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RESEARCH ARTICLE

GENETIC REGULATION OF EPIDIDYMAL MICROENVIRONMENT

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ABSTRACT

During their transit through the epididymis, spermatozoa undergo many biochemical modifications necessary to acquire flagellar motility and fertilizing ability. These modifications, collectively called sperm maturation, are well orchestrated along the epididymis. Sperm maturation requires the interaction of spermatozoa with proteins that are synthesized and secreted in a region-specific manner by principle cells of the epididymal epithelium and depend on highly regionalized gene expression patterns. But the regulatory processes that establish the discrete epididymal microenvironments remain yet largely unknown. Based on clinical observations, the role of the epididymis in human sperm maturation has been questioned but results obtained using animal models can be extrapolated to human with caution

INTRODUCTION

The epididymis consists of a single, highly coiled and convoluted tubule that Antoine De Graaf, the famous seventeenth century anatomist, compared to a thread thickening to a string. The mammalian epididymis is a segmented organ comprised of a single, highly coiled tubule conventionally divided into caput, corpus, and cauda regions in larger sections. As mammalian spermatozoa pass from the testis into the epididymis they possess a specialized and distinct morphology, but are infertile and are bathed as an they progress from siminiferous tubule to the ductus deferens. Through a number of absorptive, secretory, and contractile processes, each region of the epididymis contributes to the concentration, maturation, transport, or storage of sperm (Robaire 1988; Cornwall 2002). The hallmarks of the maturational process are the acquisition of motility and the competence to undergo capacitation, leading to the ability to fertilize an egg (Turner 1995; Visconti *et al.*, 1995). Spermatozoa are transcriptionally and translationally inactive. Therefore, extratesticular maturation of spermatozoa is not under the control of the germinal genome; rather, it is mediated by factors with in the epididymal fluid. The composition of the luminal environment of the epididymis changes continually due to both secretion and removal of specific ions, small organic molecules, and proteins from the luminal compartment of the organ. The microenvironment created along the length of the epididymal tubules are essential to the molecular modification of spermatozoa that results in fertile gametes. Each epididymal region is characterized by its own gene expression pattern encoding its specific secretome sequentially interacting with the maturing spermatozoa (Kirchhoff 1999). But the regulatory processes that establish the discrete epididymal microenvironments remain largely yet unknown.

Segment specific microenvironment

Most studies to date have used relatively large epididymal regions (caput, corpus, cauda) as boundaries for analysis of intraepididymal gene expression patterns (Cornwall *et al.*, 1992, 1999). In describing the localization of epididymal gene expression, these regions have also been further divided (e.g., proximal, mid, and distal caput). The epididymal regions can also be definitively subdivided into discrete, intraregional segments or tes. lobules of coiled tubule bounded by connective tissue septae (Vernet *et al*., 1997; Eberspaecher *et al*., 1995). Over the past decade it has become evident that many gene expression patterns within these regions are restricted to one or more segments (Eberspaecher *et al*., 1999). This implies that these segments are distinct regulatory subunits of the epididymis (Eberspaecher *et al*., 1999; Rankin *et al.*, 1992) and may play an important role in directing the tightly regulated composition of the epididymal tubule fluid. In addition to being region-specific, a number of epididymal genes are tissue-specific, expressed exclusively in the adult epididymis. Such genes include the initial segment (IS)-specific ros1 proto-oncogene (Ros1) (Sonnenberg-Riethmacher *et al.*,1996) and lipocalin 8 (Lcn8) 9 (Lareyre *et al.*, 2001) and the distal caput-specific lipocalin 5 (Lcn5) (Lareyre *et al.*,1998). In addition, there are numerous other genes that are expressed predominantly in the epididymis and only at lower levels in other tissues. IS-specific cystatin 8 (Cst8) (Cornwall *et al.*,1992, 1999), caput-specific glutathione peroxidase 5 (Gpx5) (Vernet et.al.1997), and corpus- and cauda specific cysteine-rich secretory protein 1 (Crisp1) (Eberspaecher *et al*., 1995) fall into this category. In addition to genes with sharply restricted expression in certain epididymal regions (Lareyre *et al*.,2001), some exhibit more gradual changes of expression between the different regions, leading to characteristic checkerboard-type expression patterns for many epididymal genes (Eberspaecher *et al*., 1999; Rankin *et al.*,1992; Lareyre *et al.*,1998). Furthermore, there are several epithelial cell types present in the ductus epididymis, and several epididymal genes exhibit cell specificity, most of them being expressed only in the principal cells (Blomqvist 2006). Most recently the application of gene profiling technology to the epididymis has yielded volumes of information of segment-specific gene expression that is available to the public Because the gene chips used in these studies represented sequences derived from early-stage embryos, the microarray analysis was biased

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Gene knockout in relation with different epididymal segmental microenvironment

