Pseudopregnancy induces the expression of hepatocyte nuclear factor-1 β and its target gene aminopeptidase N in rabbit endometrium via the epithelial promoter

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The rabbit endometrium is an excellent model system allowing experimental manipulation of aminopeptidase N (APN) mRNA expression *in vivo*. By RNase mapping and sequencing of cloned PCR-amplified primer-extended RNA, it was demonstrated that endometrial APN expression is directed by the epithelial APN promoter and is increased in human-choriogonadotropin-induced pseudopregnancy. Cloning and sequencing of the rabbit APN epithelial promoter revealed conservation of the upstream footprint (UF), hepatocyte nuclear factor-1 (HNF1) and Sp1 elements known to be present in the pig and human promoters

as well. The pseudopregnancy-induced APN expression was found to be accompanied by a parallel increase in the level of the transcription factor HNF1 β , whereas a much smaller increase in Sp1 and UF-binding proteins was observed. This indicates that HNF1 β acts as a switch triggering the pregnancy-induced APN expression. The sequence of the UF element suggests members of the nuclear hormone-receptor superfamily as possible UF-binding proteins, and competition experiments suggest that the chicken ovalbumin upstream promoter transcription factor functions as such in the rabbit endometrium.

INTRODUCTION

Aminopeptidase N (APN) is a well-characterized digestive enzyme present in large quantities in the microvillar membrane of the small-intestinal epithelial cell [1–3]. In addition, APN activity is found in other epithelial, epithelial-derived and haematopoietic [4] cell types. The single human APN gene is controlled by two promoters separated by approx. 8 kb. The epithelial promoter close to the coding part of the gene drives transcription in cells of epithelial origin [5], whereas the upstream myeloid promoter is active in haematopoietic cells [6]. The APN epithelial promoter contains a TATA box and binding sites for Sp1 (nucleotides –53 to –30) and hepatocyte nuclear factor-1 (HNF1) (nucleotides –85 to –58). The upstream footprint (UF) region (nucleotides –112 to –90) interacts with a heterogeneous population of unidentified nuclear proteins. The presence of the HNF1 site is critical for the function of the APN promoter, and its presence restricts the promoter activity to epithelial cells expressing one or more members of the HNF1 transcription-factor family [5,7]. This protein family consists of HNF1 α , HNF1 β (for reviews see [8–11]) and various alternatively spliced forms which differ in their transcriptional activation domains [12].

In the rabbit endometrium, APN expression is influenced by sex hormones. During pregnancy [or human chorionadotropin (hCG)-induced pseudopregnancy] the endometrium undergoes oestrogen-dependent, and especially progesterone-dependent, morphological and biochemical changes [13–16]. During this process, APN enzymic activity appears in the apical membrane of the endometrial cells, being maximal at day 5 *post coitum* [13,14]. It is the purpose of the present work to identify which APN promoter is involved in endometrial APN expression and

to determine the promoter elements and corresponding transcription factors that are involved in the regulation of this process.

MATERIALS AND METHODS

Materials

[α -³²P]dATP, [γ -³²P]ATP, Sequenase (version 2.0), and Hybond N+ filters were purchased from Amersham Life Sciences; restriction enzymes and T7 RNA polymerase were from Promega Biotech; and *Taq* DNA polymerase was from Boehringer Mannheim. The 5' AmpliFINDER RACE Kit [including avian myeloblastosis virus (AMV) reverse transcriptase and T4 RNA ligase] was from Clontech Laboratories. Oligo(dT)-coated magnetic beads (Dyna Beads) were from Dynal. Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer model 392.

Rabbit endometrium

Sexually mature nulliparous New Zealand White rabbits weighing 3.5–4.5 kg were used. The rabbits were housed in individual cages and provided with food and water *ad libitum*. Pseudopregnancy was induced by injecting 75 i.u. of hCG intravenously. Does were killed either 5 days after hCG injection or in the non-pregnant state, by cervical dislocation. The uterus was quickly removed, and the endometrium scraped out and immediately frozen in liquid N₂.

