A 5-Kilobase Pair Promoter Fragment of the Murine Epididymal Retinoic Acid-binding Protein Gene Drives the Tissue-specific, Cell-specific, and Androgen-regulated Expression of a Foreign Gene in the Epididymis of Transgenic Mice*

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The murine epididymis synthesizes and secretes a retinoic acid-binding protein (mE-RABP) that belongs to the lipocalin superfamily. The gene encoding mE-RABP is specifically expressed in the mouse mid/distal caput epididymis under androgen control. In transgenic mice, a 5-kilobase pair (kb) promoter fragment, but not a 0.6-kb fragment, of the mE-RABP gene driving the chloramphenicol acetyltransferase (CAT) reporter gene restricted high level of transgene expression to the caput epididymis. No transgene expression was detected in any other male or female tissues. Immunolocalization of the CAT protein and in situ hybridization of the corresponding CAT mRNA indicated that transgene expression occurred in the principal cells of the mid/distal caput epididymis, thereby mimicking the spatial endogenous mE-RABP gene expression. Transgene and mE-RABP gene expression was detected from 30 days and progressively increased until 60 days of age. Castration, efferent duct ligation, and hormone replacement studies demonstrated that transgene expression was specifically regulated by androgen but not by any other testicular factors. Altogether, our results demonstrate that the 5-kb promoter fragment of the mE-RABP gene contains all of the information required for the hormonal regulation and the spatial and temporal expression of the mE-RABP gene in the epididymis.

During their transit through the epididymis, spermatozoa undergo biochemical and morphological changes to acquire motility and the ability to fertilize an oocyte in vivo (1, 2). The maturation process progresses along the epididymal duct and is believed to be dependent on epididymal secretory proteins.

The epididymis displays a highly region-specific pattern of gene expression. However, little is known regarding the molecular mechanisms that are involved in the regulation of tissue- and region-specific gene expression. Gene expression in the epididymis is mainly under androgen control (for review, see Ref. 3). Indeed, androgen withdrawal, either by orchietomy or by hypophysectomy, prevents the sperm maturation process (4, 5). However, testicular factors (6), estrogen (7), growth factors (8), and retinoic acid (9) have also been demonstrated to be involved in epididymis-specific gene expression. Whether these endocrine and paracrine signal pathways cooperate to restrict gene expression to a narrow segment of the epididymal duct is unclear.

We previously described two proteins (major and minor forms) that are generated by the differential cleavage of a unique precursor, initially named mouse epididymal protein 10 (10). This protein, recently renamed murine epididymal retinoic acid-binding protein (mE-RABP) (11), is specifically synthesized and secreted by the principal cells of the distal caput epididymis. The mE-RABP protein binds active retinoids (9-cis- and all-trans-retinoic acid) but not retinol (12) and is the orthologue of two other retinoic acid-binding proteins described in the rat epididymis. These rat proteins were successively named B/C (13), EBP 1 and EBP 2 (14), E-RABP (15), and ESP I (16). Analysis of the amino acid sequence and putative threedimensional structure show that mE-RABP belongs to the lipocalin superfamily (11).

The mE-RABP protein is encoded by a single-copy gene localized to the [A3-B] region of mouse chromosome 2, a region rich in genes encoding lipocalin and displaying a similar genomic structure to that of the mE-RABP gene (17).

The mE-RABP gene expression is androgen-regulated in vivo (11). In addition, transient transfection studies have shown that a functional androgen-specific response region (ARR) is localized within the first 600 bp of the mE-RABP gene promoter (18). The androgen dependence and the strong tissue- and region-specific expression make the mE-RABP gene a good candidate to study the molecular mechanisms that restrict gene expression to a narrow segment of the epididymis under androgen control. In the absence of appropriate epididymal cell lines, cis-DNA regulatory elements involved in the tissue-specific and androgen-regulated expression of the mE-RABP gene can only be identified in transgenic mice.

In the present study, we demonstrate that 5 kb of the 5’-flanking region of the mE-RABP gene can drive the specific expression of the chloramphenicol acetyltransferase (CAT) reporter gene to the principal cells of the mid/distal caput epididymis, thereby mimicking the expression of the endogenous gene. We also demonstrate that the 5-kb DNA fragment

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§ The abbreviations used are: mE-RABP, murine epididymal retinoic acid-binding protein; ARR, androgen-specific response region; bp, base pairs; kb, kilobase pair(s); CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; PBS, phosphate-buffered saline.
contains most, if not all, of the information required for the temporal and hormonal regulation of the mE-RABP gene in vivo.

