Blackwell Publishing

Asian J Androl 2007; 9 (4): 565–573
DOI: 10.1111/j.1745-7262.2007.00302.x

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·*Review*·

Segment boundaries of the adult rat epididymis limit interstitial signaling by potential paracrine factors and segments lose differential gene expression after efferent duct ligation

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Abstract

The epididymis is divided into caput, corpus and cauda regions, organized into intraregional segments separated by connective tissue septa (CTS). In the adult rat and mouse these segments are highly differentiated. Regulation of these segments is by endocrine, lumicrine and paracrine factors, the relative importance of which remains under investigation. Here, the ability of the CTS to limit signaling in the interstitial compartment is reviewed as is the effect of 15 days of unilateral efferent duct ligation (EDL) on ipsilateral segmental transcriptional profiles. Inter-segmental microperifusions of epidermal growth factor (EGF), vascular endothelial growth factor (VEGFA) and fibroblast growth factor 2 (FGF2) increased phosphorylation of mitogen activated protein kinase (MAPK) in segments 1 and 2 of the rat epididymis and the effects of all factors were limited by the CTS separating the segments. Microarray analysis of segmental gene expression determined the effect of 15 days of unilateral EDL on the transcriptome-wide gene expression of rat segments 1–4. Over 11 000 genes were expressed in each of the four segments and over 2 000 transcripts in segment 1 responded to deprivation of testicular lumicrine factors. Segments 1 and 2 of control tissues were the most transcriptionally different and EDL had its greatest effects there. In the absence of lumicrine factors, all four segments regressed to a transcriptionally undifferentiated state, consistent with the less differentiated histology. Deprivation of lumicrine factors could stimulate an individual gene's expression in some segments yet suppress it in others. Such results reveal a higher complexity of the regulation of rat epididymal segments than that is generally appreciated. *(Asian J Androl 2007 July; 9: 565–573)*

Keywords: efferent duct ligation; dedifferentiation; proximal epididymis

1 Introduction

The epididymis has traditionally been divided the organ into the regions of caput, corpus and cauda with the "initial segment" of the caput sometimes separated for further definition. Earlier reports [1, 2] made it clear that each epididymal region of the common rodent models is made up of intra-regional segments or lobules of coiled tubule, and more recent investigations in the mouse [3] and rat [4] have demonstrated that the epididymides of those species are divided into 10 and 19 intraregional segments, respectively (Figure 1). Segmentation has been shown to exist in dogs [5], and the marmoset, a small primate species, shows histological evidence of epididymal segments as well (Terry T. Turner and D. Bomgardner, unpublished observations).

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The connective tissue septa (CTS), which separate the segments, can establish borders for epididymal gene expression, protein presence or epithelial response to lumicrine factors [3, 6, 7]. The mechanism behind the sudden turn-on-turn-off of particular gene expressions at the CTS has remained unclear, but it has been hypothesized that CTS divide the epididymis into separate interstitial compartments, which allows inter-segmental paracrine signaling that can be unique to a specific segment [7].

2 Segmentation in the rat epididymis and the partition of signaling by the mitogen activated protein kinase (MAPK) pathway

Epididymal CTS restrict diffusion of molecules from the interstitium of one segment to the next [7]. This barrier property potentially provides a physiological basis for segmented epithelial function because CTS can limit the influence of interstitial cell-epithelial signaling molecules (e.g. growth factors) to the segment in which they were secreted.

Growth factors are polypeptides that promote a variety of cell activities, usually in a paracrine or endocrine fashion [8]. Growth factors mediate their effects primarily through stimulation of the mitogen activated protein kinase (MAPK) pathway [9]. MAKP is a highly conserved kinase that upon stimulation by phosphorylation accumulates in the cell nucleus and promotes the phosphorylation of numerous proteins and downstream kinases, including transcription factors [10]. Several investigators have explored possible roles of a variety of growth factors and growth factor receptors in the epididymis [11], but more emphasis has been placed on expression and localization rather than on sorting out any specifics of regulation**.** Because previous investigators have demonstrated the presence of vascular endothelial growth factor (VEGFA), basic fibroblast growth factor 2 (FGF2), and epidermal growth factor (EGF) or their receptors in the interstitium or tubules of the epididymis, we recently verified the expression of these specific genes in the rat epididymis using real-time reverse transcription polymerase chain reaction (RT-PCR) (data not shown) and used these molecules to challenge the hypothesis that CTS restrict interstitial signaling to the segment in which the molecule first appears. The response signal used was the phosphorylation of MAPK after growth factor infusion into a specific segment *in vivo*.