RNA extraction, Northern blotting and RNase mapping

RNA was extracted from frozen rabbit tissues by the guanidinium thiocyanate/acid/phenol procedure [17]. Poly(A)⁺ RNA was

Abbreviations used: AMV, avian myeloblastosis virus; APF1, apolipoprotein factor-1; APN, aminopeptidase N; ApoCIII, apolipoprotein CIII; COUP-TF, chicken ovalbumin upstream promoter transcription factor; EMSA, electrophoretic-mobility-shift assay; ERE, oestrogen-responsive element; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hCG, human chorionadotropin; HNF, hepatocyte nuclear factor; PRE, progesterone-responsive element; RAR, retinoic acid receptor; RARE, retinoic acid response element; RXR, retinoic X receptor; UF, upstream footprint.

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The nucleotide sequence shown in Figure 1 has been submitted to the EMBL Nucleotide Sequence Database with the accession number X89921.

purified from 75 µg of total RNA by the use of Dyna Beads according to the manufacturer's recommendations, electrophoresed through formaldehyde-containing 1% agarose gel [18] and blotted to a Hybond N+ membrane. The same membrane was hybridized consecutively with selected DNA probes labelled with [α - ^{32} P]dATP by using random priming [19]. The hybridization was carried out in 50% formamide/5×SSPE [SSPE = 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA]/5×Denhardt's solution/0.2% SDS containing 100 µg/ml denatured salmon sperm DNA at 42 °C. The probes used were a 1.4 kb 5' *Eco*RI fragment from rabbit APN cDNA [20], a 1.2 kb *Pst*I glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragment [21], and a 774 bp *Sma*I HNF1 α cDNA fragment (nucleotides 118–891 [22]). The most stringent washes were with 0.1×SSPE/0.1% SDS at 65 °C (APN) or with 1×SSPE/0.1% SDS at 50 °C (HNF1 and GAPDH).

For RNase mapping of rabbit APN-coding RNA, a ^{32} P-labelled RNA probe was generated by transcription of the cloned rabbit APN promoter using T7 RNA polymerase according to the manufacturer's recommendations. The part of the probe which is complementary to rabbit APN mRNA begins at NPRIM-5 (see below). Hybridization, RNase treatment and electrophoresis were performed as described previously [5].

Cloning and sequencing of full-length rabbit APN cDNA

Based on the partial rabbit APN cDNA sequence [20], three primers (see Figure 3) were synthesized:

NPRIM-1 (5' GGTAGGGAGTGTATAATGG 3') is complementary to nucleotides 455–474;
 NPRIM-2 (5' CAGTTCCAAGGCGAGCTGGCCG 3') corresponds to nucleotides 3–24;
 NPRIM-3 (5' GCTCTAGAGCCTTCCATGTACTCGCTGCGG 3') is, following the *Xba*I site (underlined), complementary to nucleotides 44–65.

First-strand cDNA was synthesized using AMV reverse transcriptase at 50 °C with NPRIM-1 as primer and endometrial poly(A)⁺ RNA extracted from 5-days-pseudopregnant rabbits as template. For oligodeoxyribonucleotide ligation-mediated rapid amplification of 5' cDNA ends ('RACE') [23], the 5' AmpliFINDER protocol (Clontech Laboratories) was followed. The 5'-phosphorylated and 3'-amine-modified anchor was ligated to the first-strand cDNA using T4 RNA ligase. Amplification of the anchor-ligated first-strand cDNA was performed using 30 pmol of anchor primer and 30 pmol of NPRIM-3 in a standard PCR reaction [18]. The PCR products were digested with *Eco*RI and *Xba*I, size-selected by agarose-gel electrophoresis and cloned into pBluescript. APN-specific clones were identified by hybridization using NPRIM-2 as probe. The clones containing the longest inserts were sequenced, and one clone (3-1-20) had a 5' end corresponding to position +1 in the pig aminopeptidase N gene [24]. The sequence of clone 3-1-20 is, for a few ambiguities, identical with the corresponding part from the recently published full-length sequence of rabbit kidney APN cDNA [25].