Gene	Expression in epididymal region
Apolipoprotein E receptor 2 (apoer2)	Expressed in initial segment. Member of low density lipoprotein receptor gene family. Increased level of clusterin in epididymal luminal fluid of the knockout suggests apoer2 also function as a clusterin receptor (Andersen 2003).
Apolipoprotein B (apob)	Expressed in testis and epididymis. Maintain lipid absorbtion and triglyceride homeostatis. (Huang 1996)
Estrogen receptor- alpha (Es1)	Expressed in efferent duct and initial segment Transcription factor Helps in fluid reabsorption (Hess1997; Zhou 2001)
Anion exchanger 2 (slc4a2)	Expressed in initial segment caput and less expressed in cauda. Na dependent anion transporter(Medina *et al.,* 2003)
HE6(Gpr64)	Expressed in efferent ducts and initial segment. Member of G-protein coupled receptor (Davies *et al.*,2004)
c-ros tyrosine kinase receptor (Ros 1)	Expressed in initial segment Tyrosine kinase receptor (Sonnenberg-Riethmacher *et al.*, 1996; Yeung *et al.*,2002;2004 Frenoux *et al.*,2004;; Morales *et al.*,2000)
Nuclear oxysterol receptor LXR alpha and beta (lxr)	Highest in caput Transcription factor activated by oxysterol (Frenoux *et al*.,2004)
Prosaposin (psap)	Expressed in epididyal lumen Lysosomalactivator (Morales *et al.*,2000)
Nuclear phospholipids hydroperoxide glutathione (Gpx4)	Expressed in epididymal germ cells Member of glutathione peroxidase family (Conrad *et al.*,2005)
Inositol polyphosphate 5-phosphatase (Inpp5b)	Expressed in Sertoli cells, germ cells. Signaling protein in IP3 pathway. (Hellsten *et al.*,2001)
FSH receptor (Fshr)	Follicle stimulating hormone receptor Expressed in sertoli cells, decreased in caput and corpus. (Grover *et al.*,2005)
Cathepsin A (Ctsa)	Expressed in caput and corpus Lysosomal carboxypeptidae (Korah 2003)
β-hexosaminidase A and B (Hexa b)	Expressed in initial segment Lysosomal enzyme (Adamali *et al.*,1999)
Mononuclear phagocytic growth factor stimulating colony factor (Csf1)	Expressed in caput and cauda epididymis.(Pollard *et al*.,1997)
Somatic and testis specific angiotensin converting enzyme (Ace)	Regulator of the rennin-angiotensin system Help in sperm transport and zona binding (Hagaman 1998; Fuchs *et al.*,2005; Zhao *et al.*,1998)
Bone morphogenetic protein 8A (Bmp8A)	Maintain epididymal epithelium (Kondoh *et al.*,2005)
γ- Glutamyl transpeptidase (Ggtp)	Mainain structure of epidymis (Lieberman *et al.*,1996)
Hoxa 10 and Hoxa 11	Transcription factors Helps in epidymis morphogenesis during development. (Hsieh-Li *et al.*,1995; Satokata 1995)

toward identifying new subsets of genes expressed in the adult epididymis. Johnston *et al*. examined the varying expression profiles of genes from all segments of the mouse epididymis using Affymetrix mouse gene chips. This information is available in a searchable website at the Mammalian Reproductive Genetics database (http://mrg. genetics.washington.edu). Because each study used different sources of sequences, a broad representation of sequences is presented. During epididymal transit, sperm-associated proteins including ADAM2 (fertilin), ADAM3 (cyritestin), ADAM24 (testase), CE9, and others are proteolytically processed to their mature and presumably functionally active forms (Lum *et al.*,1997; Kim *et al*., 2004 Zhu *et al*., 2001; Petruszak *et al.*,1991). Although the identity of the proteases involved in the activation of these proteins is not known, furin-like proteases have been implicated for several of these processing events (Lum *et al.*, 1997). Recent studies showed that several members of the prohormone convertase family of proprotein processing enzymes including furin, PC7, PC4, and PACE4 are expressed in the epididymis in a regionalized manner and several are present in epididymal fluid suggesting a possible role for these proteases in sperm maturational events. Indeed, PC4 knockout male mice are infertile despite normal spermatogenesis and motility, suggesting that critical fertilization molecules may not be processed correctly (Mbikay *et al.*, 1997). Other proteases expressed in a segment-specific manner in the epididymis include several of the matrix metalloproteases, MMP2, MMP3, MMP9 (Metayer *et al*., 2002), ADAM28 (Oh *et al.*, 2005), and procathepsin L (Okamura *et al*.,1995).Several protease inhibitors that show segment-specific expression have also been identified. Several members of the cystatin-related epididymal spermatogenic (CRES) subgroup of family 2 cystatins of cysteine protease inhibitors including CRES, CRES2, CRES3, and cystatin E2 are highly restricted to the initial segment region of the mouse epididymis (Cornwall 1002; 2003; Hsia *et al*., 2003; Li *et al.*, 2003). Although the function of these secretory proteins in vivo is not known, in vitro CRES is an inhibitor of several members of the prohormone convertases (Cornwall *et al.*, 2003), suggesting CRES may regulate proprotein processing events in the epididymis.Other protease inhibitors expressed in the epididymis include Eppin, a member of the whey acidic protein type four–disulfide core gene family. In the human, Eppin associates with semenogelin on the surface of ejaculated spermatozoa and may provide antimicrobial activity for spermatozoa (Wang *et al*., 2005). Studies in which primates were immunized with Eppin resulted in a contraceptive effect in 78% of the monkeys that was reversible in 71%, suggesting an important function for this protein in fertility and a possible role as a male contraceptive (O'Rand *et al.*, 2004). Other gene and gene products expressed in a region-dependent manner in the epididymis include those encoding antioxidant enzymes such γ-glutamyl transpeptidase, glutathione peroxidases, and superoxide dismutase (Palladino *et al*., 1994; Ghyselinck *et al*., 1990; Schwaab *et al*.,1998; Perry *et al*.,1993). Because the epididymal lumen is oxygen-rich, unsaturated fatty acids in sperm membranes may be susceptible to oxidative damage. The expression of these enzymes may protect spermatozoa from oxidative damage. Lysosomal enzymes such as β- hexosaminidase, α-mannosidase, and α-galactosidase are secreted into the epididymal lumen, where they may affect sperm function either directly or indirectly by modifying carbohydrate moieties (Cornwall *et al.*, 2002; Hermo *et al*.,1997). A variety of signaling molecules are also expressed in a region dependent manner in the epididymal epithelium. It is likely that these proteins respond to various external stimuli in the luminal environment, ultimately affecting epithelial cell function. For

