Regulation of gene transcription in the epididymis

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The epididymis plays a crucial role in regulating the development of sperm motility and fertilizing capacity. In addition, the epididymis protects spermatozoa from reactive oxygen species and, within the cauda epididymidis, serves as a storage area for mature spermatozoa before ejaculation. These functions are carried out within the different luminal environments present along the epididymal duct. These different environments arise during development, beginning with an undifferentiated epithelium that undergoes a series of changes to become fully differentiated but with distinct regional differences in terms of morphology, gene expression activity and function. The spatial and temporal patterns of gene expression are critical to the development and maintenance of a fully functional epididymis.

Although eukaryotic gene expression is controlled at several points, tissue-specific gene expression is regulated predominantly at the level of transcription initiation. This highly complex process requires the organization of a number of different proteins responsible for the activation of the basal transcriptional machinery. A number of mechanisms have been determined to play a role in the transcriptional regulation of eukaryotic gene expression. However, little is known about the specific mechanisms involved in the control of epididymal gene expression. This review will outline some of the cis-DNA elements and associated transcription factors that have been identified in the epididymis, in addition to discussing the potential role of co-regulator molecules and changes in chromatin structure as critical control points of gene expression. Although gene expression can be controlled at several points, discussion will focus on gene regulation at the transcriptional level. The role of post-transcriptional control, with particular attention to mRNA stability, will also be discussed.

Characterization of cis-DNA regulatory elements

Some DNA elements are required for activation of the basal transcriptional machinery, whereas others are involved in the modification of transcriptional rates and specificity of promoter function. Promoters such as the TATA box are located near the transcription initiation site and direct the assembly of the pre-initiation complex. Other promoters include the Initiator, found primarily in housekeeping genes, and GC-rich promoters (Smale and Baltimore, 1989). The amount of transcription achieved from these minimal promoters tends to be low. Thus, upstream promoter elements, such as PEA3, GATA and Sp1, aid in the modulation of transcriptional activity.

The identification of cis-regulatory elements provides an insight into the regulation of a particular gene. The role of putative cis-regulatory elements can be studied in vitro using transfection assays. Owing to a lack of appropriate epididymal cell lines, these studies have used heterologous systems to investigate the control of epididymal genes. Although these initial characterization studies are important, they provide a simplistic view of gene expression and its regulation. In vivo, a plethora of factors and DNA elements work in concert and within the framework of chromatin structure to modulate the activity of gene expression. The use of transgenic mice to investigate promoters in vivo allows investigators to examine expression of a specific gene within the native environment of a specific tissue.

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Several polyomavirus enhancer activator 3 (PEA3)-binding motifs (5′-AGGAAG-3′; Lan et al., 1997) are present in the promoter region of GGT IV, a gene coding for gamma-glutamyl transpeptidase (GGT) which is highly expressed in the initial segment of the epididymis. Transient transfection studies in vitro demonstrated the ability of PEA3 to activate GGT promoter IV (Lan et al., 1999). However, only one of the PEA3 motifs was the functional site for PEA3, a member of the E26 transformation-specific transcription factor (Ets). Furthermore, an SP-1 binding motif is required for GGT-IV promoter activity (Lan et al., 1999). PEA3 response elements have also been shown to modulate the transcripational activity of the mouse epididymis caput-specific gpX5 gene (Drevet et al., 1998). Although the expression of many genes may be considered to be tissuespecific, it has become evident that cis-DNA elements do not confer tissue specificity of certain genes. Thus, other factors, or a combination of factors may be necessary for the expression of epididymis-specific genes.

Studies in transgenic mice may provide a more realistic view of how cis-acting elements control the expression of a specific gene within the context of chromatin structure. These studies have provided some interesting information regarding the segmental control of genes. A specific 5 kb fragment of the mE-RABP promoter was shown to contain all of the elements necessary for tissue-, region- and cell-specific expression of the mE-RABP gene (Lareyre et al., 1999). Further studies using this 5 kb promoter sequence may be critical for the identification of the specific elements or combination of elements that are required for the region- and cell-specific expression of the mE-RABP gene.