Sequence determination of the 5' ends of primer-extended APN mRNAs

Primer extension was carried out with AMV reverse transcriptase using NPRIM-3 and endometrial poly(A)⁺ RNA extracted from 5-days-pseudopregnant rabbits. Amplification of the primer-extended RNA was carried out by the 5' AmpliFINDER protocol as described above. NPRIM-4 and NPRIM-5 were used as 3'

primers in separate PCR reactions together with the anchor primer as 5' primer. *Eco*RI/*Xba*I-digested gel-purified PCR fragments from the NPRIM-5 PCR reaction were cloned into pBluescript SK+ and the 5' end of 13 clones determined by sequencing using the M13 primer. The sequences of the primers are:

NPRIM-4 (5' GCTCTAGACGCCAAGTCGTCGGCCAGC-TCG 3') is, following the *Xba*I site (underlined), complementary to nucleotides 14–35 of the rabbit APN cDNA [20];

NPRIM-5 (5' GGTCTAGAAGGAGGTGGCAGTGGG-GTT 3') is, following the *Xba*I site (underlined), complementary to nucleotides 192–211 of clone 3-1-20;

NPRIM-6 (5' AGGGCTTCTACATTCCAAGTCGCTGGG 3') corresponds to nucleotides 51–78 of clone 3-1-20. This primer starts at the fifth base following the initiator methionine codon.

Cloning of the rabbit APN promoter

To clone the rabbit APN promoter, DNA was extracted from frozen rabbit spleen [18] and 1 µg was used as template in a PCR reaction with 30 pmol of NPRIM-7 (5' GCGTCGACTGCA-GCCTGTAACCAGACCCTG 3'); this sequence is, following the underlined *Sa*I site, complementary to nucleotides –214 to –193 of the pig APN gene [24]) and 30 pmol of NPRIM-5. Southern blotting using the clone 3-1-20 as probe showed a hybridizing band of approx. 400 bp. This fragment was cloned in the *Sa*I and *Xba*I sites of pBluescript SK+ and sequenced. The sequence appearing in Figure 1 was completed on both strands.

Preparation of nuclear extracts

Frozen rabbit endometrium was thawed on ice, and nuclei were prepared essentially as described [26]. Nuclei from rabbit kidney were prepared directly from freshly removed kidney cortex. Nuclear protein extracts were prepared from purified nuclei as described previously [27]. The 'non-pregnant' extract was prepared from 4.7 g of endometrium obtained from 11 rabbits, and the '5-days-pseudopregnant' extract from 7.9 g of endometrium obtained from three pseudopregnant rabbits. The preparation of the nuclear extract from the enterocyte-like colon carcinoma cell line Caco-2 [28] has been described previously [27].

Electrophoretic-mobility-shift assay (EMSA)

EMSA was carried out as described [5]. The probes used were from the pig APN promoter. The UF and Sp1 elements were the same as used previously [5]. The HNF1 probe was APN HNF1:

5' TCGATCTGAGCCCTGGTTAATTTTGGCCAGTCTGCA 3'
 3' AGACTCGGGACCAATTAAAAACGGGTCAGACGTAGCT 5'

corresponding to nucleotides –88 to –56 of the pig APN gene [5].

The COUP-TF (chicken ovalbumin upstream promoter transcription factor)/HNF4 probe was apolipoprotein factor-1 (APF1):

5' TCGACCCAGGGCGCTGGGCAAGGTCACCTCG 3'
 3' GGGTCCCGGACCCGTTTCCAGTGGACGAGCT 5'

and corresponds to the HNF4/COUP-TF site in the apolipoprotein CIII (ApoCIII) promoter (APF1 [29]).

