Molecular cloning and hormonal regulation of a murine epididymal retinoic acid-binding protein messenger ribonucleic acid

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MAMMALIAN spermatozoa undergo a process of maturation in the epididymis and capacitation in the female genital tract before they are able to fertilize eggs (1). In the epididymis, spermatozoa are exposed to a microenvironment created by the absorptive and secretory activities of the epididymal cells. Epididymal secretory proteins include glycosyltransferases, glycosidases, transport proteins, as well as sperm maturation antigens (2, 3). It is believed that interaction between epididymal secretory proteins and the sperm plasma membrane is involved in the sperm maturation process that is required for normal male fertility.

Protein secretion by the male sex accessory organs is mainly under androgenic control (3, for review) but unidentified testicular factors (4), estrogens (5), and retinoic acid (6) have also been implicated. An interesting feature of epididymal secretory proteins is that, although the epididymis is composed of one primary cell type throughout its length, their secretion is that, although the epididymis is composed of one primary cell type throughout its length, their secretion is highly regionalized (3, for review).

We previously described two peptides (major and minor forms) resulting from a differential cleavage of a unique precursor that we named MEP 10 (mouse epididymal protein 10) (2). This protein is synthesized by principal cells of the distal caput epididymidis and is secreted into the lumen. MEP 10 is not tightly bound to spermatozoa because it can be easily dissociated under low-salt conditions (2). N-terminal microsequencing revealed a high homology (86%) with two rat epididymal secretory proteins (7) that were later identified as epididymal retinoic acid-binding proteins (8, 9). MEP 10 binds active retinoids (cis and all-trans retinoic acid) but not retinol (10). The gene encoding the rat epididymal retinoic acid-binding proteins has been characterized and shown to belong to the lipocalin superfamily (11). These rat epididymal secretory proteins were previously named proteins B/C (7, 12), epididymal binding proteins (EBP I and 2) (8), ESP I (11), and E-RABP (13, 14). To conform to the international nomenclature, we propose the generic term “E-RABP” (epididymal retinoic acid-binding protein) to name these proteins. MEP 10 will be referred to as mE-RABP (murine epididymal retinoic acid-binding protein).

In this report, we describe the molecular cloning of a complementary DNA (cDNA) encoding the mature form of mE-RABP. We show that mE-RABP is a new member of the lipocalin superfamily and the mouse orthologue protein of the rat epididymal retinoic acid-binding protein (ESP I). In addition, we demonstrate that the tissue-specific expression of mE-RABP is highly regionalized (3, for review).
and the hormonal regulation of the mE-RABP gene differs in the rat and the mouse.

**Materials and Methods**

**Animals**

Organs were obtained from adult mice of the Swiss strain (Hsd:ICR(CD-l), Harlan Sprague-Dawley). Castration or efferent duct ligation was performed by the abdominal route under light ether anesthesia. When required, hormone replacement began 5 days after castration with daily sc injections of testosterone propionate (TP) (2 mg/g) or dihydrotestosterone (DHT) (2 mg/g), previously dissolved in sesame oil. Treatments were conducted for 4 days and mice were killed 1 day after the last injection. Organs were excised, immediately frozen in liquid nitrogen, and stored at -80 C.

**Isolation of the cDNA encoding the mature form of mE-RABP**

Total RNA was extracted from the epididymis as described previously (15). For RT, an aliquot (2 μg) was incubated in a mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM MgCl₂, 0.5 mM each dGTP, dATP, dCTP, dTTP, 2 U/μl RNAsin (Promega, Madison, WI), 10 μg/ml oligonucleotide RACEIII (5’-CAGCTGCCAGGTACCAGTCTCGAGAAGC(T)₁₇-3’), and 200 U Moloney murine leukemia virus reverse transcriptase (MoMLV) for 1 h at 42 C. The DNA/RNA hybrids were denatured for 5 min at 90 C and stored at -80 C. Then, a PCR reaction was performed using 100 ng of single strand cDNA incubated in a mixture containing 0.2 mM each dGTP, dATP, dCTP, dTTP, 1 mM each primers (FwE-RABP: 5’-GCGAATTCACCATGGCNGTNGTNAARGAYTTYGAY-3’ and RiIII: 5’-GCAGGTACCGGATCCTCGAGAAG-3’), 13 reaction buffer II (Perkin Elmer, Foster City, CA), and 1 U AmpliTaq (Perkin Elmer). DNA was amplified for 25 cycles, each consisting of 1 min at 94 C, 2 min at 55 C, and 2 min at 72 C. Amplified fragments were purified on a 2% (wt/vol) agarose gel, digested with EcoRI and BamHI restriction enzymes (Promega), and ligated into pGEM7Zf(-) plasmid (Promega). DNA sequencing was performed as described in the thermo sequenase fluorescent labeled primer cycle sequencing kit (Perkin Elmer).