example, in the caput, several members of the retinoid signaling pathway are expressed such as epididymis-specific extracellular retinoic acid-binding protein and the related MEP17, mMUP4-L, and mEP19, cellular retinoic acid and binding protein and retinoic acid receptor (Okamura *et al.,* 1995). It is likely that these proteins participate in the delivery and trafficking of retinoids to and within the epididymal cells. The bone morphogenetic proteins belong to the transforming growth factor-superfamily of growth factors and function as signaling molecules. Bone morphogenetic protein (BMP) 7 and BMP8a are both expressed in the epididymis and the loss of Bmp8a gene function leads to epididymal degeneration that ultimately results in infertility (Orgebin-Crist *et al.,* 2003). Interestingly, although both BMPs are expressed in the initial segment, the degenerative effects observed in the Bmp8a knockout are observed in more distal epididymal regions, suggesting a possible paracrine role for BMP8a in the epididymis. Several peptides such as proopiomelanocortin, endorphin, proenkephalin, and neuropeptide Y are expressed in the epididymis and may also function in paracrine signaling pathways (Perry *et al.,* 1993). The region-dependent expression of genes implies that there are region-dependent transcription factors. Perhaps the most well studied are the members of the Etv4 subfamily including Etv4, Etv5, and Etv1. All family members are expressed in the initial segment, and their messenger RNAs (mRNAs) are profoundly reduced following the loss of signaling from the testis, suggesting these transcription factors may regulate a subset of genes dependent on testicular luminal fluid factors (Zhao *et al.,* 1998). Other transcription factors expressed in the initial segment include the androgen receptor (Yang *et al*., 2006), estrogen receptor (Viger *et al.,* 1995), B-myc (Cornwall *et al.,* 2001), C/EBP(Hsia *et al.,* 2001), and Pem (Pitman *et al.,*1998). Estrogen receptor expression is important for normal fluid resorption by the epithelium and specifically is necessary for expression of a critical transporter NA/H exchanger 3 as evidenced by examination of the knockout mouse (Huang *et al*., 1996; Hess *et al.,* 1997). C/EBP function is necessary for transactivation of the CRES gene (Hsia *et al.,* 2001). Although the epididymal gene targets for B-myc are not known, cell culture studies suggest that it is involved in the regulation of cell growth (Gregory *et al.,* 2000). Although the regional or segment-specific expression of genes has been well-documented in the epididymis, the biological roles their gene products carry out is for the most part unknown. However, recent generation of knockout mouse models have revealed the critical roles some of these epididymal expressed genes play. The loss of several genes that are involved in fluid transport or signaling in the initial segment region including that for apolipoprotein E receptor 2 (Blomqvist *et al.,* 2006), estrogen receptor (Esr1) (Huang *et al.,*1996; Hess *et al.,*1997), HE6 (Gpr64)(Medina *et al.,* 2003), or the complete loss of the initial segment region resulting from the loss of the c-ros tyrosine kinase receptor (Ros1) (Sonnenberg-Riethmacher *et al.,* 1996) leads to an inability of sperm to regulate their cell volume, resulting in a characteristic hairpin loop of the sperm flagella . Other epididymal expressed genes, including those for lysosomal proteins, such as β-hexosaminidase and cathepsin A (ctsa) or follicle-stimulating hormone receptor (Fshr), exhibit an altered epithelium and/or changes in lysosomal size (refs. Korah 2003; Grover *et al.,* 2005; Adamali *et al.,* 1999).

Testis/lumacrine regulation of the initial segment

Studies have clearly established that the epididymis is an androgen dependent organ. Indeed, following castration, epididymal weight decreases to 25% of intact after 2 wk. Restoration of circulating testosterone reverses the cellular changes in the caput, corpus, and cauda epididymis but not in the initial segment (Ezer *et al.,* 2002). Supra-physiological levels of androgens also do not reverse these changes in the initial segment. Interestingly, ligation of the efferent ducts, which connect the testis to the epididymis and are the passageway for sperm and luminal components to enter the initial segment from the testis, results in a profound regression of the initial segment region (Ezer *et al.,* 2002). Because ligation of the efferent ducts does not affect circulating androgen levels, these studies suggest that the maintenance of initial segment morphology requires components in the luminal fluid from the testis (i.e., lumacrine regulation) (Hinton *et al.,* 1998). Furthermore, gene expression studies revealed a subset of initial segment expressed genes that are down regulated following efferent duct ligation, including CRES subgroup and members, and others, suggesting that luminal factors are not only needed for the maintenance of initial segment morphology but for function as well Cornwall 1002; 2003; Hsia *et al*., 2003; Hinton *et al.,*1998). Although it is not known if one or many testis factors are required to maintain initial segment function, studies by Lan *et al.* (1998) suggest that basic fibroblast growth factor may be one such factor. Administration of fibroblast growth factor-2 but not epidermal growth factor to efferent duct-ligated rats restored GGT mRNA, protein, and activity in the initial segment to control levels. Furthermore, these investigators proposed that fibroblast growth factor may elicit its effects on Ggt_pr4 gene expression via activation of the ras–raf–mitogen-activated protein kinase pathway and downstream activation of the ETV4 transcription factor (Hinton *et al.,* 1998, 2003; Lan *et al.,* 1998). Most recently, studies by these investigators suggest that, not surprisingly, not all testis-regulated genes respond the same to changes in ETV4 transcriptional activity. The administration of an ETV5-dominant negative plasmid by in vivo electroporation to the rat initial segment resulted in the down regulation of Etv5, Etv4, and Etv1 mRNAs in the initial segment as well as putative target genes γ-glutamyl transpeptidase (Ggt_pr4), steroid 5 α reductase (Srd5a1), and glutathione peroxidase (Gpx5). However, although the testis-regulated genes CRES (cst8) and MEP17 (len8) contain ETS-binding sites within their promoters, they did not respond to the dominant negative, suggesting that there either may be several testis factors, each differentially regulating specific subsets of genes, or that one or a few testis factors may mediate different downstream effects via the activation of multiple signaling pathways and subsequent effector molecules (Robaire *et al.,* 2007).

