Regulation of gene transcription in the epididymis

Carmen M. Rodríguez, Jennifer L. Kirby and Barry T. Hinton*

*Department of Cell Biology, University of Virginia Health Sciences Center,
Charlottesville, VA, USA*

The epididymis exhibits region-specific as well as cell-specific patterns of gene expression within the epithelium. The spatial and temporal patterns of gene expression originate during development and are critical to the formation and maintenance of a fully functional epididymis. Despite the number of mechanisms reported to contribute to the regulation of eukaryotic gene expression, 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.

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. Nevertheless, the region-specific and cell-specific patterns of gene expression inherent to the epididymis indicate that an intricate number of mechanisms are involved in

regulating such complex patterns of gene expression. These mechanisms must work in concert to govern the proper development of epididymal structure and function.

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.

*Correspondence
Email: bth7c@virginia.edu

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 transcriptional activity of the mouse epididymis caput-specific *gpx5* gene (Drevet *et al.*, 1998). Although the expression of many genes may be considered to be tissue-specific, 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. Rudolph 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 (ER α ; 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 ER α . Although ER α 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 RAR α (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

Table 1. Steroid hormone receptor co-regulators

Nuclear receptor target	Co-activators	Co-repressors
Androgen receptor	ARA70, TIF2/GRIP1/NCoA-2	–
Oestrogen receptor	SRC-1/NCoA-1, TIF2/GRIP1/NCoA-2	SMRT/NCoR
Retinoic acid receptor	p/CIP/ACTR/AIB1/RAC3/TRAM-1, p/CAF, P300/CBP	SMRT/NCoR, TIF1 α , TIF β , Trip1

AIB: 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 *Prd/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), 5 α -reductase (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.*,

2000). 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 acetylase is a subunit of the TFIID 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

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

Gene	Presence of ARE
Mouse polyomavirus enhancer activator 3	None
Mouse gamma-glutamyl transpeptidase	One copy in 3' UTR
Rat gamma-glutamyl transpeptidase	None
Human HE2	Two copies in coding region; one copy in 3' UTR
Human HE5	None
Human HE6	Eight copies in coding region; five copies in 3' UTR
Rat 5 alpha reductase	Tandem AUUUUUUA in coding region
Human 5 alpha reductase 2	Two copies in coding region; six copies in 3' UTR
Human 5 alpha reductase 1	One copy in coding region; eight copies in 3' UTR
Mouse CRISP 1	Two copies in coding region; seven in 3' UTR
Human CRISP 1	One copy in 5' UTR; one copy in coding region; 11 copies in 3' UTR
Mouse ADAM 7	Four copies in coding region; one copy in 3' UTR
Rat ADAM 7	Four copies in coding region; five copies in 3' UTR
Human CRES	None

Analysis was performed using GeneRunner (Hastings Software).

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.

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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References

Key references are identified by asterisks.
Akmal KM, Dufour JM and Kim KH (1996) Region-specific localization of retinoic acid receptor- α expression in the rat epididymis *Biology of Reproduction* **54** 1111-1119
Bentvelsen FM, Brinkmann AO, van der Schoot P, van der Linder JE, van der Kwast ThH, Boersma WJA, Schröder FH and Nijma JM (1995) Developmental pattern and regulation by androgens of androgen receptor expression in the urogenital tract of the rat *Molecular and Cellular Endocrinology* **113** 245-253
***Bomgardner D, Hinton BT and Turner TT** Hox transcription factors may play a role in regulating segmental function of the adult epididymis *Journal of Andrology* (in press)
***Collingwood TN, Urnov FD and Wolffe AP** (1999) Nuclear receptors:

- coactivators, corepressors and chromatin remodeling in the control of transcription *Journal of Molecular Endocrinology* **23** 255–275
- Cooke PS, Young P, Hess RA and Cunha GR (1991) Estrogen receptor expression in developing epididymis, efferent ductules, and other male reproductive organs *Endocrinology* **128** 2874–2879
- Costa SL, Boekelheide K, Vanderhyden BC, Seth Rahul and McBurney MW (1997) Male infertility caused by epididymal dysfunction in transgenic mice expressing a dominant negative mutation of retinoic acid receptor α *Biology of Reproduction* **56** 985–990
- de Haan G, Chusacutanachai S, Mao C, Katzenellenbogen BS and Shapiro DJ (2000) Estrogen receptor–KRAB chimeras are potent ligand-dependent repressors of estrogen-regulated gene expression *Journal of Biological Chemistry* **275** 13 493–13 501
- Drevet JR, Lareyre JJ, Schwaab V, Vernet P and Dufaure JP (1998) The PEA3 protein of the Ets oncogene family is a putative transcriptional modulator of the mouse epididymis-specific glutathione peroxidase gene *gpx5* *Molecular Reproduction and Development* **49** 131–140
- Fisher JS, Turner KJ, Fraser HM, Saunders PTK, Brown D and Sharpe RM (1998) Immunoeexpression of aquaporin-1 in the efferent ducts of the rat and marmoset monkey during development, its modulation by estrogens, and its possible role in fluid resorption *Endocrinology* **139** 3935–3945
- Garrett SH, Garrett JE and Douglass J (1990) A spermatozoa-associated factor regulates proenkephalin gene expression in the rat epididymis *Molecular Endocrinology* **4** 108–118
- Gaspar ML, Meo T, Bourgairel P, Guenet JL and Tosi M (1991) A single base deletion in the Tfm androgen receptor gene creates a short-lived messenger RNA that directs internal translation initiation *Proceedings of the National Academy of Sciences USA* **88** 8606–8610
- Gehring WJ, Affolter M and Bürglin T (1994) Homeodomain proteins *Annual Review of Biochemistry* **63** 487–526
- Ghyselinck NB, Sufaure I, Lareyre J-J, Rigaudière N, Mattéi M-G and Dufaure J-P (1993) Structural organization and regulation of the gene for the androgen-dependent glutathione peroxidases-like protein specific to mouse epididymis *Molecular Endocrinology* **7** 258–272
- Hess RA, Gist DH, Bunick D, Lubahn DB, Farrel A, Bahr J, Cooke PS and Greene GL (1997) Estrogen receptor (alpha and beta) expression in the excurrent ducts of the adult male rat reproductive tract *Journal of Andrology* **18** 602–611
- Hess RA, Bunick D, Lubahn DB, Zhou Q and Bouma J (2000) Morphologic changes in efferent ductules and epididymis in estrogen receptor-alpha knockout mice *Journal of Andrology* **21** 107–121
- Hsieh-Li HM, Witte DP, Weinstein M, Branford W, Li H, Small K and Potter SS (1995) Hoxa 11 structure, extensive antisense transcription, and function in male and female fertility *Development* **121** 1373–1385
- Jackson RJ and Wickens M (1997) Translational controls impinging on the 5'-untranslated region and initiation factor proteins *Current Opinion in Genetics and Development* **7** 233–241
- Keilhack H, Müller M, Böhmer S-A, Carsten F, Weidner KM, Birchmeier W, Ligensa T, Berndt A, Kosmehl H, Günther B, Müller T, Birchmeier C, Böhmer FD (2001) Negative regulation of Ros receptor tyrosine kinase signaling: an epithelial function of the SH2 domain protein tyrosine phosphatase SHP-1 *Journal of Cell Biology* **152** 325–334
- Kuschert S, Rowitch DH, Haenig B, McMahon AP and Kispert A (2001) Characterization of Pax-2 regulatory sequences that direct transgene expression in the Wolffian duct and its derivatives *Developmental Biology* **229** 128–140
- Lan Z-J, Palladino MA, Rudolph DB, Labus DB and Hinton BT (1997) Identification, expression, and regulation of the transcriptional factor polyomavirus enhancer activator 3, and its putative role in regulating the expression of gamma-glutamyl transpeptidase mRNA-IV in the rat epididymis *Biology of Reproduction* **57** 186–193
- Lan Z-J, Labus JC and Hinton BT (1998) Regulation of gamma-glutamyl transpeptidase catalytic activity and protein level in the initial segment of the rat epididymis by testicular factors: role of basic fibroblast growth factor *Biology of Reproduction* **58** 197–206