EXPERIMENTAL PROCEDURES

Animals—All experiments were conducted in accordance with the National Institutes of Health Guidelines for Care and Use of Animals in the Laboratory. Castration or efferent duct ligation was performed by the abdominal route under light methoxyflurane (Mallinckrodt, Mundelein, IL) anesthesia. When required, hormone replacement was begun 30 days after castration with daily subcutaneous injections of testosterone propionate (2 mg/kg), ß-estradiol-3-benzoate (2 mg/kg), hydrocortisone 17-butryate (2 mg/kg) dissolved in sesame oil. Treatments were conducted for 9 days, and mice were killed 1 day after the last injection.

Organs were excised, immediately frozen in liquid nitrogen, and stored at −80 °C.

Cloning Constructs—DNA fragments encompassing the mE-RABP gene promoter were generated from the pHindIII genomic clone (17) using appropriate restriction enzymes. DNA fragments were purified on a 1% (w/v) agarose gel and then ligated into the promoterless pBLCAT3 plasmid (19) using standard methods (20) in such a way that the mE-RABP promoter DNA fragment would drive the CAT reporter gene.

Transgenic Mice—The m6.9kb-CAT and m5kb-CAT DNA fragments, containing 0.6 or 5 kb, respectively, of the mE-RABP gene promoter driving the CAT reporter gene were excised from the pUC18 vector by restriction enzyme digest. DNA fragments were purified on a 1% (w/v) agarose gel using the AgarACE™ enzyme (Promega, Madison, WI). Transgenic mice (strain B6D2, Harlan Sprague-Dawley) were generated by microinjection of the DNA into the male pronucleus of a fertilized oocyte using standard techniques (21). Transgenic animals were identified by PCR-based screening assay using isolated tail DNA. Approximately 1 cm of the tail was digested overnight at 55 °C in a Proteinase K digestion mix (10 mM Tris–Cl, pH 7.5, 75 mM NaCl, 25 mM EDTA, 1% SDS, 0.5 mg/ml Proteinase K). Then DNA was extracted with 1 volume of phenol/chloroform/isomyl alcohol (25/24/1) and precipitated in 70% ethanol, centrifuged at 10,000 × g for 10 min, and then dissolved in 20 µl of DNAase-free water.

RESULTS

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5-kb Fragment of mE-RABP Gene Drives Foreign Gene Expression

In Situ Hybridization—The epididymis was fixed overnight in freshly prepared 4% (v/v) paraformaldehyde/phosphate-buffered saline (pH 7.4), rinsed with PBS, and then dehydrated through a series of increasing concentrations of ethanol for a period of 3–5 h prior to embedding in paraplast. Tissues were sectioned at 7 µm. The CAT reporter gene was excised from the pBLCAT3 vector (19) and ligated into the pGEM7Zf(−) vector (Promega) to obtain the pGEM-CAT construct. CAT mRNAs were detected from 5-kb Fragment of mE-RABP and the CAT probe labeled with 35SUTTP to a specific activity of 1.2×108 cpm/ml using the in vitro transcription kit MAXiscript™ (Ambion Inc., Austin, TX). Hybridization was carried out at 55 °C with 2×106 cpm/ml riboprobe overnight in 50% (v/v) formamide, 300 mM NaCl, 10 mM Tris (pH 7.4), 10 mM NaH2PO4 (pH 6.8), 5 mM EDTA (pH 8.0), 0.2% (v/v) Fixoll 400, 0.2% (v/v) polyvinyl pyrrolidone, 10% (v/v) dextran sulfate, 200 µg/ml yeast transfer RNA, and 50 µM dithiothreitol (DTT). Excess of riboprobe was removed by washing in 2× SSC, 20 µM mercaptoethanol for 15 min at 50 °C, once followed by two washes in 4× SSC, 50% (v/v) formamide, 20 µM mercaptoethanol for 30 min each at 55 °C, and two washes in 4× SSC, 20 mM Tris–Cl, pH 7.5, 2 mM EDTA for 10 min each at 37 °C. Single-stranded RNA was digested in 4× SSC, 20 mM Tris–Cl, pH 7.5, 2 mM EDTA, and 20 µg/ml RNase A for 30 min at 37 °C. The reaction was stopped by two washes in 4× SSC, 20 mM Tris–Cl, pH 7.5, 2 mM EDTA for 10 min each at 37 °C and two washes in 4× SSC, 50% (v/v) formamide, 20 µM β-mercaptoethanol for 30 min each at 55 °C. Slides were quickly rinsed twice in H2O and air-dried. Slides were dipped in NTB-2 Kodak emulsion and exposed for 23 days at 4 °C, developed, fixed, and mounted with Permount (Fisher) for photography.
the standard protocols of staining for 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (28).

