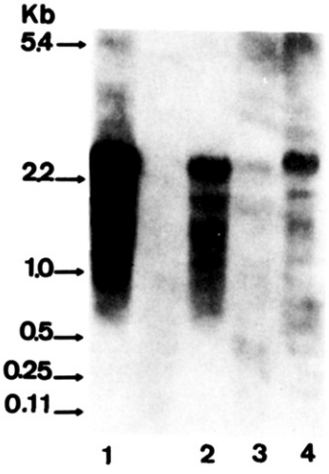

FIGURE 2: Presence of rEPIP messenger RNA in various organs of the rabbit. Total RNA (40 μg) was analyzed by Northern blot. The probe was nick-translated $rEPIP_1$ plasmid. RNAs were prepared from the following tissues of prepubertal (lanes 1 and 2) and mature (lanes 3 and 4) rabbits injected for 6 days with 5 mg of progesterone/day: lane 1, endometrium; lane 2, liver; lane 3, ovary; lane 4, Fallopian tube. The film was overexposed to show the band in lane 3. Arrows indicate the molecular weight of marker nucleic acids (Kb = 10^3 nucleotides).

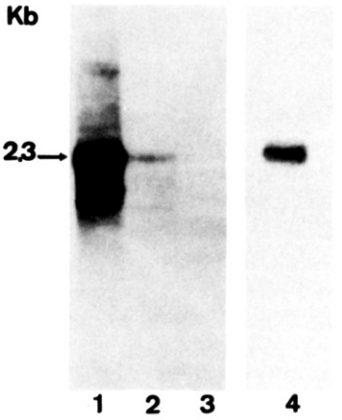

FIGURE 3: Presence in human endometrium of a messenger RNA cross-hybridizing with rEPIP probe. Northern blots were performed with endometrial poly(A+) RNAs (2 μg) from lane 1; 5 day pregnant rabbits; lane 2, women on day 21 of the menstrual cycle; lane 3, woman on day 10 of the menstrual cycle. Lane 4, Northern blot performed with total RNAs (40 μg) from the endometrium of a woman on day 21 of the menstrual cycle. Lanes 1, 2, and 3 correspond to the same experiment. Lane 4 corresponds to a separate experiment. Samples on lanes 2 and 4 were prepared from different patients. The probe consisted of three fragments of the coding region of $rEPIP_2$ (*Hin*dIII–*Pst*I, nucleotides 134–420; *Pst*I–*Hin*dIII, nucleotides 699–1029; and *Hin*dIII–*Hin*dIII, nucleotides 1029–1379) subcloned into M13 vector (specific activity 1.5×10^6 cpm/ng). The hybridization was performed at the same stringency as in the case of rabbit RNAs. Washings were performed up to a stringency of 0.5 standard saline citrate (SSC) at 50 °C (for experiments using rabbit RNAs alone, final washing was in 0.1 SSC at 50 °C).

a strong difference in signal intensity between the two probes, while six clones exhibited less pronounced differences.

Characteristics of the Messenger RNA Corresponding to Clone $rEPIP_1$. Frequency of Messenger RNA in Endometrium of 5 Day Pregnant Rabbits. A *Hpa*II–*Sac*I fragment from the insert of clone $rEPIP_1$ was used as a probe to screen the cDNA library and found to hybridize to 0.2% of the colonies. The frequency of the messenger is thus approximately 15-fold lower than that of uteroglobin mRNA.

Tissue Distribution. Northern blotting (Thomas, 1980) detected a major mRNA species of ~2300 nucleotides in rabbit uterus. The same messenger was found in the Fallopian tube and in the ovary but at a lower concentration than in the endometrium (Figure 2). This mRNA was also present in the liver but was constitutively expressed since no difference in messenger concentration was observed between control and progesterone-treated rabbits (not shown).