However, specific cis-DNA elements may be involved in modulating transcription in specific tissues. For example, even though glutamine synthetase is synthesized in several tissues, expression of its gene is high in tissues such as the liver and caput epididymidis. Analysis in vitro identified several cis-acting elements, including a basal promoter, an enhancer element in the 5′ region as well as a second enhancer element located within the first intron. Studies in transgenic mice have shown that the 5′ enhancer region plays a role in directing the high rates of transcription observed in the liver and epididymis (Lie-Venema et al., 1997).

Gene expression studies in the epididymis have focused on the activation of gene expression. However, a critical mechanism of gene regulation is the active repression of gene expression. Silencers or negative regulatory elements are cis-elements that actively repress the expression of genes (Ogbourne and Antalis, 1998). Although such elements have not received much attention in epididymal studies, they must contribute to the regulation of epididymal gene expression.

**Transcription factors**

Transcription factors are responsible for the activation or repression of transcription. Structural domains in these factors enable them to interact with specific DNA sequences and regulate gene expression. One mechanism by which region-specific gene expression may be regulated in the epididymis is the repertoire of transcription factors present along the length of the epididymis. An interesting example is the observation of a decrease in PEA3 message from the most proximal to distal regions of the epididymis (D. B. Rudyolph and B. T. Hinton, unpublished).

**Steroid hormone receptors**

Steroid hormone receptors (SRs) belong to a large family of ligand-inducible transcription factors. These receptors contain a number of functional domains critical for modulating gene expression, including a DNA-binding domain (DBD) as well as a C-terminal ligand-binding domain (LBD) responsible for high affinity ligand binding and dimerization. The N-terminal domain is highly variable in sequence and length and contains a hormone-independent transactivation function domain (AF-1). This domain is thought to interact with the basal transcriptional machinery as well as other transactivator proteins. A second activation function (AF-2) domain is located within the ligand-binding domain and is responsible for hormone-dependent activation (Tsai and O’Malley, 1994; Torchia et al., 1998).

Steroid hormone receptors that have been localized to different regions of the epididymis include the androgen receptor (AR; Takeda et al., 1990; Bentvelsen et al., 1995; You and Sar, 1998), oestrogen receptor α (ERα; Cooke et al., 1991; Hess et al., 1997; Nielsen et al., 2000) and the retinoic acid receptor (RAR; Akmal et al., 1996). Moreover, the expression of these receptors has been shown to be critical for epididymal development. A frame shift mutation in the AR gene of the testicular feminization (Tfm) mouse was found to produce a shorter protein devoid of both the DNA- and hormone-binding domains (Gaspar et al., 1991). Despite having adequate concentrations of testosterone, Tfm mice are unable to undergo male differentiation and consequently develop as females (Lyon and Hawkes, 1970). Efferent duct differentiation requires the presence of oestrogen receptor, specifically ERα. Although ERα knockout mice appear normal at birth, upon reaching puberty, the testes of these animals begin to degenerate and eventually atrophy. Analysis of sperm concentration detected a reduced number of spermatozoa, rendering the mice infertile (Lubahn et al., 1993). Histological evaluations of these mice revealed major lesions in the efferent ductules that interfered with fluid reabsorption critical for sperm concentration and maturation (Hess et al., 2000). An abnormal epididymal epithelium, accompanied by a loss of or a reduction in fertility, was also observed in transgenic mice expressing a dominant negative mutation of the RARα (Costa et al., 1997).