The chimeric genes, named m0.6Kb-CAT and m5Kb-CAT, respectively, were excised from the pUC18 plasmids using the appropriate restriction enzymes (Fig. 1A). After purification, the fusion genes were injected into the male pronuclei of B6D2 mouse embryos to generate transgenic mice. Two m5Kb-CAT (one male and one female) and four m0.6Kb-CAT (two males and two females) founder animals were detected by PCR using DNA extracted from the tail and primers 1 and 2 (Fig. 1B). All founder animals passed on the transgene to their offspring in a Mendelian fashion. Analysis of the genomic DNA on Southern blot was carried out to confirm random insertion of both transgenes into the mouse genome and to determine the number of transgene copies in each transgenic line (Fig. 1C). A probe encompassing the first 600 bp of the mE-RABP gene promoter was used to detect both the endogenous mE-RABP gene as well as the transgenes (Fig. 1A). In the nontransgenic control animal (wild type), a 9-kb HindIII restriction fragment corresponding to the endogenous mE-RABP gene was detected as described previously (17). In addition to the endogenous 9-kb DNA fragment, other DNA fragments of different lengths were detected for each transgenic mouse line, indicating that the transgenes were inserted in a random fashion. Transgene copy number was determined by calculating the ratio between the hybridization signals of the transgenes and the endogenous mE-RABP gene (Fig. 1C). The presence of 18 copies and 1 copy of the m5Kb-CAT transgene was detected in mouse lines 4 and 19, respectively. In transgenic mouse lines 143, 148, 149, and 391, 9, 6, 2, and 4 copies of the m0.6Kb-CAT transgene were

![Diagram](image_url)
inserted, respectively. The mouse line 4 carrying the m5Kb-CAT transgene exhibited a smaller 5.5-kb band in addition to the full size of the m5Kb-CAT transgene of interest (6.7 kb). The 5.5-kb band probably corresponds to the insertion of a truncated transgene and was not taken into account for determination of copy number. Another probe, specifically designed to detect only the CAT reporter gene (Fig. 1A), was also used (Fig. 1D). The CAT probe detected the same restriction fragments described above with the exception of the endogenous mE-RABP gene. Only in mouse line 4, a 9-kb restriction fragment was still detectable, suggesting again that a complete or truncated m5Kb-CAT transgene had been inserted at multiple sites.

The 5-kb Promoter Fragment, but Not the 0.6-kb Fragment, of the mE-RABP Gene Targets High Level of Transgene Expression to the Caput Epididymis—We have previously shown that the mE-RABP gene is specifically expressed in the principal cells of the mid/distal caput epididymidis (11). Therefore, to detect the expression of the m0.6Kb-CAT and m5Kb-CAT transgenes, adult male mice were killed and the CAT activity was first measured in cell extracts of the caput epididymidis (Fig. 2). When compared with the wild type control animals, no significant CAT activity was detectable in mouse lines 143, 148, 149, and 391 carrying the m0.6Kb-CAT transgene. However, high and moderate levels of CAT activity were found in the caput epididymidis from lines 4 and 19 carrying the m5Kb-CAT transgene. The m5Kb-CAT transgene expression appeared to be correlated with transgene copy number, since line 4 expressed 11-fold greater CAT activity than line 19, and carried the highest copy number (18 versus 1, respectively). In addition, homozygous transgenic mice expressed on average a 2-fold higher level of CAT activity than the heterozygous transgenic males (Table I).