**In vitro transcription/translation assays**

Sense or antisense RNA were transcribed from the E7 clone using the T7 or the SP6 RNA polymerase, respectively. All transcription and translation reactions were performed according to the manufacturer’s instructions (Promega). Briefly, 5 μg of linearized E7 plasmid were incubated with 10 mM dithiothreitol (DTT), 5 μg RNasin RNase inhib-
in lipo]propanesulfonic acid (MOPS) (pH 7), 5 mM sodium acetate, 1 mM EDTA, 6% (vol/vol) formaldehyde, and then transferred to a Hybond N+ nylon membrane (Amersham) by blotting overnight in 20 ¥ SSC. The membrane was washed once in 2 ¥ SSC, dried, and baked 2 h at 80°C. The prehybridization was carried out for 3 h at 42°C in 50% (vol/vol) formamide, 6 ¥ SSC, 5% (vol/vol) Denhardt’s, 100 µg/ml salmon sperm DNA, 0.1% (wt/vol) SDS, and then random primed [32P]-labeled mE-RABP cDNA, prepared with “Random prime” kit (Amersham), was added and incubated overnight. The filter was washed once in 2 ¥ SSC for 15 min, once in 2 ¥ SSC, 0.1% (wt/vol) SDS for 15 min, once in 2 ¥ SSC, 0.1% (wt/vol) SDS for 30 min, once in 0.2 ¥ SSC, 0.1% (wt/vol) SDS for 15 min, and once in 0.1 ¥ SSC, 0.1% (wt/vol) SDS for 15 min at 65°C before being autoradiographed with a Hyperfilm MP (Amersham).

Northern blots were reprobed with a cloned 18S cDNA to standardize the loaded RNA samples. The relative absorbance of the mE-RABP and 18S RNA was determined using an imaging densitometer (model GS-670, Bio-Rad, Hercules, CA) and the Multi Analyst software (Bio-Rad).

Western blotting

Tissues were homogenized in 10 mM Tris-HCl, pH 7.4, 150 mM NaCl in the presence of protease inhibitors (leupeptin 1 µg/ml, chymostatin 1 µg/ml, aprotinin 1 µg/ml, antipain 2 µg/ml, benzamidine 10 µg/ml). Samples were centrifuged at 40,000 ¥ g, and the supernatants were stored at −80°C before use. Twenty micrograms of total protein were separated on a SDS-PAGE (17% polyacrylamide gel) and transferred to a Protran nitrocellulose membrane as described previously (18). Nitrocellulose membranes were incubated overnight at 4°C in PBS/1% (wt/vol) BSA, washed five times in PBS/0.1% (wt/vol) BSA, 0.1% (vol/vol) Tween-20, incubated for 1 h with the immune rabbit IgG anti-mE-RABP (2), washed, incubated for 1 h at room temperature with biotinylated antirabbit IgG (“Vectastain kit”, Vector Laboratories, Inc.), washed, and incubated for 1 h at room temperature in the ABC-peroxidase reagent (Vector Laboratories, Inc.). After washing five times in PBS/0.1% (vol/vol) Tween-20 and once in PBS 1×, reactive bands were visualized using a solution containing 0.5 mg/ml diaminobenzidine (DAB), 0.02% H2O2, 0.04% NiCl2 in 0.05 M Tris-HCl, pH 7.5. The reaction was stopped with H2O, and the membrane was air dried. The relative absorbance of mE-RABP was determined using an imaging densitometer (model GS-670, Bio-Rad) and the Multi Analyst software.