- Lan Z-J, Lye RJ, Holic N, Labus JC and Hinton BT (1999) Involvement of polyomavirus enhancer activator 3 in the regulation of expression of gamma-glutamyl transpeptidase messenger ribonucleic acid-IV in the rat epididymis *Biology of Reproduction* **60** 664–673
- Lareyre JJ, Claessens F, Rombauts W, Dufaure JP and Drevet JR (1997) Characterization of an androgen response element within the promoter of the epididymis-specific murine glutathione peroxidase 5 gene *Molecular and Cellular Endocrinology* **129** 33–46
- Lareyre JJ, Thomas TZ, Zheng W-L, Kasper S, Ong DE, Orgebin-Crist M-C and Matusik RJ (1999) A 5-kilobase pair promoter fragment of the murine epididymal retinoic acid-binding protein gene drives tissue-specific, cell-specific, and androgen-regulated expression of a foreign gene in the epididymis of transgenic mice *Journal of Biological Chemistry* **274** 8282–8290
- Lareyre JJ, Reid K, Nelson C, Kasper S, Rennie PS, Orgebin-Crist M-C and Matusik RJ (2000) Characterization of an androgen-specific response region within the 5' flanking region of the murine epididymal retinoic acid binding protein gene *Biology of Reproduction* **63** 1881–1892
- Le Mouellie H, Lallemand Y and Phillippe B (1992) Homeosis in the mouse induced by a null mutation in the Hox-3.1 gene *Cell* **69** 251–264
- Levine TD, Gao F, King PH, Andrews LG and Keene JD (1993) Hel-N1, an autoimmune RNA-binding protein with specificity of 3' uridyate-rich untranslated regions of growth factor mRNAs *Molecular and Cellular Biology* **13** 3494–3504
- Lie-Venema H, de Boer PA, Moorman AF and Lamers WH (1997) Role of the 5' enhancer of the glutamine synthetase gene in its organ-specific expression *Biochemical Journal* **323** 611–619
- Lindsey JS and Wilkinson MF (1996) An androgen-regulated homeobox gene expressed in the rat testis and epididymis *Biology of Reproduction* **55** 975–983
- Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS and Smithies O (1993) Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene *Proceedings of the National Academy of Sciences USA* **90** 11 162–11 166
- *Luo RX and Dean DC (1999) Chromatin remodeling and transcriptional regulation *Journal of the National Cancer Institute* **91** 1288–1294
- Lyon MF and Hawkes SG (1970) X-linked gene for testicular feminization in the mouse *Nature* **227** 1217–1219
- Mitchell P and Tollervey D (2000) mRNA stability in eukaryotes *Current Opinion in Genetics and Development* **10** 193–198
- Nielsen M, Björnsdóttir S, Høyer PE, and Byskov AG (2000) Ontogeny of oestrogen receptor α in gonads and sex ducts of fetal and newborn mice *Journal of Reproduction and Fertility* **118** 195–204
- *Ogbourne S and Antalís TM (1998) Transcriptional control and the role of silencers in transcriptional regulation in eukaryotes *Biochemical Journal* **331** 269–282
- Palladino MA and Hinton BT (1994) Expression of multiple γ -glutamyl transpeptidase messenger ribonucleic acid transcripts in the adult rat epididymis is differentially regulated by androgens and testicular factors in a region-specific manner *Endocrinology* **135** 1146–1156
- Peng H, Begg GE, Harper SL, Friedman JR, Speicher DW and Rauscher FJ (2000) Biochemical analysis of the Kruppel-associated box (KRAB) transcriptional repression domain *Journal of Biological Chemistry* **275** 18 000–18 010
- Podlasek CA, Seo RM, Clemens JQ, Ma L, Maas RL and Bushman W (1999) Hoxa-10 deficient male mice exhibit abnormal development of the accessory sex organs *Developmental Dynamics* **214** 11–12
- Rudolph DB and Hinton BT (1997) Stability and transcriptional regulation of gamma-glutamyl transpeptidase mRNA expression in the initial segment of the rat epididymis *Journal of Andrology* **18** 501–512
- Schwidetzky U, Schleuning W-D and Haendler B (1997) Isolation and characterization of the androgen-dependent mouse cysteine-rich secretory protein-1 (CRISP-1) gene *Biochemical Journal* **321** 325–332
- Shaw G and Kamen R (1986) A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation *Cell* **46** 659–667
- Shibata H, Spencer TE, Oñate SA, Jenster G, Tsai SY, Tsai M-J and O'Malley BW (1997) Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action *Recent Progress in Hormone Research* **52** 141–165
- Shyu AB, Greenberg M and Belasco J (1989) The c-fos transcript is targeted