The pattern of tissue-specific expression of the m5Kb-CAT and m0.6Kb CAT transgenes was then determined. No CAT activity was detected in the cauda epididymidis and in 12 other tissues, including testis, vas deferens, seminal vesicles, prostate, spleen, kidney, heart, lung, brain, small intestine, liver, and muscle, of adult male mice carrying the m5Kb-CAT transgene (Fig. 3) or the m0.6Kb-CAT transgene (data not shown). In addition, CAT reporter gene expression was undetectable in adult female tissues, including ovary, oviduct, uterus, spleen, kidney, liver, heart, lung, brain, small intestine, and muscle (data not shown). This demonstrates that 5 kb of the 5′-flanking region of the mE-RABP gene is required to target high levels of gene expression to the caput epididymidis.

The m5Kb-CAT Transgene Expression Is Restricted to the Principal Cells of the Mid/Distal Caput Epididymidis—We have previously shown that the mE-RABP gene expression is limited to the caput epididymidis with a highly region- and cell-specific pattern (11). To address the question of whether the m5Kb-CAT transgene contained all of the information required for region- and cell-specific gene expression, in situ hybridization of CAT mRNA was carried out (Fig. 4). Serial tissue sections of the epididymis from homozygous m5Kb-CAT transgenic mouse line 4 were incubated either with a CAT antisense or mE-RABP antisense probe. Both CAT (Fig. 4C) and mE-RABP mRNA (Fig. 4E) were detected only in the principal cells of the mid/distal caput epididymidis. No CAT mRNA was detected in the efferent ducts, the initial segment, the corpus, or cauda epididymidis of m5Kb-CAT transgenic mice. In addition, no hybridization signal was detected in the epididymis of wild type mice using the antisense CAT probe (Fig. 4A) or line 4 mice using the sense CAT probe (Fig. 4B).

The region- and cell-specific expression of the m5Kb-CAT transgene was further studied by the immunohistochemical localization of the CAT protein (Fig. 5). Sections of the epididymis, from wild type and heterozygous transgenic mouse lines, were incubated in the presence of a rabbit polyclonal antibody directed against CAT (5 Prime – 3 Prime, Inc.). No CAT expression was detected in the efferent ducts and initial segment of the epididymis from heterozygous mouse line 4 (Fig. 5A). A faint staining was first detected in the cytoplasm and nucleus of principal cells in segment 2 (Fig. 5E) (classification of Abou-Haila and Fain-Maurel (29)). Levels of CAT gene expression progressively increased in segment 3, reaching a maximum in the distal portion of segment 3 and segment 4 (Fig.

### Table I

| Transgenic Line | CAT Activity (dpm/min/mg protein) | Fold Increase |
|----------------|-----------------------------------|--------------|
| Wild type      | 2560 ± 610                       | 1.0          |
| Line 4         | 3772581 ± 151596                 | 2.6          |
| Line 19        | 251387 ± 52236                   | 1.8          |

The table presents the CAT activity in the epididymis of heterozygous and homozygous m5Kb-CAT transgenic mice, with values expressed as the mean ± S.E. Note that CAT activity increases twice on average between heterozygous and homozygous transgenic mice.
Finally, expression decreased in segment 5 and ended with a checkerboard pattern, i.e. some principal cells expressed CAT and others did not, at the boundary between the distal caput and the proximal corpus (Fig. 5). No staining was observed in the apical cells, the clear cells, the basal cells of the epithelium, the myoid cells surrounding the tubule, or cells of the connective tissue between the tubules (Fig. 6). No transgene expression was seen in the corpus, the cauda or the vas deferens (data not shown). Thus, the pattern of transgene expression was identical to that described previously for the endogenous mE-RABP gene (11).

In agreement with our previous results, no CAT staining was detected in the caput epididymidis of wild type mice and transgenic mice carrying the m0.6Kb-CAT transgene (Fig. 5, C and D). Corpus and cauda epididymidis of wild type or heterozygous mouse line 4 were also negative (not shown).

Altogether, these results demonstrate that the 5-kb promoter fragment contains all of the cis DNA regulatory elements required for tissue-, region-, and cell-specific mE-RABP gene expression.

Temporal Expression of the Endogenous mE-RABP Gene and m5Kb-CAT Transgene Is Identical during Postnatal Development—The m5Kb-CAT transgene expression was further studied by monitoring CAT activity in epididymal cell extracts during development (Fig. 7). No CAT activity was found in the epididymis of heterozygous mice from 1 to 20 days of age. The CAT activity was first detected at 30 days and continued to increase until 50 days of age. Finally, a 3-fold increase of CAT activity occurred between day 50 and 60. This temporal expression pattern of the m5Kb-CAT transgene was identical to that of the endogenous mE-RABP gene as demonstrated by Western blot analyses (Fig. 7).