Androgenic regulation of epididymal microenvironment

The epididymis is critically dependent on the presence of testis. A variety of epididymal functions are regulated directly or indirectly by androgens. For example, epididymal histology, intermediary metabolism, ion transport, synthesis and secretion of a number of epididymal proteins, and activity of certain enzymes have been shown to be under the control of androgens. Furthermore, transport, acquisition of fertilizing capacity, and storage of spermatozoa are dependent on androgens (Orgebin-Crist *et al.,* 1996; Robaire *et al*., 1998). Androgenic control is mainly mediated by 5a-dihydrotestos terone (DHT) and less clearly by testosterone [T] (Robaire *et al*., 1998; 2000), which is avidly converted to DHT by steroid 5α-reductase isoenzymes, type 1 and 2, in the epididymis. The expression patterns of the two steroid 5α- reductases (Srd5a) in the epididymis exhibit a gradient. While the IS expresses high levels of both isoforms, their expression decreases in the more distal segments (Viger *et al.,* 1991; 1996). DHT acts via binding to the androgen receptor (AR), which is expressed in all epididymal regions and in most of the epididymal cell types (Zhou *et al.,* 2002). Although the expression of many epididymal genes is regulated by androgens, only a few of them have been shown to contain androgen response elements (AREs) in their promoter region. However, the 5'-flanking region of murine Crisp1 contains several putative AREs (Roberts *et al.,* 2001); also, the murine Gpx5 (Ghyselinck *et al.,* 1993; Lareyre *et al*., 1997), Lcn5 (Lareyre *et al.,* 2000), and reproductive homeobox 5 (Rhox5) (Barbulescu *et al.,* 2001) promoter regions contain functional AREs. Interestingly, certain epididymal genes with a wider tissue distributions, such as gamma-glutamyltransferase 1 (Ggt1), are androgen regulated only in the epididymis; furthermore, Ggt1 transcripts are regulated differentially by androgen in the different epididymal regions (Palladino *et al.,*1994), which suggests the possibility that they play a role as AR co-regulators in the regulation of Ggt1 expression. Examples of tissue-specific co-regulators of AR are known from other tissues (Puigserver *et al.,*1998; Muller

et al., 2000), and the findings on Ggt1 expression after gonadectomy suggest that a tissue-, segment-, and cell-specific combination of transcription factors and co-regulators mediates the androgen regulation of epididymal genes. In addition to androgens, mostly unknown testicular factors present in efferent duct fluid have been shown to regulate the maintenance of the epithelial structure and gene expression in the IS (Nicander *et al.*, 1983; Abe *et al.*, 1984). These genes include Cst8 (Cornwall *et al.*, 1992), v-raf murine sarcoma 3611 viral oncogene homolog (Araf) (Winer *et al.*, 1995), Gpx5 (Vernet *et al.*, 1997; Rigaudiere *et al.*, 1992), a disintegrin and metallopeptidase domain 7 (Adam7) (Cornwall *et al.*,1997) ,and Lcn8 (Lareyre *et al.*, 2001). Similar to androgens, these testicular factors can also act as inhibitors of transcription (Brooks *et al.*, 1983), and in addition, they have been shown to stabilize the mRNA of Ggt1 (Rudolph *et al.*,1997). Several testicular factors have been suggested, such as the androgen binding protein, which regulates the expression of Srd5a1 (Robaire *et al.*,1995), the basic fibroblast growth factor that regulates the expression of Ggt1 (Lan *et al.*,1998), and the germ cells themselves, or germ cell associated factors, that regulate the expression of preproenkephalin 1 (Penk1) (Garrett *et al.*,1980).

Conclusion

While there are numerous forms of contraception available worldwide, it is clear that there is no single method that fulfills the requirements, preferences and intentions of all individuals during their reproductive lifetime (Harrison *et al.*, 1996). Men, in particular, have few contraceptive choices, though it has been estimated that male methods of contraception account for 37% of all contraceptive use (Darroch 2000). The primary targets for male-based contraceptives are the testis and the epididymis. Intervention in the testis has potential endocrine sequelae, may have problems posed by the blood–testis barrier, and must avoid teratogenic effects arising from sperm that may have completed spermatogenesis but with damaged DNA. An alternative strategy is to direct attention to the processes of sperm maturation, which occurs in the epididymis. Inhibition of these post-testicular maturation events in the sperm by modulating normal epididymal microenvironment represents an attractive option for contraceptive intervention. The challenge is to identify novel targets which, if inhibited, would prevent sperm maturation and successful fertilization but are also devoid of the side effects commonly encountered with current contraceptive products or strategies (T.T. Turner *et al.*, 2006). This implies that any potential target would optimally have a tissue-distribution pattern limited to the epididymis, though this is not required. The development of a drug addressing such a target would be novel and would move the field away from therapeutics that affect multiple genes towards therapeutics that act on a single gene product. Comparison of the gene expression data from this study to a Wyeth internal microarray database allowed for the identification of genes that exhibit a higher level of expression in the epididymis than in 22 other tissues. At the microarray level of sensitivity these genes were categorized as either epididymis selective (307 genes) or epididymis-specific (75 genes). A number of genes had distinctive patterns of expression in the epididymis but were neither selective nor specific, e.g. ATP binding cassette, subfamily B, member 9 (*Abcb9*), while others had distinctive, segmental patterns of expression and were epididymal specific, e.g. *Gpx-5*. A number of other genes previously studied in the epididymis, e.g. *cres2* (cystatin-11), *lcn5* (lipocalin5, ERABP) and *Gpx-5* were epididymal-specific at this level of sensitivity, Mining of human genome databases has revealed that of these epididymal-specific mouse genes, 41 have homologues in the human epididymis. To speak of absolute specificity of the homologues in human tissues is complicated for technical reasons, but many of these genes have their highest expression in human epididymal tissue. Selected members of this gene set can be characterized with regard to protein synthesis and localization as discussed in this review. Proteins judged suitable for further study can be carried further in the pathway for contraceptive development or /and can be used to assess different pathological conditions.