Within the epididymis, SRs may modulate the expression patterns of a number of hormone-regulated genes. Indeed, the expression of many epididymal proteins and mRNAs has been described as androgen-dependent. However, few
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Table 1. Steroid hormone receptor co-regulators

<table>
<thead>
<tr>
<th>Nuclear receptor target</th>
<th>Co-activators</th>
<th>Co-repressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen receptor</td>
<td>ARA70, TIF2/GRIP1/NCoA-2</td>
<td>–</td>
</tr>
<tr>
<td>Oestrogen receptor</td>
<td>SRC-1/NCoA-1, TIF2/GRIP1/NCoA-2</td>
<td>SMRT/NCoR</td>
</tr>
<tr>
<td>Retinoic acid receptor</td>
<td>p/CIP/ACTR/AIB1/RAC3/TRAM-1, p/CAF, P300/CBP</td>
<td>TIFβ, Trip1</td>
</tr>
</tbody>
</table>

AAIB: amplified in breast cancer; ACTR: activator of the thyroid hormone receptor and retinoic acid receptor; ARA70: androgen receptor associated protein 70; GRIP: glucocorticoid receptor interacting protein; NCoA: nuclear receptor co-activator; NCoR: nuclear hormone receptor co-repressor; p/CIP: p300/CBP co-integrator associated protein; p/CAF: p300/CBP associated factor; p300/CBP: p300 cyclic AMP-responsive element binding (CREB) protein; RAC: receptor associated co-activator; SMRT: silencing mediator of retinoid and thyroid hormone receptor; SRC: steroid receptor co-activator; TIF: transcription intermediary factor; TRAM: thyroid hormone receptor activator molecule; TRIP: thyroid hormone receptor interacting protein.

Information summarized from Shibata et al., 1997; Torchia et al., 1998; and Collingwood et al., 1999.

studies have demonstrated that the expression of these androgen-dependent proteins is controlled at the transcriptional level by androgen receptor via hormone response elements. Most studies have determined androgen dependence by examining gene expression before and after orchidectomy. The presence of an androgen response element (ARE) in the promoter region is an indicator that a gene may be regulated by androgens at transcription. The mouse CRISP-1 gene contains several motifs similar to those of an ARE consensus sequence (Schwidetzky et al., 1997). Nonetheless, further studies must be conducted to determine whether these putative AREs are indeed involved in the androgen-dependent expression of CRISP-1. Lareyre et al. (2000) identified two androgen-specific response regions within the 5′ flanking region of the mE-RABP. Further experiments determined that only one of these regions, ARBS-1, was the major cis-element responsible for androgen responsiveness. Functional AREs are also present in the arMEP24 promoter (Ghyselinck et al., 1993) and the gpx5 promoter (Lareyre et al., 1997).

Oestrogen- and retinoic acid-regulated genes have not been identified in the epididymis. However, oestrogen receptors may modulate the expression of genes involved in the regulation of fluid resorption in the efferent ductules. For example, oestrogens regulate the expression of aquaporin-1 (Fisher et al., 1998) but further studies must be conducted to determine whether this regulation takes place at transcription via oestrogen response elements.

Transcription factors as repressors

In addition to acting as activators, transcription factors can also function as repressors of transcription initiation. For example, PEA3 has been shown to act as a repressor of GGT-IV (Lan et al., 1999) and gpx5 (Drevet et al., 1998) transcriptional activity. Specific domains present in transcription factors mediate repression of gene expression. The KRAB (Krüppel-associated box) transcription domain of the KOX1 protein is a conserved motif present in zinc finger proteins, and is responsible for suppressing transcription activation mediated by a number of transcription factors (Peng et al., 2000). The KRAB domain has been reported to repress the expression of oestrogen-regulated genes (de Haan et al., 2000).