Our results demonstrate that the endogenous mE-RABP gene expression is developmentally regulated and that the 5-kb DNA promoter fragment contains all of the information required for correct temporal expression of the mE-RABP gene.

The m5Kb-CAT Transgene Expression Is Specifically Regulated by Androgens—The appearance of the m5Kb-CAT transgene expression at puberty suggested that androgens might be involved in its regulation. To confirm this hypothesis, adult male mice carrying the m5Kb-CAT transgene were castrated...
for 5, 10, 20, and 30 days (Fig. 8A). The m5Kb-CAT transgene expression dramatically decreased during the first 10 days following castration (47% and 5.8% of the intact heterozygous mice, 5 and 10 days after castration, respectively) and then decreased progressively to reach background level 30 days after castration (not shown). Western blot analyses showed that endogenous mE-RABP protein expression decreased during the first 10 days after castration (47% and 5.8% of the intact heterozygous mice, 5 and 10 days after castration, respectively) and then decreased progressively to reach background level 30 days after castration (5.7 and 3.4% of the intact heterozygous mice, 20 and 30 days after castration).

To study whether transgene expression was regulated by testicular factors present in the luminal fluid, efferent duct ligation was carried out (Fig. 8B). No difference of the m5Kb-CAT transgene and mE-RABP gene expression was observed 30 days after efferent duct ligation in the intact heterozygous transgenic mice. This strongly suggests that the 5-kb mE-RABP promoter, but not the 0.6-kb fragment, contains all of the information needed for the epididymis-specific expression of the mE-RABP gene.

Numerous studies have reported that promoter fragments are able to target transgene expression to the epididymis. For instance, the 0.4-kb promoter fragment of the probasin gene (30, 31) and the 6-kb promoter region of the prostate-specific antigen gene (28) are able to target transgene expression to the mouse prostate. However, gene expression was not correlated with transgene copy number, suggesting that, although these promoter fragments contain tissue-specific cis-DNA regulatory elements, they may lack important elements such as matrix attachment regions or locus control regions. Matrix attachment regions are believed to facilitate gene expression and may serve as specific sequence landmarks as they anchor DNA to the nuclear scaffold (32–34). On the other hand, locus control regions are strong enhancers that bind multiple transcription factors that function in a cooperative manner (for review, see Ref. 34). Both of these DNA elements are believed...
while expression of the superoxide diamutase gene is restricted to the cauda epididymidis (36). Our results show that all of the cis-DNA regulatory elements necessary to confer mid/distal caput-specific expression reside within the 5 kb of the 5′-flanking region of the mE-RABP gene. Therefore, we feel that this 5-kb DNA fragment provides a unique tool to identify region-specific transcription factors that may regulate epididymal genes.

Within the caput epididymidis, the m5Kb-CAT transgene was expressed specifically in the cytoplasm and nuclei of the principal cells as shown by in situ hybridization and by immunohistochemistry. No staining was observed in other cell types either in the epithelium or in the connective tissue between the tubules. The nuclear immunolocalization of the CAT protein may be due to a cryptic nuclear localization signal. Indeed, the CAT protein contains the motif KKKN in its primary amino acid sequence, which is homologous to the nuclear localization signal (KKRK) of the DNA helicase Q1 (37). This cell-specific expression of the m5Kb-CAT transgene mimicked that previously reported for the endogenous mE-RABP gene (11). In addition, as with mE-RABP, there was a checkerboard pattern of expression at the proximal and distal borders of the distal caput epididymidis such that some principal cells exhibited strong expression, whereas adjacent principal cells displayed no or very low levels of expression. A similar pattern has also been observed for other epididymal genes (for review, see Ref. 3). These observations imply that the 5-kb mE-RABP promoter contains all of the information necessary for this highly cell-specific gene expression. A single transcription factor can function as a trigger to determine tissue-specific gene expression. For instance, the critical role of the Pit-1 transcription factor in cell differentiation of the pituitary somatotropes, lactotropes, and thyrotropes is well documented (for review, see Ref. 38).

However, to achieve cell-specific gene expression, the tissue-specific transcription factor may combine with other regulatory proteins to control gene expression (39, 40). On the basis of a such model, it is reasonable to anticipate that the m5Kb-CAT transgene contains several cis-DNA elements that bind ubiquitous, tissue- and/or cell-specific transcription factors.