The retinoic acid response element (RARE) probe was β RARE:

5' TCGACGGGTAGGGTTTACCGAAAGTTCACTCGC 3'
3' GCCCATCCCAAGTGGCTTTTACGTGAGCGAGCT 5'

and corresponds to the RARE in the retinoic acid receptor β 2 (RAR β 2) promoter [30]. Annealed oligonucleotides were end-labelled with T4 polynucleotide kinase using [γ - ^{32} P]ATP and then purified by PAGE. As unspecific competitor, either 800 ng of poly(dI-dC) (HNF1) or 1500 ng of poly(dI-dC) + 100 ng of sonicated salmon sperm DNA (UF, Sp1, APF1, β RARE) was included in each reaction. When antibodies were included, the reaction was incubated for 7 min with the indicated antibody at 37 °C before the addition of the labelled probe, followed by a 25 min incubation at 37 °C. The antisera to HNF1 α and HNF1 β [31] were diluted 10-fold, and the antisera to RAR and retinoic acid X receptor (RXR) 20-fold, in Tris-buffered saline + 0.1 % BSA.

RESULTS

APN promoter utilization in the rabbit endometrium

A 378 bp fragment of the rabbit APN gene surrounding the transcription initiation site was amplified by PCR and sequenced (Figure 1). The sequence has a 74 % sequence identity with the pig APN promoter. The TATA box as well as the UF, HNF1 and Sp1 sites are very well conserved (Figure 1). The EMSA

5' G C C C C C A T C A G G C G C C C C T G A G C C

ACATTCCCAACACCGAGCCTGGACCAG

UF

CCCAGTTCAGTGACCTT

CCCCTGCCC GGTGCAGTGACCTTCGCC

HNF1

GAGCCCTGGTTAATTTTGGCC

TGTCTGAGCCTTGGTTAATTTTGGCC

Sp1

GGGGCTCCTCCCT

AGTTGAAAGGCACGGGGCTCCTCCCT

T C A G G TATA box

TGGGGATATAAGCCTGGCCCCCGGGCT

INT

GCTCCGGTCCCTGCCTGGCCTGAGCTG

CCCCAGCTCCCTCCCTGCCTCGCCATG

PSE PSE

GCCAAGGG

Figure 1 Sequence of the rabbit APN promoter

The rabbit APN promoter was cloned by use of PCR and sequenced. The indicated sequence was derived from three independent PCR clones. The locations of UF, HNF1 and Sp1 sequences are shown and compared with the similar sequences of the pig APN promoter. The arrows represent the transcriptional initiation sites as determined by RNase mapping of endometrial (PSE) and small-intestinal (INT) RNA (Figure 2). The 5' end of amplified cDNA (Figure 3) is indicated by vertically arranged dots, where the number of dots represents the number of clones with a cDNA insert having the particular 5' end.

Figure 2 RNase mapping of rabbit APN mRNA

RNA extracted from small intestine, spleen and endometrium of 5-days-pseudopregnant rabbits (5 days P.) was used to protect the RNA probe from the coding part of the APN gene (position +181 in the rabbit APN cDNA sequence published by Yang et al. [25]) into the promoter region. The indicated major protected fragments correspond to initiation 8 bp, 18 bp and 58 bp upstream of the initiator methionine codon. A longer exposure (72 h) of the last three lanes of the autoradiograph is shown to the right, and the positions of known DNA fragments used as size markers (M) are also indicated.

analyses presented below were therefore carried out with the oligonucleotides spanning the elements in the pig APN promoter [5].

RNase mapping of RNA extracted from endometrium of 5-days-pseudopregnant rabbits (Figure 2) with a RNA probe spanning the presumed transcription initiation site resulted in two major protected fragments, of approx. 175 nt and 185 nt. This indicates that there are two major transcription initiation sites, 8 bp and 18 bp upstream of the initiator methionine codon (Figure 1). A longer exposure of the autoradiograph (Figure 2) reveals the presence of additional protected fragments with a size distribution between 185 nt and 250 nt, indicating the presence of multiple minor initiation sites. RNase mapping of rabbit small-intestinal RNA (Figure 2) generated a single major protected fragment of approx. 225 nt, indicating the presence of a single transcription initiation site 58 bp upstream of the initiator methionine codon. No protected fragments were observed when using rabbit spleen RNA.