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REFERENCES

Abe K, Takano H, Ito T. Interruption of the luminal flow in the epididymal duct of the corpus epididymidis in the mouse, with special reference to differentiation of the epididymal epithelium. Arch Histol Jpn 1984; 47:137–147.

Adamali HI, Somani IH, Huang JQ, Gravel RA, Tralser JM, Hermo L. Characterization and development of the regional- and cellular-specific abnormalities in the epididymis of mice with beta-hexosaminidase A deficiency. J Androl 1999; 20:803–824.

Andersen OM, Yeung C-H, Vorum H, *et al*. Essential role of the apolipoprotein E receptor-2 in sperm development. J Biol Chem 2003; 278:23,989–23,995.

Andonian S, Hermo L. Immunocytochemical localization of the Ya, Yb1, Yc, Yf, and Yo subunits of glutathione S-transferases in the cauda epididymidis and vas deferens of adult rats. J Androl 1999; 20:145–157

Barbulescu K, Geserick C, Schu¨ttke I, Schleuning W-D, Haendler B. New androgen response elements in the murine Pem promoter mediate selective transactivation. Mol Endocrinol 2001; 15:1803–1816.

Blomqvist. S.R., Vidarsson.H, Soder O, and Euerback.S. Foxi-1(forehead transcription factor) 2006, 25:4131-4141

Brooks DE. Effect of androgens on protein synthesis and secretion in various regions of the rat epididymis, as analysed by two-dimensional gel electrophoresis. Mol Cell Endocrinol 1983; 29:255–270.

Conrad M, Moreno SG, Sinowatz F, *et al*. The nuclear form of phospholipids hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. Mol Cell Biol 2005;25:7637–7644.

Cornwall GA, Cameron A, Lindberg I, Hardy DM, Cormier N, Hsia N. The cystatin-related epididymal spermatogenic protein inhibits the serine protease prohormone convertase 2. Endocrinology 2003;144:901–908

Cornwall GA, Collis R, Xiao Q, Hsia N, Hann SR. B-Myc, a proximal caput epididymal protein, is dependent on androgens and testicular factors for expression. Biol Reprod 2001;64:1600–1607.

Cornwall GA, Hsia N, Sutton HG. Structure, alternative splicing and chromosomal localization of the cystatin-related epididymal spermatogenic gene. Biochem J 1999; 340(pt 1):85–93

Cornwall GA, Hsia N. A new subgroup of the family 2 cystatins. Mol Cell Endocrinol 2003;200:1–8.

Cornwall GA, Hsia N. ADAM7, a member of the ADAM (a disintegrin and metalloprotease) gene family is specifically expressed in the mouse anterior pituitary and epididymis. Endocrinology 1997; 138:4262–4272.

Cornwall GA, Lareyre J, Matusik R, Hinton BT, Orgebin-Crist MC. Gene expression and epididymal function. In: Robaire B, Hinton BT (eds.), The Epididymis: From Molecules to Clinical Practice. New York: Kluwer Academic Press/Plenum Press Publishers; 2002:169– 199

Cornwall GA, Lareyre JJ, Matusik RJ, Hinton BT, Orgebin-Crist M-C. Gene expression and epididymal function. In: Robaire B, Hinton BT, eds. The Epididymis: From molecules to clinical practice, A comprehensive survey of the efferent ducts, the epididymis and the vas deferens. Kluwer Academic/Plenum Publishers, New York; 2002:169–199.

Cornwall GA, Orgebin-Crist M-C, Hann SR. The CRES gene: a unique testisregulated gene related to the cystatin family is highly restricted in its expression to the proximal region of the mouse epididymis. Mol Endocrinol 1992;6: 1653–1664.

Cornwall GA, Orgebin-Crist MC, Hann SR. The CRES gene: a unique testis-regulated gene related to the cystatin family is highly restricted in its expression to the proximal region of the mouse epididymis. Mol Endocrinol 1992; 6:1653–1664.

Cornwall GA, Orgebin-Crist MC, Hann SR. The CRES gene: a unique testis-regulated gene related to the cystatin family is highly restricted in its expression to the proximal region of the mouse epididymis. Mol Endocrinol 1992; 6:1653–1664.

Darroch, J.E., 2000. The pill at 40—a new look at a familiar method. The Pill and Men's Involvement in Contraception. Fam. Plann. Perspect. 32, 90–91.

Davies B, Baumann C, Kirchhoff C, *et al*. Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. Mol Cell Biol 2004;24:8642–8648.

Eberspaecher U, Roosterman D, Kra¨tzschmar J, Haendler U-F, Becker A, Quensel C, Petri T, Schleuning W-D, Donner P. Mouse androgendependent epididymal glycoprotein CRISP-1 (DE/AEG): isolation, biochemical characterization, and expression in recombinant form. Mol Reprod Dev 1995; 42:157–172.