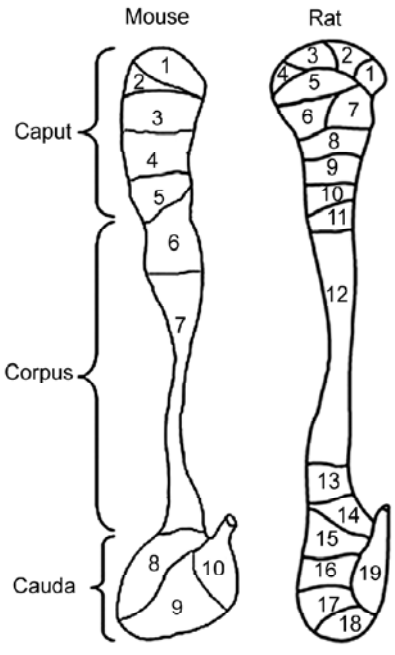

Figure 1. Schematic representation of mouse and rat epididymides (not to scale) showing typical segment patterns. The rat epididymis is approximately 2.5 times the length and 40 times the mass of the mouse epididymis. Segment 1 of the mouse epididymis and segments 1–3, sometimes 4, of the rat epididymis are often referred to as the initial segment. Modified with permission from Jelinsky *et al*. [4].

The experiments were done using segments 1 and 2 of the rat epididymis [12] (Figure 2A). Preliminary, *in vitro* experiments determined that MAPK phosphorylation occurred in both segments in response to all three growth factors and that the timing of the response to growth factors was both immediate (within 2 min) and prolonged (lasting out to 90 min; data not shown). Following those experiments, *in vivo* microperifusion experiments were performed wherein rats were killed and prepared for *in vivo* micropuncture as previously described [13].

Microperifusions (i.e. infusions into the interstitial space surrounding the tubules of a specific segment) were carried out using sharpened glass micropipettes filled with approximately 20 μL growth factor solution (10^{-6} mol/L EGF, FGF2, or VEGFA in phosphate-buffered saline [PBS] + 0.1% lissamine green as a tracking dye). The loaded pipette was attached to a micromanipulator and to an infusion pump via a PE50 cannula. Under a dissecting microscope the tunica albuginea of either segment 1 or segment 2 of the rat epididymis was punctured with the micropipette. The pipette tip was left in the segment's interstitial space and 15 μL of the selected growth factor solution was infused over an approximate 5-min time period. The pipette was left in place until the end of the experiment and the epididymis was covered with mineral oil to prevent dehydration and to help maintain proper tissue temperature. Control experiments were carried out using vehicle alone as the