The RNase mapping results were complemented by sequencing of cloned PCR-amplified primer-extended RNA, as depicted in Figure 3. The amplification with the 5' anchor primer and two different 3' primers (NPRIM-4 and NPRIM-5), followed by Southern blotting using the NPRIM-6 primer, resulted in two hybridizing smears which differ in their mean size, corresponding to the different locations of the 3' primer (Figure 3). The 5' ends of the cloned amplified primer-extension products are shown in Figure 1 and confirm the presence of several initiation sites. These results show that the cloned rabbit epithelial promoter is the active promoter in rabbit endometrium.

Figure 3 Amplification of APN 5' cDNA ends from pseudopregnant-rabbit endometrial mRNA

First-strand cDNA was synthesized using NPRIM-3 as primer. Following ligation of the 5' anchor primer, PCR was performed using either NPRIM-4 or NPRIM-5 as 3' primers. The resulting products were separated on a 1.5% NuSieve agarose gel and blotted to a Hybond N+ nylon filter. APN coding fragments were identified by hybridization with the NPRIM-6 primer, which is located 5 bp downstream of the initiator methionine codon. The sequences from the 5' ends of 13 hybridizing clones were determined and used to identify the transcriptional initiation sites shown in Figure 1. The locations of the primers are indicated relative to the first 1000 bp of the APN mRNA.

Northern-blot analysis of uterine RNA from non-pregnant or 5-days-pseudopregnant rabbits shows that the increase in APN activity in 5-days-pseudopregnant rabbits is due to an increase in APN-coding mRNA (Figure 4). By using a probe for rat HNF1 α , an increase in HNF1-coding mRNA is also demonstrated (Figure 4). The hybridization conditions used do not allow a distinction between HNF1 α and HNF1 β , due to their high degree of sequence identity, and similar results (not shown) were obtained by using a probe for HNF1 β .

Pseudopregnancy induces HNF1 β in the rabbit endometrium

By EMSA, the pseudopregnancy-induced changes in the expression of HNF1 and Sp1 were analysed. As seen in Figure 5, the amount of HNF1 is markedly increased following pseudopregnancy. A less pronounced increase in the amount of Sp1 is

Figure 4 Northern-blot analysis of endometrial RNA

Poly(A)⁺ RNA was purified from 75 μ g of total RNA extracted from the endometrium of non-pregnant (Non-p.) or 5-days-pseudopregnant (5 days p.) rabbits, separated by agarose-gel electrophoresis, blotted to a Hybond N+ nylon filter and hybridized consecutively using probes for rabbit APN (APN) and rat HNF1 α (HNF1). The sizes of the hybridizing mRNAs were approx. 3.5 kbp (APN) and 3.0 kbp (HNF1). Hybridization with a rat GAPDH probe was also carried out to estimate the variation in the amounts of mRNA applied to the gel.

Figure 5 Pseudopregnancy stimulates HNF1 expression in the rabbit endometrium

Nuclear extracts were prepared from the endometrium of non-pregnant (Non-p.) or 5-days-pseudopregnant (5 days p.) rabbits. Samples (3 μ g of protein) were used in EMSA with the pig APN HNF1 or Sp1 sites. Reactions without nuclear extract (—) are shown for comparison.

also observed. Both the HNF1- and Sp1-protein/DNA complexes represent specific interactions, as they are competed out by using excess unlabelled specific oligonucleotides, but not by using oligonucleotides with different sequences (results not shown).

The identity of the HNF1-binding proteins in endometrium was determined by using polyclonal antisera raised against mouse HNF1 α and HNF1 β (Figure 6). The mobility of the HNF1-protein/DNA complex observed with 5-days-pseudo-

Figure 6 HNF1 β is the major HNF1-binding protein in rabbit endometrium

EMSA was performed using Caco-2 nuclear extract, 5-days-pseudopregnant rabbit endometrial nuclear extract (5 days p.) or rabbit kidney nuclear extract with the pig APN HNF1 probe. Antisera raised against bacterially produced mouse HNF1 α and HNF1 β [31] were included as indicated. The uterine extract generates a faster-migrating protein/DNA complex as compared with the Caco-2 and kidney nuclear extracts, and most of the endometrial HNF1-binding activity is supershifted by addition of the anti-HNF1 β sera.