Ezer N, Robaire B. Androgenic regulation of the structure and functions of the epididymis. In: Robaire B, Hinton BT, eds. The Epididymis: from molecules to clinical practice. A comprehensive survey of the efferent ducts, the epididymis and the vas deferens. Kluwer Academic/Plenum Publishers, New York; 2002:297–316.

Frenoux JM, Vernet P, Volle DH, *et al*. Nuclear oxysterol receptors, LXRs, are involved in the maintenance of mouse caput epididymidis structure and functions. J Mol Endocrinol 2004;33:361–375.

Fuchs S, Frenzel K, Hubert C, *et al*. Male fertility is dependent on dipeptidase activity of testis ACE. Nature Med 2005;11:1140–1142.

Garrett JE, Garrett SH, Douglass J. A spermatozoa-associated factor regulates proenkephalin gene expression in the rat epididymis. Mol Endocrinol 1990; 4:108–118.

Ghyselinck NB, Dufaure I, Lareyre JJ, Rigaudiere N, Mattei MG, Dufaure JP. Structural organization and regulation of the gene for the androgendependent glutathione peroxidase-like protein specific to the mouse epididymis. Mol Endocrinol 1993; 7:258–272.

Ghyselinck NB, Jimenez C, Lefrancois AM, Dufaure J-P. Molecular cloning of a cDNA for androgen-regulated proteins secreted by the mouse epididymis. Mol Endocrinol 1990;4:5–12.

Gregory MA, Xiao Q, Cornwall GA, Lutterbach B, Hann SR. B-Myc is preferentially expressed in hormonally-controlled tissues and inhibits cellular proliferation. Oncogene 2000;19:4886–4895.

Grover A, Smith CE, Gregory M, Cyr DG, Sairam MR, Hermo L. Effects of FSH receptor deletion on epididymal tubules and sperm morphology, numbers, and motility. Mol Reprod Dev 2005;72:135–144.

Hagaman JR, Moyer JS, Bachman ES, *et al*. Angiotensin-converting enzyme and male fertility. Proc Natl Acad Sci USA 1998;95:2552–2557.

Harrison, P.F., Rosenfield, A., 1996. Contraceptive Research and Development. National Academy Press, Washington, DC, p. 31.

Hellsten E, Evans JP, Bernard DJ, Janne PA, Nussbaum RL. Disrupted sperm function and fertilin□□processing in mice deficient in the inositol polyphosphate 5- phosphatase Inpp5b. Develop Biol 2001;240:641–653.

Hermo L, Adamali HI, Mahuran D, Gravel RA, Tralser JM. beta-Hexosaminidase immunolocalization and alpha- and beta-subunit gene expression in the rat testis and epididymis. Mol Reprod Dev 1997;46:227–242.

Hess RA, Bunick D, Lee KH, *et al*. A role for oestrogens in the male reproductive system. Nature 1997;390:509–512.

Hinton BT, Kirby JL, Rodriguez CM, Lye RJ, Troan BV,Yang L. Signal transduction pathways to gene expression. In: Hinton BT, Turner TT, eds. The Third International Conference on the Epididymis. Van Doren Company, Charlottesville; 2003:103–113.

Hinton BT, Lan ZJ, Rudolph DB, Labus JC, Lye RJ. Testicular regulation of epididymal gene expression. J Reprod Fertil Suppl 1998;53:47–57.

Hsia N, Cornwall GA. CCAAT/enhancer binding protein beta regulates expression of the cystatin-related epididymal spermatogenic (Cres) gene. Biol Reprod 2001;65: 1452–1461.

Hsia N, Cornwall GA. Cres2 and Cres3: new members of the cystatin-related epididymal spermatogenic subgroup of family 2 cystatins. Endocrinology 2003; 144:909–915.

Hsieh-Li HM, Witte DP, Weinstein M, *et al*. Hoxa 11 structure, extensive antisense transcription, and function in male and female fertility. Development 1995;121: 1373–1385.

Huang LS, Voyiaziakis E, Chen HL, Rubin EM, Gordon JW. A novel functional role for apolipoprotein B in male infertility in heterozygous apolipoprotein B knockout mice. Proc Natl Acad Sci USA 1996;93:10,903–10,907.

Johnston DS, Jelinsky SA, Bang HJ, *et al*. The mouse epididymal transcriptome: transcriptional profiling of segmental gene expression in the epididymis. Biol Reprod 2005;73:404–413.

Kim E, Nishimura H, Iwase S,Yamagata K, Kashiwabara S, Baba T. Synthesis, processing, and subcellular localization of mouse ADAM3 during spermatogenesis and epididymal sperm transport. J Reprod Dev 2004;50:571–578.

Kirchhoff C Gene expression in the epididymis . International Review of Cytolology 1999 188:133-202

Kondoh G, Tojo H, Nakatani Y, *et al*. Angiotensin-converting enzyme is a GPIanchored protein releasing factor crucial for fertilization. Nature Med 2005; 11:160–166.

Korah N, Smith CE, D'Azzo A, Mui J, Hermo L. Characterization of cell- and region-specific abnormalities in the epididymis of cathepsin A deficient mice. Mol Reprod Dev 2003;66:358–373.

Lan ZJ, Labus JC, Hinton BT. 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. Biol Reprod 1998; 58:197–206.

Lareyre JJ, Claessens F, Rombauts W, Dufaure JP, Drevet JR. Characterization of an androgen response element within the promoter of the epididymis-specific murine glutathione peroxidase 5 gene. Mol Cell Endocrinol 1997; 129:33–46.

Lareyre JJ, Reid K, Nelson C, Kasper S, Rennie PS, Orgebin-Crist MC, Matusik RJ. Characterization of an androgen-specific response region within the 5' flanking region of the murine epididymal retinoic acid binding protein gene. Biol Reprod 2000; 63:1881–1892.