Presence of rEPIP Messenger RNA in Human Endometrium. RNAs were extracted from human endometria at various phases of the menstrual cycle and between weeks 6 and 10 of pregnancy. Northern blots were performed with clone $rEPIP_1$ as a probe (Figure 3). An ~2300-nucleotide mRNA hybridizing with the probe was found in three of four biopsies taken at the luteal phase (days 20–21 of the menstrual cycle), in zero of two biopsies taken at follicular phase (days 8 and 10 of the menstrual cycle), and, surprisingly in neither of two pools of biopsies (each pool consisted of 10 biopsies)

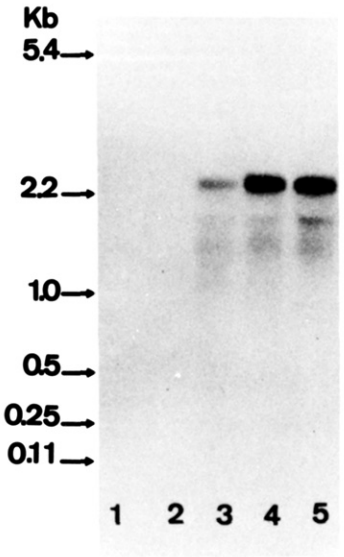

FIGURE 4: Effect of varying the dose of progesterone on the induction of rEPIP messenger RNA. Total endometrial RNA (40 μg) was studied by Northern blot analysis using nick-translated plasmid $rEPIP_1$ as a probe. Animals were treated with hormone for 6 days. Lane 1, control animals; lane 2, animals treated with 0.1 mg of progesterone/day; lane 3, animals treated with 0.2 mg of progesterone/day; lane 4, animals treated with 5 mg of progesterone/day; lane 5, 5 day pregnant animals.

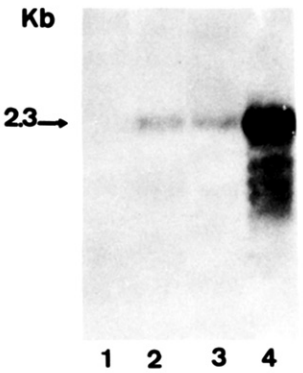

FIGURE 5: Time course of induction by progesterone or rEPIP messenger RNA. After a single administration of 5 mg of progesterone, rabbit endometria were excised at various times and total RNAs were extracted. Northern blot hybridization was performed with a probe made from the *Hin*dIII–*Hin*dIII (nucleotides 1029–1379) fragment of $rEPIP_2$ subcloned into M13 vector (specific activity 1.5 $\times 10^6$ cpm/ng). Lane 1, control animals not treated by hormone; lanes 2, 3, and 4, animals having received progesterone 2, 6 and 20 h, respectively, before sacrifice.

taken during pregnancy (data not shown).

Hormonal Induction of rEPIP Messenger RNA. A dose-dependent effect of progesterone administration was observed when rabbits were injected with varying amounts of hormone [0.1–5 mg/(day·kg of weight)] and mRNA concentration was studied by Northern blots (Figure 4). The maximal effect observed was obtained with 5 mg of progesterone/day, the messenger concentration being then equivalent to that observed in 5 day pregnant rabbits.

The time course of hormonal induction was studied by injecting the animals with progesterone (5 mg) and excising the uteri 2, 6, and 20 h after hormone administration. Northern blot analysis indicated that the mRNA concentration had already increased 2–5-fold at 2 h and had reached a 35-fold increase after 20 h (Figure 5). Progesterone assays showed that maximal blood concentrations were reached after 1 h, and thus there was no apparent time lag.