Figure 7 UF-binding activity in rabbit endometrium

Nuclear extracts were prepared from the endometrium of non-pregnant (Non-p.) or 5-days-pseudopregnant (5 days p.) rabbits and used in EMSA with either the UF probe or the β RARE probe. Where indicated, monoclonal antibodies against mouse RAR or RXR were included.

pregnant-rabbit nuclear extract is not affected by the addition of anti-HNF1 α serum, whereas the mobility of practically all HNF1-binding activity is markedly decreased by the addition of the anti-HNF1 β serum, demonstrating the presence of HNF1 β homodimers. Nuclear extracts from Caco-2 cells and rabbit kidney cortex were similarly analysed for comparison. The mobility of the 5-days-pseudopregnant-rabbit HNF1-protein/DNA complex is higher than the mobility of the HNF1-protein/DNA complexes observed with the Caco-2 and kidney nuclear extracts, suggesting that no or very little HNF1 β homodimers are present in the latter extracts. In agreement with this, most of the protein/DNA complexes obtained with kidney

Figure 8 COUP-TFs are possible candidates as UF-binding proteins

Nuclear extracts prepared from the endometrium of 5-days-pseudopregnant rabbits were used in EMSA with the pig UF probe or the COUP-TF/HNF4 (APF1) probe from the apoCIII promoter. No (—) or increasing amounts (12-, 36- and 108-fold molar excess respectively) of unlabelled UF or APF1 competitor was included as indicated. Unlabelled Sp1 oligonucleotide (108-fold molar excess) was used as non-specific competitor.

and Caco-2 nuclear extracts are supershifted by addition of the anti-HNF1 α serum. However, it can also be concluded that the kidney nuclear extract contains more HNF1 β than the Caco-2 nuclear extract, as more material is supershifted on addition of the anti-HNF1 β serum.

Nuclear proteins in the endometrium bind to the UF element

EMSA with a probe spanning the pig UF element (Figure 7) results in closely migrating bands with the same mobility for both non-pregnant and 5-days-pseudopregnant-rabbit nuclear extracts. A weak increase in the amount of UF-binding proteins is observed following the change from the non-pregnant to the pregnant state. The sequence of the UF element suggests that it may bind members of the steroid-hormone nuclear receptor family (see the Discussion section). However, the mobility of the UF-protein/DNA complex was not altered by the addition of monoclonal antibodies raised against the mouse RAR- α or the RXR. EMSAs using the retinoic acid response element (β RARE) from the RAR β 2 promoter [30] show the presence of two protein/DNA complexes. The addition of the anti-RAR antibody has no effect on the mobility of either of the complexes, whereas the anti-RXR antibody induces a new slower-migrating complex in parallel with a concomitant decrease in the intensity of the faster-migrating protein/DNA complex. This demonstrates the presence of RXR, but not RAR, in rabbit endometrium.

To investigate whether the UF-binding proteins might be related to the proteins binding to the UF-related sequence in the ApoCIII promoter [5], the UF-protein/DNA complexes were compared with those obtained by using a double-stranded oligonucleotide spanning this element. As seen in Figure 8, the complexes migrate with the same mobility and the unlabelled ApoCIII probe (APF1) competes as efficiently as the unlabelled UF probe for the UF-binding protein, suggesting that the same or closely related proteins from rabbit endometrium bind to the two sites.

DISCUSSION

The APN epithelial promoter is active in the endometrium

In the rabbit endometrium, two major transcription initiation

sites are used. Additional minor initiation sites are also present; however, the RNase mapping (Figure 2) shows that these additional initiation sites only constitute a small fraction of the total APN mRNA pool. In the small-intestinal epithelium only a single, different, initiation site is found. The TATA box and initiator elements (Inr) together determine the transcription initiation sites of RNA polymerase II promoters (for reviews see [32,33]). Thus the observed difference in transcription initiation from the APN promoter between the endometrium and the small intestine might be due to tissue-specific differences in Inr binding proteins.