Co-regulators

The transcriptional activity of SRs is modulated further by a number of proteins. Co-regulators, or more specifically, co-activators and co-repressors are proteins that cooperate with traditional transcription factors to promote or repress the expression of particular genes. Co-regulators do not bind to DNA, yet act as molecular bridges between the cis-acting elements and the basal transcriptional machinery. Most of the studies on these molecules have been in the context of their modulation of steroid hormone receptor responses; however, co-regulators modulate the function of a wide range of transcriptional regulators. In the absence of ligand, steroid receptors associate with co-repressor proteins. These proteins bind to the C-terminal ligand-binding domain of steroid receptors to modulate repression of the basal transcription machinery. In the presence of hormone, co-activator complexes replace the co-repressor complex. The histone acetylase activity of some co-activators de-stabilizes the chromatin, allowing the activation of transcription (for review, see Collingwood et al., 1999). Co-regulators have been shown to be involved in the fine-tuning of gene expression. A number of co-regulators that have been found to associate with AR, ER and RAR are listed (Table 1). Although these co-regulators have not been identified in the epididymis, their presence in a number of systems indicates that they may play a role in the epididymis. Moreover, the repertoire of co-activator and co-repressor molecules in the epididymis may determine the functional activity of steroid hormone receptors within this organ.

Homeodomain proteins

Transcriptional regulation is critical for proper development of the epididymis. The activation of genes
must be orchestrated in a precise manner to allow for proper gene expression at critical times. In fact, expression of certain genes during early development dictates the fate of later gene expression and the subsequent development of an organism. Studies in Drosophila melanogaster have provided insight into some of the genetic elements involved in the control of gene expression during development and differentiation. These genes encode a homeodomain containing a highly conserved 180 bp sequence that codes for the 60 amino acid DNA-binding domain of homeodomain proteins. Mutations in any of these genes result in homeotic transformations (that is, replacement of one body structure for another) or alteration in the number of body segments (for review, see Gehring et al., 1994). Homeodomain proteins are critical regulators of transcription and their role in segmental development is of unique interest in epididymal research.

Hox genes belong to a family of homeobox-containing genes. Expression of Hox genes in the epididymis indicates that these genes code for transcription factors necessary for correct epididymal development. Hoxc-8 (previous nomenclature Hox-3.1) is expressed in the developing epididymis of mice (Le Mouellic et al., 1992) and rats (Lindsey and Wilkinson, 1996) and Hoxa-10 is required for normal development of the Wolffian duct (Podlasek et al., 1999). Targeted mutations of Hoxa-11 in mice resulted in male and female infertility, and the males exhibited abnormal development of the vas deferens. Not only were the vasa deferentia of the mutant mice smaller but also their structure resembled that of the epididymis indicating that the vas deferens had undergone a homeotic transformation (Hsieh-Li et al., 1995). Another family of genes that appear to be critical for Wolffian duct development are the Pax genes, characterized by a 128 amino acid domain known as the paired domain. In addition to the identification of homeodomain protein in the epididymis, research has been directed at evaluating the molecular mechanisms responsible for the expression pattern of these genes. Recent studies identified an 8.5 kb fragment critical for Pax-2 expression in the Wolffian duct (Kuschert et al., 2001).

Finally, homeodomain proteins may be involved in the segmentation of the adult epididymis (Bomgardner et al., in press). The Pem homeobox gene, which is distantly related to the Pdr/pax homeobox gene family, is expressed in the epididymis of adult rats and may be partly androgen-dependent (Lindsey and Wilkinson, 1996). Further studies will elucidate the specific roles of homeodomain proteins on the transcriptional regulation of the gene expression patterns necessary for proper epididymal development and function.

Role of signal transduction pathways

Extracellular signals play a key role in the transcriptional regulation of gene expression. In the epididymis, several mechanisms are likely to govern the expression of necessary genes. Androgen-dependent regulation is a critical way to control gene expression within the epididymis. Indeed, without androgens, the epididymis fails to develop. But in addition to androgens, several genes expressed in the proximal regions of the epididymis are controlled by testicular factors such as testicular-derived growth factors (Lan et al., 1998) or possibly spermatozoa-associated factors (Garret et al., 1990). Furthermore, autocrine regulation by the epididymal epithelium or paracrine regulation by the supporting cells may also play a role. All of these mechanisms together will result in a repertoire of gene expression patterns that are the handprint of the epididymis. Unfortunately, signal transduction mechanisms have not been widely studied in the epididymis in part due to the lack of an adequate tissue culture model system.