Effect of the Anti-Progestin RU38486. RU38486 inhibited

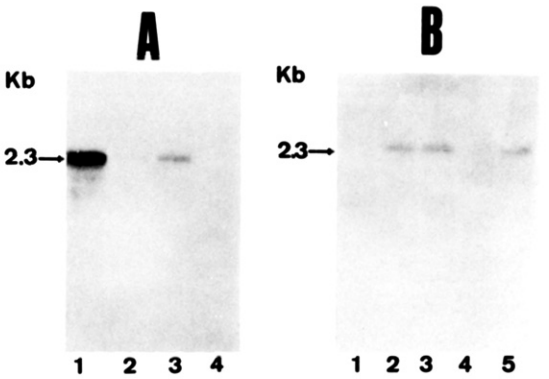

FIGURE 6: Effects of the anti-progestin RU38486 (A), estradiol, and the anti-estrogen tamoxifen (B) on the concentration of rEPIP messenger RNA. Total endometrial RNA (50 μg in A and 40 μg in B) was analyzed by Northern blot. The probes were in A, a fragment *Hin*dIII–*Hin*dIII, (nucleotides 1029–1379) of clone $rEPIP_2$ subcloned into M13 vector; in B, the nick-translated plasmid $rEPIP_1$. Rabbits were treated with hormone for 6 days. (a) Lane 1, rabbits treated with 0.1 mg of progesterone/day; lane 2, rabbits treated with 0.1 mg of progesterone and 10 mg of RU38486/day; lane 3, rabbits treated with 10 mg of RU38486/day; lane 4, control rabbits, not treated by hormone. (B) Lane 1, control rabbits, not treated with hormone; lane 2, rabbits treated with 5 μg of estradiol/day; lane 3, rabbits treated with 5 μg of estradiol and 75 μg of tamoxifen/day; lane 4, rabbits treated with 5 μg of estradiol and 500 μg of tamoxifen/day; lane 5, rabbits treated with 500 μg of tamoxifen/day.

the induction by progesterone of rEPIP messenger RNA (Figure 6A). At the same dosage it exerted a weak agonistic activity when administered alone.

Effect of Estradiol and of the Anti-Estrogen Tamoxifen. Administration of estradiol elicited a small increase of rEPIP messenger RNA concentration (Figure 6B). Tamoxifen (at high dosage), inhibited this effect and, when administered alone, had a small agonistic activity.

Sequence of rEPIP Messenger RNA and of the Deduced Protein (Figure 8). The three overlapping clones that were used and the strategy of sequencing are shown in Figure 7.

To demonstrate that the cDNA insert in $rEPIP_3$ contained the complete 5′ end of the mRNA, a primer extension experiment was performed with a 51-bp fragment extending from nucleotide 88 to nucleotide 138 (see Materials and Methods). An elongation of 90 nucleotides was found, thereby confirming that a full-length cDNA had been obtained.

The first ATG found in the open reading frame was considered as the translation initiation codon (Figure 8). This interpretation was strengthened by the fact that the nucleotides which encompassed the ATG closely matched the Kozak consensus sequence (Kozak, 1984). The open reading frame was 1110 nucleotides. The 5′ noncoding region was 88 nucleotides long, and it contained a high proportion of G-C nucleotides. It has been shown for several mRNAs that the 5′ noncoding region may form a hairpin structure that is complementary by its loop to the 3′ end of 18S ribosomal RNA (Hagenbuchle et al., 1978). Such a structure may be found at the 5′ end of rEPIP messenger RNA (Figure 9).

The 3′ noncoding region is 959 nucleotides long. It contains a modified polyadenylation signal (ATTAAA) 21 nucleotides upstream from the poly(A) tail. This modified signal has been described in other mRNAs (Unterman et al., 1981). It may perhaps explain the use of other polyadenylation sites as suggested by the existence of minor mRNA species of lower molecular weight. In the 3′ noncoding region a short open reading frame (nucleotides 1848–2130) is present.

From the DNA sequence analysis, it was deduced that the protein comprises 370 amino acids and has a calculated mo-

FIGURE 7: Sequencing strategy of the cDNA clones. Three clones (rEPIP$_1$, rEPIP$_2$, rEPIP$_3$) were used. Horizontal arrows show the direction and extent of sequence determination. $(A)_n$ = poly(A) tail. $(C)_n$ and $(G)_n$ = homopolymers added to the cDNA. Main restriction sites are shown. The coding region is indicated by a thick line.

lecular weight of 40 800. The hydropathy plot (Figure 10), established according to Hopp and Woods (1981), is indicative of a hydrophobic protein.

There are two strikingly hydrophobic regions (A and B in Figure 10) for which the predicted secondary structures (Chou & Fasman, 1974) are of β pleated sheets surrounded by β turns.