Lareyre JJ, Winfrey VP, Kasper S, Ong DE, Matusik RJ, Olson GE, Orgebin-Crist MC. Gene duplication gives rise to a new 17-kilodalton lipocalin that shows epididymal region-specific expression and testicular factor(s) regulation. Endocrinology 2001; 42:1296–1308.

Lareyre JJ, Winfrey VP, Kasper S, Ong DE, Matusik RJ, Olson GE, Orgebin-Crist MC. Gene duplication gives rise to a new 17-kilodalton lipocalin that shows epididymal region-specific expression and testicular factor(s) regulation. Endocrinology 2001; 142:1296–1308.

Lareyre JJ, Zheng WL, Zhao GQ, Kasper S, Newcomer ME, Matusik RJ, Ong DE, Orgebin-Crist MC. Molecular cloning and hormonal regulation of a murine epididymal retinoic acid-binding protein messenger ribonucleic acid. Endocrinology 1998; 139:2971–2981.

Li Y, Friel PJ, McLean DJ, Griswold MD. Cystatin E1 and E2, new members of male reproductive tract subgroup within cystatin type 2 family. Biol Reprod 2003;69:489–500

Lieberman MW,Wiseman AL, Shi ZZ, *et al*. Growth retardation and cysteine deficiency in gamma-glutamyl transpeptidase-deficient mice. Proc Natl Acad Sci USA 1996;93:7923–7926.

Lum L, Blobel CP. Evidence for distinct serine protease activities with a potential role in processing the sperm protein fertilin. Dev Biol 1997;191:131–145.

Mbikay M, Tadros H, Ishida N, *et al*. Impaired fertility in mice deficient for the testicular germ cell protease PC4. Proc Natl Acad Sci USA 1997;94:6842–6846.

Medina JF, Recalde S, Prieto J, *et al*. Anion exchanger 2 is essential for spermiogenesis in mice. Proc Natl Acad Sci USA 2003;100:15,847–15,852.

Metayer S, Dacheux F, Dacheux J-L, Gatti J-L. Comparison, characterization, and identification of proteases and protease inhibitors in epididymal fluids of domestic mammals. Matrix metalloproteinases are major fluid gelatinases. Biol Reprod 2002;66:1219–1229.

Morales CR, Zhao Q, El-Alfy M, Suzuki K. Targeted disruption of the mouse prosaposin gene affects the development of the prostate gland and other male reproductive organs. J Androl 2000;21:765–775.

Mu¨ ller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schule R. FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J 2000; 19:359–369.

Nicander L, Osman DI, Ploen L, Bugge HP, Kvisgaard KN. Early effects of efferent ductule ligation on the proximal segment of the rat epididymis. Int J Androl 1983; 6:91–102.

O'Rand MG,Widgren EE, Sivashanmugam P, *et al*. Reversible immunocontraception in male monkeys immunized with eppin. Science 2004;306:1189–1190.

Oh J,Woo J-M, Choi E, Kim T, *et al*. Molecular, biochemical, and cellular characterization of epididymal ADAMs, ADAM7 and ADAM28. Biochem Biophys Res Comm 2005;331:1374–1383.

Okamura N, Tamba M, Liao H-J, *et al*. Cloning of complementary DNA encoding a 135-kilodalton protein secreted from porcine corpus epididymis and its identification as an epididymis-specific alpha-mannosidase. Mol Reprod Dev 1995;42: 141–148.

Okamura N, Tamba M, Uchiyama Y, *et al*. Direct evidence for the elevated synthesis and secretion of procathepsin L in the distal caput epididymis of boar. Biochim Biophys Acta 1995;1245:221–226.

Orgebin-Crist MC. Androgens and epididymal function. In: Bhasin S, Gabelnick HL, Spieler JM, Swerdloff RS, Wang C, Kelly C, (eds.), Pharmacology, Biology, and Clinical Applications of Androgens: Current Status and Future Prospects: Wilmington, DE: Wiley-Liss Inc.; 1996:27–39.

Orgebin-Crist M-C. The epididymis in a post-genome era. In: Hinton BT, Turner TT, eds. The Third International Conference on the Epididymis. Van Doren Company, Charlottesville; 2003:2–22.

Palladino MA, Hinton BT. Expression of multiple gamma-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 1994;135:1146–1156.

Perry AC, Jones R, Hall L. Isolation and characterization of a rat cDNA clone encoding a secreted superoxide dismutase reveals the epididymis to be a major site of its expression. Biochem J 1993;293:21–25.

Petruszak JAM, Nehme CL, Bartles JR. Endoproteolytic cleavage in the extracellular domain of the integral plasma membrane protein CE9 precedes its redistribution from the posterior to the anterior tail of the rat spermatozoon during epididymal maturation. J Cell Biol 1991;114:917–927.

Pitman JL, Lin TP, Kleeman JE, Erickson GF, MacLeod CL. Normal reproductive and macrophage function in Pem homeobox gene-deficient mice. Dev Biol 1998;202:196–214.

Pollard JW, Dominguez MG, Mocci S, Cohen PE, Stanley ER. Effect of the colony-stimulating factor-1 null mutation, osteopetrotic (csfm(op)), on the distribution of macrophages in the male mouse reproductive tract. Biol Reprod 1997;56:1290–1300.

Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998; 92:829–839.

Rankin TL, Tsuruta KJ, Holland MK, Griswold MD, Orgebin-Crist MC. Isolation, mmunolocalization, and sperm-association of three proteins of 18, 25, and 29 kilodaltons secreted by the mouse epididymis. Biol Reprod 1992; 46:747–766.