A number of studies have provided evidence that factors originating from the testis and travelling via rete testis fluid regulate gene expression in the epididymis, especially in the proximal regions. GGT-IV expression in the initial segment of the epididymis is under the control of testicular factors (Palladino and Hinton, 1994) and studies indicate that basic fibroblast growth factor (bFGF) may be the testicular factor responsible for this regulation. Western blot analysis showed the presence of bFGF-like proteins in initial segment homogenates as well as in epididymal fluid (Lan et al., 1998). Moreover, bFGF was able to restore GGT protein and catalytic activity in the initial segment after 3 day efferent duct ligation. Basic FGF has been hypothesized to regulate GGT-IV expression in the initial segment by the activation of the Ras-dependent ERK pathway (Lan et al., 1998). This pathway has been found to be a general signalling pathway used by a number of growth factors to regulate gene expression.

In addition to GGT IV, several other genes have been described to be under the control of testicular factors. The list increases rapidly with new publications, but several interesting examples in the context of the transcriptional control mechanisms discussed include retinoic acid-binding protein (Zwain et al., 1992), S Asians (Viger and Robaire, 1994), A-raf (Winer and Wolgemuth, 1995) and PEA3 (Lan et al., 1997). Other epididymal genes may also be controlled by growth factors of testicular origin.

The most proximal region of the epididymis, the initial segment, appears to be less dependent on androgens for its development and maintenance of function, as evidenced by the failure of male c-ros knockout mice to develop an initial segment (Sonnenberg-Riethmacher et al., 1996). c-ros is a member of the receptor tyrosine kinase family, the ligand of which is unknown at present. Keilhack et al. (2001) showed that SHP-1, a tyrosine phosphatase, binds to the c-ros cytoplasmic tail and downregulates tyrosine phosphorylation. An incomplete knockout of SHP-1 in mice leads to a similar phenotype in the initial segment as that observed in c-ros knockout mice (Keilhack et al., 2001).

The ApoER receptor, which is expressed in the caput epididymidis has been shown to have not only endocytic capacity, but also signalling capacity via reelin signalling through the c-jun kinase (JNK) cascade (Stockinger et al.,...
Because JNKs are known to activate the AP-1 transcriptional machinery, this new signalling pathway may play a role in gene expression in the epididymis. Hence, studies are needed to determine whether the ApoER receptor has signalling capacity in the epididymis. Clearly, signal transduction cascades act at several levels to control gene expression. Studies aimed at identifying novel roles for these signalling pathways in the epididymis will enhance understanding of gene expression in this tissue and may shed light on the development and maintenance of the unique segmentation that exists within the epididymis.

**Chromatin structure**

Chromatin structure plays an integral role in the regulation of transcription (for reviews, see Struhl, 1998; Luo and Dean, 1999; Spencer and Davie, 1999). Nucleosomes act as transcriptional repressors by preventing access of transcription factors to promoter regions. A number of mechanisms have been found to alter chromosomal structure allowing access of transcription factors to DNA regions. The role of histone modification in transcriptional regulation, specifically the role of histone acetylation, has been of particular interest. The N-terminal domain of core histones contains a number of positively charged lysine residues, sites of histone acetylation. Acetylation of histones is thought to cause the dissociation of these proteins from the DNA, thereby exposing DNA-binding sites that are recognized by transactivator proteins. Examples of histone acetylases include p300/CBP, P/CAF, activator of the thyroid and retinoic acid receptor (ACTR), steroid receptor co-activator (SRC-1) and TAF130/250 (Luo and Dean, 1999). Many of these proteins were implicated as having a role in the regulation of transcription before their identification as histone acetylases (Struhl, 1998; Luo and Dean, 1999). For example, the TAF 130/250 histone acylase is a subunit of the TFIIID complex involved in the basal transcriptional machinery. Besides the presence of histone acetylases, a number of histone deacetylases involved in transcriptional repression via the stabilization of the chromatin structure have also been described.