There is not hydrophobic region at the N terminus that would correspond to the signal peptide of a secreted protein. The C-terminal region is highly hydrophilic with a predicted α-helical structure.

The protein contains seven cysteines, which suggests the possibility of at most three intramolecular disulfide bridges.

Potential glycosylation sites Asn-X-Thr/Ser (Hubbard & Ivatt, 1981) are found at positions 54–56, 154–156, and 297–299, and a potential phosphorylation site for cAMP-dependent kinase on serine is present at positions 268–271 (Lys-Lys-Leu-Ser) (Krebs & Beavo, 1979). Tyrosines with basic or acidic amino acids on their NH_2 side, which are possible substrates for phosphorylation, are observed at positions 32, 101, and 230 (Patschinsky et al., 1982).

Comparison of rEPIP sequence with the nucleotide and amino acid sequences present in the Genbank and National Biomedical Research Foundation libraries revealed no homology with previously described nucleic acids or proteins.

Discussion

The differential hybridization method enabled us to detect in the rabbit endometrium several mRNAs, different from uteroglobin mRNA, that were induced by progesterone. Furthermore, it allowed us to identify a cDNA clone corresponding to the messenger that displayed the largest increase in concentration after administration of the hormone.

These experiments also showed the existence of mRNAs repressed by the administration of progesterone. Such a situation has been extensively studied in *Xenopus laevis* liver, where estradiol stimulates the transcription of the vitellogenin gene and inhibits the transcription of the albumin gene (Wolffe et al., 1985).

The time course of induction, in the first 20 h, by progesterone, of rEPIP messenger RNA is very similar to that of uteroglobin RNA, and this suggests that the induction may be a primary effect of the hormone. However, the concentration of rEPIP mRNA is about 15-fold lower than that of uteroglobin mRNA. As in the case of uteroglobin, the anti-progesterone RU38486 is an antagonist (Rauch et al., 1985), but unlike in the case of uteroglobin it exerts a small agonistic activity when administered alone. Since it has been shown that RU38486 promotes receptor activation (Rauch et al., 1985) and even binding to putative regulatory sites on the uteroglobin gene (Bailly et al., 1986), an explanation for its activity will have to be found at other steps of hormone action, perhaps in the reactions following interaction of the receptor with the gene. Isolation of the corresponding genomic clones will allow the regulatory regions of the rEPIP gene to be studied and compared with those of the uteroglobin gene in order to define better the general features of the DNA sequences recognized by the progesterone receptor. Estradiol exerts a small stimulatory effect on the accumulation of rEPIP messenger RNA; however, this estrogenic stimulation is markedly weaker than that observed in the case of uteroglobin mRNA (Loosfelt et al., 1981). Tamoxifen is an antagonist with some agonistic activity.

Cross-hybridization with human endometrial RNAs shows the presence of a messenger having the same size as rEPIP mRNA. Preliminary results indicate that rEPIP mRNA is also progesterone regulated in human endometrium. If these results are confirmed in a larger group of patients, this mRNA could be an interesting marker of progestative activity in humans. Apparently, rEPIP messenger RNA is not detected in 8–12-week pregnancies. This observation is similar to the disappearance of uteroglobin after day 12 of pregnancy in the rabbit (Beier, 1982).

The identity and subcellular localization of rEPIP are unknown. Its amino acid sequence, derived from the analysis of the cDNA, suggests that it is not a secretory protein since it lacks a signal peptide. rEPIP may be a membrane or a transmembrane protein as it contains several hydrophobic domains and hydrophilic regions similar to those described in transmembrane proteins (stretches of 15 amino acids in β sheet or 21 amino acids in α helix (Nicholson et al., 1981; Eisenberg, 1984; Schneider et al., 1984; Russel et al., 1984). Recently, ^{125}I labeling of membrane proteins in the rabbit endometrium, at day 5 of pregnancy, has shown the presence of a specific 38-kilodalton protein (Ricketts et al., 1984).