Results

Identification of a cDNA encoding the minor form of mE-RABP

The N-terminal microsequences of the minor and major form of mE-RABP were aligned and a forward degenerate oligonucleotide (FwmE-RABP) encompassing ten conserved amino acids was designed. An EcoRI cloning site and an initiation translation consensus sequence were also integrated into the oligonucleotide. Total RNA, extracted from adult mice epididymis, was reverse transcribed using the primer RACEIII containing 18 thymine residues and an anchored region. Then, the PCR was performed using the FwmE-RABP oligonucleotide and the RIII anchored primer. The PCR product analysis was performed on a 1% agarose gel and two bands (0.6 kb and 0.4 kb) were obtained. These DNA fragments were digested with EcoRI and BamHI restriction enzymes, purified, and then ligated into the pGEM7Zf(-) cloning vector (Promega). The DNA sequencing of these inserts showed that the 0.4-kb DNA fragment was homologous to the LTR of the mouse endogenous retrovirus (MuRVY). However, the 0.6-kb DNA fragment showed a 495-bp open reading frame (ORF) encoding 164 amino acids of an estimated 18.1-kDa peptide (Fig. 1). The first thirteen amino acids at the N-terminal end of the peptide were identical to the published microsequence of the minor form of
mE-RABP (2). To confirm that this peptide was immunologically related to mE-RABP, in vitro transcription/translation assays were carried out (Fig. 2). The sense RNA transcribed from the T7 RNA polymerase promoter encoded an 18 kDa peptide which was specifically immunoprecipitated with a rabbit polyclonal antibody raised against mE-RABP (2). This antibody was unable to immunoprecipitate any peptide produced in presence of antisense RNA. Altogether, these results demonstrate that the clone E7 containing the 0.6 kb DNA fragment encoded the minor form of mE-RABP.

mE-RABP is a new member of the lipocalin family

The mE-RABP primary amino acid sequence was compared with the GenBank database and was found homologous with members of the lipocalin superfamily (Fig. 3). The highest match (75% identity, 90% homology) was with rat ESP I (ESP I:11), lizard epididymal secretory protein IV (LESP IV: 36), mouse major urinary protein (MUP: 19), sheep β-Lactoglobulin (BLG: 21), human α-1-acid glycoprotein (AGP: 22) and rat retinol binding protein (RBP: 20) are aligned (Matrix: BLOSUM62). The conserved amino acids between mE-RABP and other lipocalin proteins are shaded. Boxed regions indicate the conserved motifs G-X-W and T-D-Y and the two cysteine residues.

Tissue-specific and region-specific expression of mE-RABP messenger RNA (mRNA)

Total RNA was extracted from brain, liver, spleen, kidney, seminal vesicle, vas deferens, testis, and epididymis. Hybridization of Northern blots with radiolabeled E7 cDNA revealed the presence of a mRNA of approximately 950 bases only in the epididymis (Fig. 6A). The region-specific expression of mE-RABP mRNA was first studied by Northern blot analyses (Fig. 6B). mE-RABP mRNA was detected in the caput but not in the corpus or the cauda.

In situ hybridization experiments showed the highly restricted expression of mE-RABP mRNA within the caput (Fig. 7). No expression was detected in the efferent ducts and initial segment (1). A low level of expression, detectable only by dark field microscopy, was seen in segment 2 [classification of Abou-Haila and Fain-Maurel (24)]. Expression progressively increased in segment 3, reaching a maximum in the distal portion of segment
3 and segment 4. mE-RABP mRNA expression abruptly decreased in segment 5 where, interestingly, a checkerboard pattern was observed, i.e. some principal cells expressed mE-RABP mRNA and others did not. No expression was seen in the corpus, the cauda or the vas deferens.