In addition to histone acetylation–deacetylation, a second system has been implicated in the transcriptional regulation of gene expression via chromatin remodelling. Chromosome-remodelling complexes that contain adenosine triphosphatase (ATPase) activity have been shown to disrupt histone–DNA interactions. Chromatin remodelling complexes include the SWI/SNF complex described in yeast and the BRG1/Brm complex described in higher eukaryotes (Luo and Dean, 1999).

The roles of chromatin remodelling by histone acetylation–deacetylation and ATP-dependent chromatin remodelling complexes in the epididymis have yet to be examined. However, the alteration of chromosomal structure is a critical step for initiation of transcription and is therefore likely to play a role in the regulation of epididymal gene expression. In fact, steroid hormone receptors have been implicated in chromosome remodelling via the recruitment of co-activators, which alter the histone acetylation status. The role of chromatin remodelling in regulating epididymal gene expression warrants future study.

**Post-transcriptional regulation**

Studies have focussed on the regulation of transcription; however, regulation of gene expression can also occur at RNA processing, nuclear export of each RNA species, RNA localization, mRNA decay, translation and post-translational events. Although discussion of the regulation of each post-transcriptional step is beyond the scope of this review, this aspect of gene regulation is clearly relevant. Thus, the role of mRNA stability will be discussed briefly.

The steady-state level of any gene that is being expressed is a balance between transcript synthesis and degradation. Hence, the regulation of mRNA turnover plays a major role in the overall control of gene expression. Messenger RNAs must be protected from endogenous nucleases as they move out of the nucleus to the cytoplasm for translation. At the same time, cells need to degrade their mRNAs in a regulated manner. Therefore, mRNAs with long half-lives may be necessary when there is constant demand for protein (for example, housekeeping genes), whereas mRNAs with short half-lives are probably involved in situations in which a cell needs to respond rapidly to a stimulus. Hence, half-lives of messages can range from minutes to days, although the average half-life of many mRNAs is about 12–24 h.

Each mRNA that is transcribed is flanked by 3′ and 5′ untranslated regions (UTRs). Both regions contain important cis elements that ultimately control translation, localization and stability. Furthermore, the ends of the 3′ and 5′ regions contain specializations, such as the 5′ cap, 3′ poly (A) tail and secondary structure, that play important roles in the translation of mRNA and its stability. Messenger RNA decay is a consequence of 3′ deadenylation followed by decapping and 5′–3′ decay by exonucleases or endonucleases. Decay can also occur from 3′ to 5′; however, cis-acting elements that bind specific RNA-binding proteins together with the structure (for example, stem loops) of the 3′ UTR tend to regulate the stability of many messages. The open reading frames of some genes contain cis elements, which may also affect stability (Shyu et al., 1989). For more detailed information on the function of the 3′ and 5′ UTRs, see reviews by Jackson and Wickens (1997), Wickens et al. (1997), Mitchell and Tollervey (2000) and Staton et al. (2000).

Although the 3′ UTR region plays a critical role in the regulation of mRNA stability, the 5′ UTR of epididymal GGT mRNAs may be important in maintaining stability of these mRNAs. Previous studies have shown that three of the four GGT mRNAs (II–IV) transcribed from a single copy rat GGT gene are expressed and regulated differentially in the rat epididymis (Palladino and Hinton, 1994). After 12 h loss of testicular factors, there is an approximately 50% decline in the steady-state concentrations of GGT mRNA IV. This
decline is due primarily to a reduction in the rate of transcription, although mRNA stability appeared to contribute to the decline in the steady-state concentrations (Rudolph and Hinton, 1997). The rate of decay for GGT mRNA IV differs from the rates of decay for GGT mRNAs II and III, yet all three transcripts contain a common 3′ UTR, coding region and -144 bp 5′ of the coding region. These three transcripts differ only in their 5′ UTRs. Hence, the differences noted in the stability of each mRNA species are accounted for by their differences in their 5′ UTRs. Rudolph and Hinton (1997) speculated that the differences in the secondary structure of the 5′ UTRs were responsible for the differences in decay rates. However, the interaction among the 3′ UTR, 5′ UTR and RNA-binding proteins may also contribute to the differences in decay.