Alternatively, rEPIP could be an intracytoplasmic protein with several internal hydrophobic domains. Many progesterone-induced endometrial enzymatic activities have been described: proteases involved in implantation (Denker, 1982) and steroid enzymes involved in estrogen metabolism (Tseng

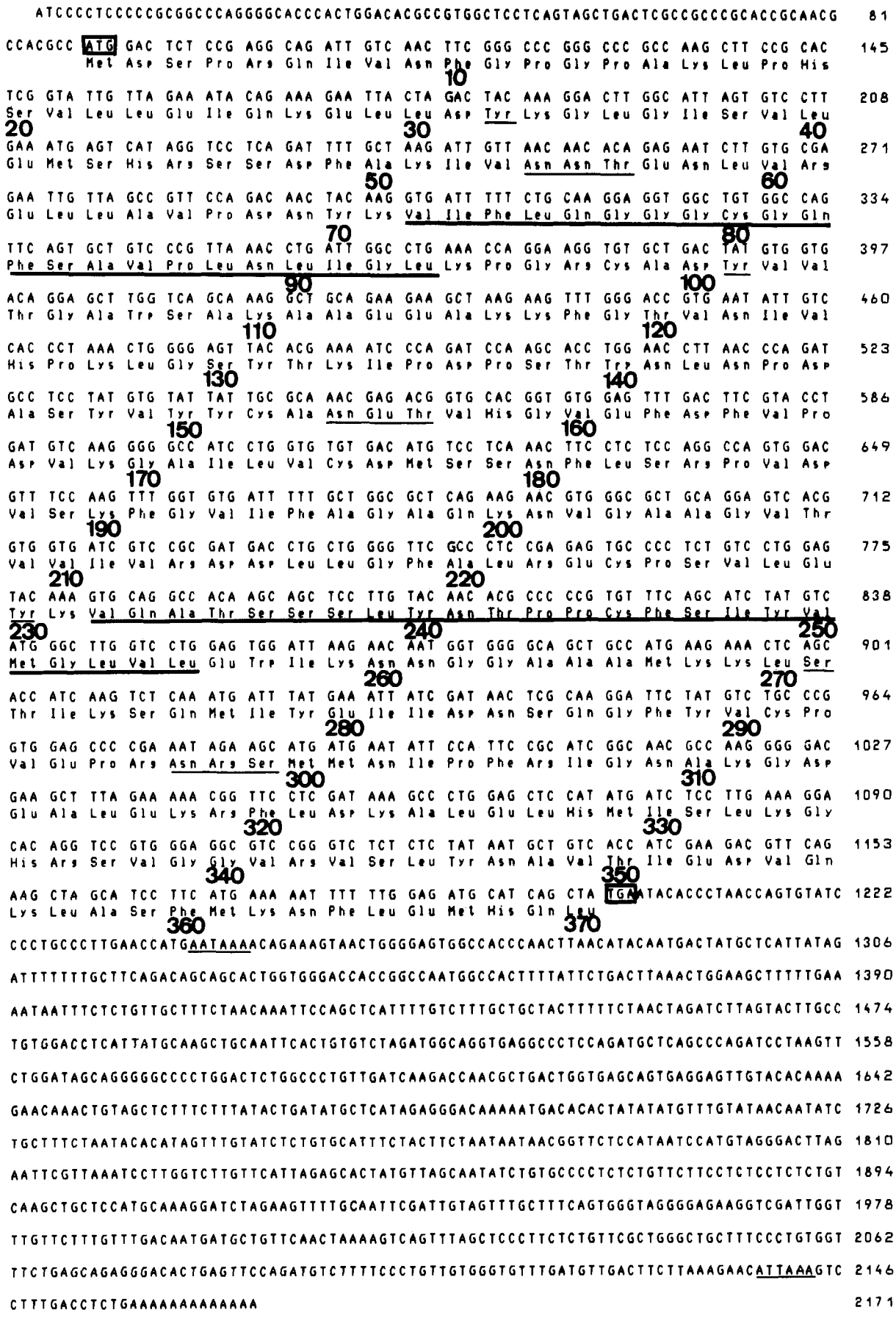

FIGURE 8: Nucleotide sequence of rEPIP messenger RNA and deduced protein sequence. The putative initiation codon (ATG) and the stop signal (TGA) are boxed. The hydrophobic domains A and B (see Figure 7) are underlined with a thick line; potential sites for glycosylation and phosphorylation are underlined with a thin line. The ATTAAA signal corresponding to the major polyadenylation site and another AATAAAA site are underlined.