- for rapid decay by two distinct mRNA degradation pathways *Genes and Development* **3** 60–72
- Smale ST and Baltimore D** (1989) The 'Initiator' as a transcription control element *Cell* **57** 103–113
- Sonnenberg-Riethmacher E, Walter B, Riethmacher D, Godecke S and Birchmeier C** (1996) The c-ros tyrosine kinase receptor controls regionalization and differentiation of epithelial cells in the epididymis *Genes and Development* **10** 1184–1193
- Spencer VA and Davie JR** (1999) Role of covalent modifications of histones in regulating gene expression *Gene* **240** 1–12
- Staton JM, Thomson AM and Leedman PJ** (2000) Hormonal regulation of mRNA stability and RNA-protein interactions in the pituitary *Journal of Molecular Endocrinology* **25** 17–34
- Stockinger W, Brandes C, Fasching D, Hermann M, Gotthardt M, Herz J, Schneider WJ and Nimpf J** (2000) The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2 *Journal of Biological Chemistry* **275** 25 625–25 632
- Struhl K** (1998) Histone acetylation and transcriptional regulatory mechanism *Genes and Development* **12** 599–606
- Takeda H, Chodak G, Mutchnik S, Nakamoto T and Chang C** (1990) Immunohistochemical localization of androgen receptors with mono- and polyclonal antibodies to androgen receptor *Journal of Endocrinology* **126** 17–25
- *Torchia J, Glass C and Rosenfeld MG** (1998) Co-activators and co-repressors in the integration of transcriptional responses *Current Opinion in Cell Biology* **10** 373–383
- Tsai M-J and O'Malley BW** (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members *Annual Review of Biochemistry* **63** 451–486
- Viger RS and Robaire B** (1994) Immunocytochemical localization of 4-ene steroid 5 alpha-reductase type 1 along the rat epididymis during postnatal development *Endocrinology* **134** 2298–2306
- Wickens M, Anderson P and Jackson RJ** (1997) Life and death in the cytoplasm: messages from the 3' end *Current Opinion in Genetics and Development* **7** 220–232
- Winer MA and Wolgemuth DJ** (1995) The segment-specific pattern of A-raf expression in the mouse epididymis is regulated by testicular factors *Endocrinology* **136** 2561–2572
- You L and Sar M** (1998) Androgen receptor expression in the testis and epididymides of prenatal and postnatal Sprague–Dawley rats *Endocrine* **9** 253–261
- Zwain IH, Grima J and Cheng CY** (1992) Rat epididymal retinoic acid-binding protein: development of a radioimmunoassay, its tissue distribution, and its changes in selected androgen-dependent organs after orchiectomy *Endocrinology* **131** 1511–1526