Hormonal regulation of mE-RABP mRNA and protein

The effect of castration on mE-RABP mRNA expression was investigated by Northern blot (Fig. 8). The steady state level of mE-RABP mRNA decreased to 11% of control intact animals by 5 days after castration and became almost undetectable (0.7%) by 10 days. However, a rebound from 0.7% to 5% of the mE-RABP mRNA was noted in mice castrated for 15 days. The mE-RABP mRNA expression increased progressively from day 15 to day 60 when it reached 18% of the control level. Western blot analysis showed that mE-RABP was still present 5 days after castration (0.5% of control) in the epididymis but was not detectable 20 days after castration despite a similar level of mE-RABP mRNA being present 5 days and 20 days after castration (11% and 13.6% of control, respectively). Therefore, the rebound of the mE-RABP mRNA did not lead to a recovery of the protein.

To study androgen regulation of mE-RABP gene expression, testosterone propionate (TP) (150 μg/day), dihydrotestosterone (DHT) (150 μg/day), or sesame oil (100 μl/day) was injected for 4 days into mice that had been castrated 5 days earlier. These concentrations of androgen are known to restore the physiological concentration of androgens in the mouse epididymis (25). The mE-RABP mRNA expression was partially restored with short-term TP or DHT treatment (77 and 69% of control intact animals, respectively). Efferent duct ligation for 10 days did not alter mE-RABP expression (109% of control intact animals).

In situ hybridization was used to determine the localization of the mE-RABP mRNA after androgen withdrawal and testosterone replacement (Fig. 9 and 10). mE-RABP mRNA expression was not detectable 45 days after castration due to...
the limited time of film exposure (1 day). However, injections of testosterone propionate for 10 days to 45-day castrated animals restored mE-RABP mRNA expression. Although testosterone replacement did not restore the size of the organ, a similar segment-specific expression of mE-RABP mRNA to that seen in the intact animal was observed. The major differences were a loss of the checkerboard pattern of mE-RABP expression at the junction of the caput and the corpus seen in the intact animal and expression in the proximal corpus while in the intact animal there was an abrupt disappearance of expression in the corpus (Fig. 10). Altogether, these results indicate that the steady state level and region-specific expression of the mE-RABP mRNA are not dependent on testicular factor(s) present in the luminal fluid but are dependent on testicular androgens present in the blood.

Discussion

The mE-RABP is a new member of the lipocalin superfamily and is the mouse orthologue of rat ESP I

A degenerate oligonucleotide corresponding to 10 amino acids of mE-RABP N-terminal microsequence was used in 3’ RACE experiments to isolate a 594 bp cDNA. In vitro transcription/translation assays showed that this cDNA encoded a 18 kDa peptide which was preferentially immunoprecipitated using a rabbit polyclonal antibody raised against mE-RABP (2). Moreover, the first 13 amino acids predicted by the cDNA open reading frame matched the mature mE-RABP N-terminal microsequence. These observations confirmed that the 594-bp cDNA encodes the minor form of mE-RABP (Fig. 1).
Analysis of the derived primary amino acid sequence revealed that mE-RABP was a new member of the lipocalin superfamily (Fig. 3). This family includes a large number of secretory proteins (26, for review), but statistical analysis showed that the identity between any pair may be as low as 20–30%. Despite the low overall amino acid sequence identity (26.5% identity, 48% similarity), mE-RABP contains two motifs Gly-X-Trp and Thr-Asp-Tyr as well as two cysteine residues that are highly conserved among the members of the lipocalin family (27) and are believed to be important for the three-dimensional structure of lipocalins. Indeed, all available crystal structures show the lipocalins to have a similar three-dimensional structure (human plasma retinol binding protein (RBP) (28), human β-lactoglobulin (Blg) (29), insecticyanin (30), bilin-binding protein (BBP) (31), mouse major urinary protein (MUP) and rat α2u-globulin (32), bovine odorant-binding protein (OBP) (33, 34), and rat epididymal retinoic acid binding protein (E-RABP) (13). All lipocalins...
have an eight stranded up-and-down β-barrel closed at one end by a single turn of the α-helix and a hydrophobic binding cavity. The ligand binding domain is well adapted for non-covalent binding of small hydrophobic ligands (retinol, retinoic acid, biliverdin, porphyrin, cholesterol derivates, pheromones, or prostaglandins) (26, for review).