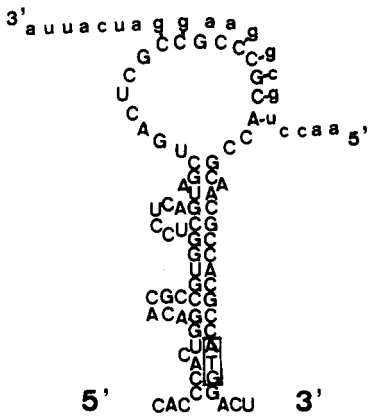

FIGURE 9: Possible secondary structure of the 5′ end of rEPIP messenger RNA. The structure shown is predicted to be the most stable. The first nucleotide corresponds to position +25 in the sequence (Figure 8). The putative initiator ATG is boxed. The 3′ end of 18S ribosomal RNA is shown in small letters (Hagenbuchle et al., 1978).

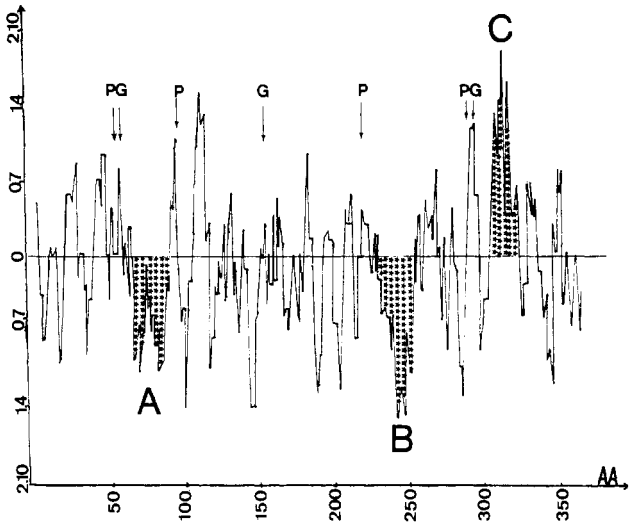

FIGURE 10: Hydropathy plot according to Hopp and Woods (1981) of the primary structure of rEPIP. Hydrophilicity (above the 0) or hydrophobicity of different amino acids (AA) is shown. A window of eight successive amino acids was employed. Regions A and B are hydrophobic regions; C is the most hydrophilic region. Arrows indicate possible phosphorylation (P) and glycosylation (G) sites.

& Mazella, 1980; Holinka & Gurpide, 1981). In human endometrium several progesterone-induced proteins have been reported: a 24-kilodalton (-kDa) protein observed on the superficial epithelium during the secretory phase by the immunohistochemical method (Ciocca et al., 1983); the PEP protein composed of two subunits of 27 kDa which decline rapidly after week 18 of pregnancy (Sutcliffe et al., 1982; Joshi, 1983; Julkunen et al., 1986); PP12 with a molecular weight estimated between 25 000 and 51 000 (Wahlstrom & Seppala, 1984; Rutanen et al., 1986) identified in the glandular epithelium of the secretory-phase endometrium as well as in the stromal cells of the decidua. PAPPA is a tetrameric protein of 620 kDa that can be detected in the glandular epithelium of the endometrium only 4 days after ovulation (Bischof et al., 1984; Sjoberg et al., 1984). Prolactin is also a progesterone-induced protein in human endometrium (Maslar & Riddick, 1979), and its cDNA has been cloned from this source (Takahashi et al., 1984).

rEPIP appears to be a potentially interesting molecular marker for progesterone action in the rabbit and perhaps in the human. Further studies will be necessary to discover its cellular and subcellular localizations and its function.

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