Rigaudiere N, Ghyselinck NB, Faure J, Dufaure JP. Regulation of the epididymal glutathione peroxidase-like protein in the mouse: dependence upon androgens and testicular factors. Mol Cell Endocrinol 1992; 89:67–77.

Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E, Neill J, (eds.), The Physiology of Reproduction, vol. 1. New York: Raven Press Ltd.; 1988: 999–1080.

Robaire B, Seenumdum S,Hamzch M and Lamour S .Androgenic regulation of novel genes in the epididymis 2007, 9:545-553

Robaire B, Syntin P, Jervis K. The coming age of the epididymis. In: Jegou B, (ed.), Testis, Epididymis and Technologies in the Year 2000. New York: Springer-Verlag; 2000:229–262.

Robaire B, Viger RS. Regulation of epididymal epithelial cell functions. Biol Reprod 1995; 52:226–236.

Robaire B. Efferent ducts, epididymis, and vas deferens: structure, functions and their regulation. In: Knobil E, Neil J (eds.), Physiology of Reproduction, vol. 1. New York: Raven Press; 1988:999–1080

Roberts KP, Hoffman LB, Ensrud KM, Hamilton DW. Expression of CRISP-1 mRNA splice variants in the rat epididymis, and comparative analysis of the rat and mouse CRISP-1 gene regulatory regions. J Androl 2001; 22:157–163.

Rudolph DB, Hinton BT. Stability and transcriptional regulation of gamma-glutamyl transpeptidase mRNA expression in the initial segment of the rat epididymis. J Androl 1997; 18:501–512.

Satokata I, Benson G, Mass R. Sexually dimorphic sterility phenotypes in Hoxa10- deficient mice. Nature 1995;374:460–463.

Schwaab V, Faure J, Dufaure J-P, Drevet JR. GPx3: the plasma-type glutathione peroxidase is expressed under androgenic control in the mouse epididymis and vas deferens. Mol Reprod Dev 1998;51:362–372.

Sonnenberg-Riethmacher E, Walter B, Riethmacher D, Godeche S, Birchmeier C. C-ros: the vertebrate homolog of the sevenless tyrosine kinase receptor is tightly regulated during organogenesis in mouse embryonic development . 1996 Genes Dev ; 10:1184–1193.

Sonnenberg-Riethmacher E, Walter B, Riethmacher D, Godecke S, Birchmeier C. The c-ros tyrosine kinase receptor controls regionalization and differentiation of epithelial cells in the epididymis. Genes Dev 1996;10:1184–1193.

Turner T.T. a,□, D.S. Johnston b, S.A. Jelinsky cEpididymal genomics and the search for a male contraceptive Molecular and Cellular Endocrinology 2006,250 :178–183

Turner TT. On the epididymis and its role in the development of the fertile ejaculate. *J Androl 1995 16:292*-298

Vernet P, Faure J, Dufaure J-P, Drevet JR. Tissue and developmental distribution, dependence upon testicular factors and attachment to spermatozoa of GPX5, a murine epididymis-specific glutathione peroxidase.Mol Reprod Dev 1997; 47:87–98.

Viger RS, Robaire B. Differential regulation of steady state 4-ene steroid 5 alpha-reductase messenger ribonucleic acid levels along the rat epididymis. Endocrinology 1991; 128:2407–2414.

Viger RS, Robaire B. Gene expression in the aging brown Norway rat epididymis. J Androl 1995;16:108–117.

Viger RS, Robaire B. The mRNAs for the steroid 5 alpha-reductase isozymes, types 1 and 2, are differentially regulated in the rat epididymis. J Androl 1996; 17:27–34.

Visconti PE, Bailey JL, Moore GD, Pan D, Olds-Clarke P, Kopf GS. Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation. Development 1995 121:1129-1137

Wang Z, Widgren EE, Sivashanmugam P, O'Rand MG, Richardson RT. Association of eppin with semenogelin on human spermatozoa. Biol Reprod 2005;72:1064–1070.

Winer MA, Wolgemuth DJ. The segment-specific pattern of A-raf expression in the mouse epididymis is regulated by testicular factors. Endocrinology 1995; 136:2561–2572.

Yang L, Fox SA, Kirby JL, Troan BV, Hinton BT. Putative regulation of expression of members of the ETS variant 4 transcription factor family and their downstream targets in the rat epididymis. Biol Reprod 2006;74:714–720.

Yeung CH, Anapolski M, Sipila P, *et al*. Sperm volume regulation: maturational changes in fertile and infertile transgenic mice and association with kinematics and tail angulation. Biol Reprod 2002;67:269–275.

Yeung CH, Breton S, Setiawan I, Lang F, Cooper TG. Increased luminal pH in the epididymis of infertile c-ros knockout mice and the expression of sodium-hydrogen exchangers and vacuolar proton pump H□-ATPase. Mol Reprod Dev 2004;68: 159–168.

Zhao GQ, Liaw L, Hogan BL. Bone morphogenetic protein 8A plays a role in the maintenance of spermatogenesis and the integrity of the epididymis. Development 1998;125:1103–1112.

Zhou Q, Clark L, Nie R, *et al*. Estrogen action and male fertility: roles of the sodium/hydrogen exchanger-3 and fluid reabsorption in reproductive tract function. Proc Natl Acad Sci USA 2001;98:14,132–14,137.

Zhou Q, Nie R, Prins GS, Saunders PT, Katzenellenbogen BS, Hess RA. Localization of androgen and estrogen receptors in adult male mouse reproductive tract. J Androl 2002; 23:870–881.

Zhu GZ, Myles DG, Primakoff P. Testase 1 (ADAM 24) a plasma membraneanchored sperm protease implicated in sperm function during epididymal maturation or fertilization. J Cell Sci 2001;114:1787–1794.

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