In the pituitary gland, hormones such as testosterone, oestrogen and progesterone regulate the stability of mRNA for other hormones including TSH, FSH, GH and LH. Hence, hormones may play an important role in the stability of many epididymal genes. Shaw and Kamen (1986) examined the 3′ UTR for AU-rich elements (AREs) and showed that the expression of some epididymal genes may also be controlled post-transcriptionally and in terms of their mRNA stability. AREs (for example, AUUUA) and their binding proteins (for example, HuR; Levine et al., 1993) may influence mRNA stability. Several epididymal genes contain AREs primarily in the 3′ UTR, although AREs may also be present in the coding region and the 5′ UTR (Table 2). Hence, epididymal mRNAs containing multiple AREs may also be regulated by stability of message. However, the lack of AREs is not an indication that an mRNA is not regulated at the level of mRNA stability since the 5′ UTR and the secondary structure of the 3′ UTR are also responsible for message stability.

### Table 2. Analysis of the 3′ untranslated regions (UTR) of a number of epididymal genes for the presence of AU-rich elements (ARE)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Presence of ARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse polyomavirus enhancer activator 3</td>
<td>None</td>
</tr>
<tr>
<td>Mouse gamma-glutamyl transpeptidase</td>
<td>One copy in 3′ UTR</td>
</tr>
<tr>
<td>Rat gamma-glutamyl transpeptidase</td>
<td>None</td>
</tr>
<tr>
<td>Human HE2</td>
<td>Two copies in coding region; one copy in 3′ UTR</td>
</tr>
<tr>
<td>Human HE5</td>
<td>None</td>
</tr>
<tr>
<td>Human HE6</td>
<td>Eight copies in coding region; five copies in 3′ UTR</td>
</tr>
<tr>
<td>Rat 5 alpha reductase</td>
<td>Tandem AUUUAUUUA in coding region</td>
</tr>
<tr>
<td>Human 5 alpha reductase 2</td>
<td>Two copies in coding region; six copies in 3′ UTR</td>
</tr>
<tr>
<td>Human 5 alpha reductase 1</td>
<td>One copy in coding region; eight copies in 3′ UTR</td>
</tr>
<tr>
<td>Mouse CRISP 1</td>
<td>Two copies in coding region; seven in 3′ UTR</td>
</tr>
<tr>
<td>Human CRISP 1</td>
<td>One copy in 5′ UTR; one copy in coding region; 11 copies in 3′ UTR</td>
</tr>
<tr>
<td>Mouse ADAM 7</td>
<td>Four copies in coding region; one copy in 3′ UTR</td>
</tr>
<tr>
<td>Rat ADAM 7</td>
<td>Four copies in coding region; five copies in 3′ UTR</td>
</tr>
<tr>
<td>Human CRES</td>
<td>None</td>
</tr>
</tbody>
</table>

Analysis was performed using GeneRunner (Hastings Software).

### Conclusion

Region-specific and cell-specific patterns of gene expression in the epididymis indicate that an intricate number of mechanisms are involved in regulating these complex patterns of gene expression. Epididymal studies are currently dominated by efforts to identify key DNA elements and associated transcription factors responsible for the expression of specific genes. Clearly, a number of other players, such as co-regulators, are critical for the activation and repression of gene expression. Therefore, further studies are needed to identify such molecules in the epididymis. The regulatory mechanisms operating in the epididymis must be understood within the framework of chromatin structure since, ultimately, these mechanisms must work in concert to govern the proper development of epididymal structure and function.

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