The m5Kb-CAT Transgene Expression Is Specifically Regulated by Androgens—Our study clearly demonstrated that the m5Kb-CAT transgene expression was developmentally regulated and mimicked that of the endogenous mE-RABP gene. The m5Kb-CAT gene expression was well correlated with the increase of DHT and androgen receptor content that occurs in the mouse epididymis during development (41, 42). Castration, efferent duct ligation, and hormonal replacement studies confirmed that androgens, but not other testicular factors, were required to maintain m5Kb-CAT transgene expression in adult animals. Moreover, in vitro transient transfection assays had shown that the 5-kb promoter fragment of the mE-RABP gene directing the CAT reporter gene was highly androgen-responsive, indicating that androgens may control mE-RABP gene expression at the transcriptional level (18). In addition, only androgens, but not glucocorticoids or estradiol, were able to increase reporter gene expression in vitro. Therefore, the m5Kb-CAT transgene expression during development and its androgen-specific regulation are consistent with our previous in vitro studies, demonstrating that the 5-kb promoter fragment of the mE-RABP gene confers androgen-specific responsiveness and that androgens may act directly at the transcriptional level to modulate mE-RABP gene expression in vivo.

We have demonstrated in transient transfection studies using HeLa cells that a functional ARR was localized within the first 600 bp of the mE-RABP gene promoter (18). A similar androgen response region was also identified within the 400-bp...
promoter region of the probasin gene, which encodes another androgen-regulated lipocalin in the rat prostate (43). In transgenic mice, this short 400-bp promoter fragment was sufficient 1) to restrict gene expression to the prostate and 2) to confer androgen control (30, 31). In the present study, the m0.6Kb-CAT transgene was not sufficient to direct detectable levels of the CAT reporter gene expression in four independent transgenic mouse lines. A similar result has been reported for the human prostate-specific antigen gene encoding an androgen-regulated prostatic secretory protein and belonging to the kalikrein gene family. The first 600 bp of the prostate-specific antigen gene promoter did not target gene expression to the prostate, although two distinct functional androgen receptor binding sites that cooperate were localized within the first 600 bp of the prostate-specific antigen gene promoter (44). However, a 6-kb promoter fragment containing an upstream enhancer was able to place reporter gene expression under androgen control in the prostate of transgenic mice (28). Therefore, it is probable that the ARR localized within the first 600 bp of the mE-RABP gene promoter requires an upstream enhancer present in the m5Kb-CAT transgene to drive gene expression in vivo. Further deletions between 0.6 and 5 kb upstream from the transcription initiation site of the mE-RABP gene will be required to identify important cis-DNA elements required for promoter activity in vivo. This enhancer may bind to tissue-specific transcription factors, but our results do not exclude the possibility that the 600-bp promoter fragment of the mE-RABP gene may also be required for androgen-regulated and tissue-specific gene expression.

Concluding Remarks—Despite the fact that the epididymis is the site of an important physiological event, the maturation of the male gamete, little is known about the molecular mechanisms regulating its function. The highly region-specific pattern of gene expression observed in the epididymis may be required to coordinate the functions of the different epididymal regions to ensure the efficient post-testicular maturation of spermatozoa. In the present study, we demonstrate that the 5-kb promoter fragment of the mE-RABP gene restricts high levels of gene expression to the principal cells of the mid/distal caput epididymis in transgenic mice. To our knowledge, this is the first demonstration of targeted expression by an epididymal gene promoter. It is now possible to further dissect and identify the cis-DNA regulatory elements and their associated proteins (steroid receptor coactivators, tissue- and/or cell-specific transcription factors) that are involved in the regulation of the tissue region- and cell-specific expression of epididymal genes.

The identification of an androgen-regulated and epididymis-specific gene promoter is not only relevant to identifying individual regions of the gene involved in cell specificity and hormonal regulation but also enables one to study the function of epididymal secretory proteins that are involved in the sperm maturation process. It is possible to disrupt gene function by targeted mutation (‘knockout’), but it is also possible to use the antisense RNA or protein engineering technologies as alternative strategies to inhibit gene function. Due to the high level of gene expression achieved with the m5Kb-CAT transgene, the mE-RABP gene promoter will be an appropriate tool to over-express an antisense-ribozyme RNA or a dominant negative protein to inhibit the expression and/or the function of target proteins in vivo.

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