An upstream myeloid promoter is present in both the human and pig APN genes, and exon sequences transcribed only from the myeloid promoter contribute to the mature mRNA in tissues where this promoter is active [6]. Therefore an alternative interpretation of the RNase mapping result would be that the position(s) defined by the mapping in the rabbit promoter could represent an intron/exon junction, as myeloid exon sequences cannot protect the RNA probe. However, since no exon sequences from a putative upstream promoter were detected following PCR amplification of primer-extended APN mRNA, this possibility can be ruled out. None of the PCR clones has 5' sequences corresponding to the positions defined by RNase mapping. However, this is probably due to the inherent difficulty in measuring the exact size of the protected RNA fragments. It is also important to note that, as no attempts were made to secure a linear amplification of the primer-extended cDNA, the relative distribution of the initiation sites defined by the RACE procedure does not represent the relative abundance of the mRNA molecules serving as templates in the reverse-transcription procedure.

HNF1 β expression is increased in pseudopregnancy

The most dramatic change in the binding of nuclear proteins to the APN promoter during pseudopregnancy was clearly the increase in HNF1 β . The trans-activation potential differs between the different forms of HNF1 α and HNF1 β [12]. HNF1 β has been reported as an inefficient activator through HNF1 sites of the sucrase/isomaltase promoter (SIF2 and SIF3; [34]) and the β -fibrinogen promoter [35]. In the latter case, however, efficient activation through the same HNF1 site has been observed in another cell type [12]. Furthermore, HNF1 β efficiently activates through the HNF1 elements of the albumin promoter [36] and the C-reactive-protein promoter [37]. We therefore find it highly probable that the observed increase in the amounts of endometrial HNF1 β in the 5-days-pregnant stage results in a stimulation of APN promoter activity.

In 8-days-pseudopregnant rabbits, which express no APN enzymic activity [13] or mRNA, we have observed the presence of HNF1 β at a level comparable with that in the 5-days-pseudopregnant rabbits (results not shown), suggesting that the endometrial APN expression is also influenced by other positively and negatively acting factors. The finding of HNF1 β , but not HNF1 α , expression in the endometrium is in agreement with similar findings in 6.5- and 7.5-days-pregnant mice [31]; the up-regulation of the uterine HNF1 β expression by pregnancy has not, however, been reported previously.

UF-binding proteins: possible members of the nuclear hormone receptor superfamily

The sequence of the UF element

5' AGTTCAGTGACCT 3'

3' TCAAGTCACTGGA 5'

can be described as an imperfect palindrome of the half-site

sequence 5' AGGTCA 3' with a 1 bp spacing between the two half-sites. This sequence is the half-site consensus sequence in binding sites for the nuclear hormone receptor superfamily [38,39], which includes receptors for steroid hormones, retinoic acids, vitamin D, thyroid hormone, as well as some orphan receptors (receptors with no known ligands). Within this protein family, good candidates for binding to the palindromic UF sequence are the RXRs and the COUP-TFs. RXR is known to form heterodimers with a multitude of other nuclear hormone receptors [40]. The COUP-TFs show promiscuous DNA binding, with the ability to bind efficiently to various direct repeats and palindromes [41]. Despite the presence of RXR in rabbit endometrium, this protein is not a part of the UF-protein/DNA complex (Figure 7). The EMSA and competition experiment with the HNF4/COUP-TF element from the ApoCIII promoter (APF1) presented in Figure 8 strongly suggests that the same proteins from the rabbit endometrium bind to the UF and APF1 oligonucleotides. The APF1 sequence is a strong binding site both for COUP-TFs [42] and for HNF4 [29]. However, since HNF4 has a direct repeat as consensus binding sequence [29], the result suggests the binding of COUP-TFs to the UF element rather than HNF4. Whether or not COUP-TFs bind to the UF element, no effect on transcription from the APN promoter by the UF-binding proteins has been observed in either HepG2 or Caco-2 cells [5,7], and the importance of the UF binding still needs to be defined.

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