The mE-RABP had the highest amino acid sequence identity (75%) with a rat androgen-regulated epididymal secretory protein named protein B/C (7), also known as EBP (8, 9), E-RABP (13, 14), and ESP I (11). This protein, identified in the luminal fluid of the rat epididymis (7, 12), is a retinoic acid binding protein (8) encoded by a single-copy gene (11). Rat E-RABP, like mE-RABP, binds both all-trans and 9-cis retinoic acid (13) but not retinol (8). A comparison of the putative mE-RABP three-dimensional structure to that of ESP I showed that some amino acids in the ligand binding domain were different. However, these minor changes do not affect the space required to bind retinoic acid (Fig. 5). The amino acid sequence homology, the ligand-binding specificity, and the epididymis-specific expression of a single-copy gene (35) confirm that mE-RABP is the mouse orthologue of the rat epididymal secretory protein.

Another androgen-dependent lipocalin related to mE-RABP (26.5% identity, 48% similarity) was described in the lizard epididymal fluid (23, 36). This suggests that an E-RABP related to the lipocalin superfamily is conserved during evolution and may have an important function in male fertility.

Tissue-and region-specific expression of the mE-RABP gene

Although the epididymis consists of a single convoluted duct composed of one major cell type, it displays a high degree of region-specific expression of a large number of genes (3, for review). Northern blot analyses have shown that mE-RABP mRNA expression is restricted to the caput epididymidis (Fig. 8). This observation is in agreement with previous studies showing that mE-RABP was synthesized and secreted into the luminal fluid from the mid/distal caput epididymidis (2, 37, 38). mE-RABP was also found by immunohistochemistry in the cytoplasm of clear cells in the corpus and cauda epididymidis (2). In situ hybridization showed that mE-RABP mRNA was gradually expressed in the principal cells from the proximal to the distal caput epididymidis (Fig. 7). However, no mE-RABP mRNA was detected in clear cells located in the corpus and cauda epididymidis (data not shown). This result is in agreement with our previous report suggesting that clear cells endocytosed mE-RABP from the luminal fluid (38). The mE-RABP is related to retinol binding protein that binds retinol and forms a complex with another plasma protein named Transtherithin (39). This complex is able to deliver retinol to cells expressing a specific receptor on their surface (40). Therefore, it is reasonable to expect that a mE-RABP-specific receptor exists on clear cell plasma membranes allowing the endocytosis of mE-RABP from the luminal fluid.

The increase in mE-RABP mRNA expression in the caput was gradual within all principal cells. In contrast, the disappearance of expression at the junction of the caput and the corpus was abrupt, but some principal cells continued to exhibit strong expression, whereas adjacent cells display none, giving a checkerboard appearance to segment 5. Such a cell-specific gene expression has been observed for several epididymal mRNA and/or proteins (4, for review).

Although mE-RABP and rat proteins B/C are homolo-
FIG. 9. *In situ* hybridization of mE-RABP mRNA was done using an $[^{35}S]$-radiolabeled antisense RNA probe as described in materials and methods. I, Intact animal; C, mouse castrated for 45 days and then injected with sesame oil for 10 days; TP, mouse castrated for 45 days and then injected with testosterone propionate (TP) (150 µg/day) for 10 days. There is no mE-RABP mRNA expression in the efferent ducts (ED), and the testis (T) in intact animal. The mE-RABP mRNA expression disappears in the castrate epididymis. The mE-RABP mRNA is expressed at low level in segment 2 and at a higher level in segment 3 and 4 after androgen replacement. Note that there is a high level of expression in segment 5 and a low level of expression in the upper portion of segment 6 that are not observed in the intact animal.

FIG. 10. *In situ* hybridization of mE-RABP mRNA in region 5 (a–d) and the upper part of region 6 (e–h) of an intact mouse (a, b, e, f) and a mouse castrated for 45 days and then injected with testosterone propionate (TP) for 10 days (c, d, g, h). Note 1) the checkerboard pattern of high and no expression in the epithelium of region 5 of the intact animal and the uniform high expression in the same region of the castrate + TP animal, 2) the lack of mE-RABP expression in the upper region of segment 6 of the intact animal, and the low level of expression in the same region of the castrate + TP animal.
gous, the mRNA expression patterns are different. In the rat, proteins B/C mRNA are expressed uniformly in the efferent duct, and in the initial, proximal and distal caput epididymidis (41). In the mouse, mE-RABP mRNA is expressed in a gradient pattern from the mid to the distal caput epididymidis but is undetectable in the efferent ducts and the initial segment (Fig. 7). Although it is difficult to compare the segments of the caput epididymidis from different species, our result suggests that the regulation of region-specific expression of E-RABP may be different in mouse and rat epididymis.

Androgen regulation of the mE-RABP gene expression

Androgens are required for epididymal sperm maturation (42, 43). Previous studies have shown that castration of adult mice results in the loss of the mE-RABP expression (37). Androgens could regulate mE-RABP translation or stability, but Northern blot analyses showed that castration, but not efferent duct ligation, led to a rapid decrease of mE-RABP mRNA expression. Moreover, testosterone propionate or DHT injections to castrated mice restored mE-RABP mRNA expression. These results suggest that mE-RABP mRNA expression is dependent only on circulating androgens. When mE-RABP expression reappeared after androgen replacement, the same overall region-specific pattern of expression seen in the intact mice was observed. However, the transition between the caput and the corpus was not as abrupt and the mRNA expression patterns are different. In the rat, the same overall region-specific pattern of expression, the checkerboard pattern of expression seen in the intact animals was not present, and all principal cells expressed similar levels of mRNA. This suggests that the factors involved in the region-specific and cell-specific expression of mE-RABP may be affected by castration and testosterone replacement.

Androgens bind to a nuclear receptor that belongs to the ligand-inducible transcription factors superfamily (44). The binding of the androgen receptor to specific cis-acting DNA regulatory elements named the androgen response element (ARE) results in the modulation of the target gene transcription (45). A nucleotide sequence homologue to this regulatory element is present in the rat ESP I gene promoter (11). Therefore, mE-RABP gene expression may be directly regulated by androgens at the transcriptional level. However, in the prostate, androgens regulate mitochondrial aspartate aminotransferase (mAAT) (46) and prostatic binding protein (PBP) C3(1) gene expression (47) primarily by controlling the stability of the mRNA. Thus, androgens may also regulate the turn over of mE-RABP mRNA.

mE-RABP mRNA expression progressively reappeared between 15 and 60 days after castration (Fig. 8). In the prostate, an androgen-dependent mRNA encoding another lipocalin protein named probasin was observed to rebound to precastrated levels 12 days after castration (48). However, to our knowledge, the rebound of an androgen-regulated gene never has been reported before in the epididymis. Moreover, rat proteins B/C gene expression does not rebound after long-term castration (49), suggesting that this rebound of mE-RABP is unique to the mouse. It is unlikely that adrenal androgens are involved because the level of epididymal androgens stay low during long-term castration in the mouse (25). This atypical behavior suggests that other transcription factor(s) (repressor or activator) may cooperate with the androgen receptor to control mE-RABP gene expression at a transcriptional and/or posttranscriptional level. Further studies will be required to elucidate this phenomenon.

At 5 days, but not at 30 days post castration, a significant amount of mE-RABP protein was present in the epididymis despite a similar level of mE-RABP mRNA. This suggests that the synthesis or the stability of mE-RABP protein, in vivo, may be dependent on testicular factor(s) and/or androgens. Such posttranscriptional regulation has been described for other epididymal genes (25, 50). For example, the transcription and the steady-state levels of the GPX5 mRNA are fully restored after castration and testosterone replacement but not the translation of the GPX5 mRNA suggesting that testicular factors exert a posttranscriptional control for GPX5 synthesis (25).

In summary, mE-RABP belongs to the lipocalin superfamily. Because the lipocalins are mainly extracellular transport proteins involved in the delivery of small lipophilic molecules (26), the most plausible function for mE-RABP is as transport protein for retinoic acid within the epididymis. The conservation of this protein during evolution suggests that it has an important function in male fertility.

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References

12. Newcomer ME 1993 Structure of the epididymal retinoic acid binding protein at 2.1 A resolution. Structure 1:7–18
of a protein-binding site for both all-trans- and 9-cis-retinoic acid. Proc Natl Acad Sci USA 90:9223–9227