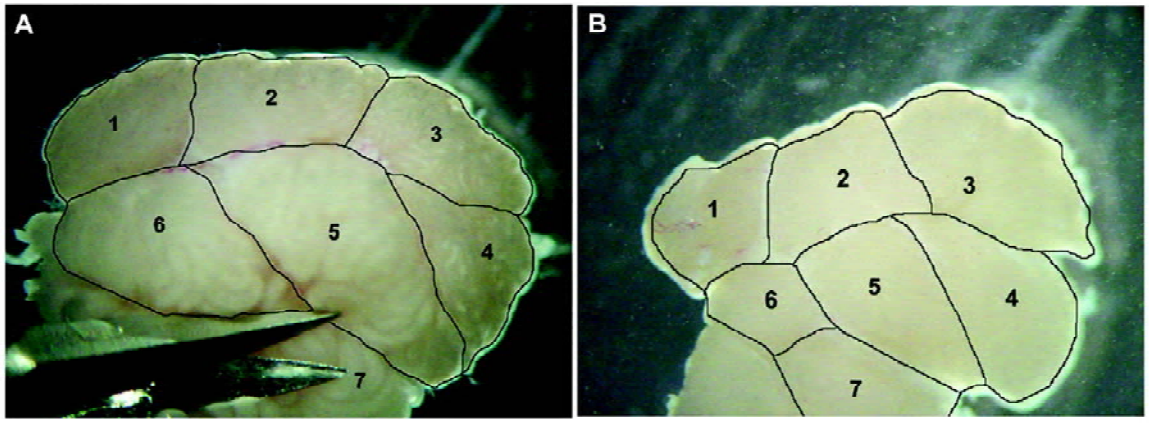

Figure 2. The proximal caput epididymis of the rat with its tunica albuginea removed. The epididymal segments are outlined with a black line and numbered. (A): Control caput. (B): Caput 15 d after efferent duct ligation (EDL). Magnification, 6.7 ×. Modified with permission from Turner *et al.* [12].

perifusion medium. To test the effect of CTS disruption on the effect of perifused growth factors, 0.1% collagenase type I was added to the VEGFA solution and used for *in vivo* perifusion into segments 1 and 2 in different epididymides for 90 min, as described above. Perifusions with medium lacking VEGFA but containing 0.1% collagenase were included as negative controls.

After each *in vivo* perifusion, segments 1 and 2 were collected separately and analyzed for MAPK phosphorylation by Western blot using an antibody to active MAPK (kindly provided by Dr Thomas Sturgill, Department of Pharmacology, University of Virginia, VA, USA) that recognizes both the MAPK1 and MAPK3 isoforms [14]. For the detection of total MAPK (i.e. phosphorylated and nonphosphorylated forms), a rabbit anti-MAPK antibody was used (Santa Cruz, CA, USA). Tissue samples (50 μg protein/lane) were electrophoresed in 12% PAGE gels, transferred to nitrocellulose membranes, and stained with Ponceau S to verify the transfer. Membranes were blocked with 3% non-fat dry milk in PBS and probed with the primary antibodies according to standard protocols. Membranes were washed and incubated with peroxidase-labeled, goat anti-rabbit antibody and detection was carried out using a commercial chemiluminescent substrate.

Both the MAPK1 and MAPK3 isoforms were detected in both segments 1 and 2 of the rat epididymis [15] (Figure 3). The average molecular masses of these two isoforms were approximately 44.5 kDa and 43.8 kDa for MAPK1 and MAPK3, respectively (n = 5, each). No other bands were revealed by the anti-active MAPK antibody or by the anti-total MAPK antibody in either nonstimulated or growth factor-stimulated epididymides.

Mitogen activated kinase activation was observed only

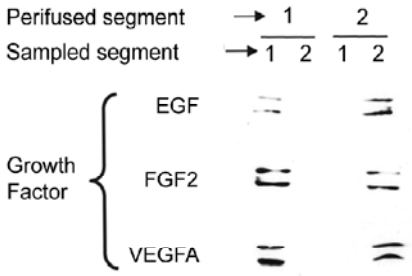

Figure 3. Western blot detection of activated (phosphorylated) mitogen activated protein kinase (MAPK) isoforms in proteins from segments 1 and 2 of the rat epididymis 90 min after segment-specific perifusion with either epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), or vascular endothelial growth factor (VEGFA). Phosphorylated MAPK was detected only in the segment in which the growth factors originated via microperifusion. This implies a restraint to signaling by growth factors which is imposed by the segment boundaries. Reprinted with permission from Tomsig *et al.* [15].

in the specific segment perifused with either of the three growth factors, EGF, FGF2 or VEGFA, not in the adjacent, non-perifused segment (Figure 3). MAPK activation persisted through the entire 90-min experimental period, but whether the segment perifused was segment 1 or 2, the adjacent segment did not typically show detectable levels of MAPK phosphorylation (Figure 3). Total MAPK, as detected by western blot, was present with equivalent signal in both segments irrespective of perfusate (not shown). Preliminary experiments at 30 and 60 min after growth factor perifusion gave the same result (i.e. at no time after growth factor presentation was MAPK phosphorylation detected in the non-perifused segment). As with the *in vitro* experiments, no MAPK

phosphorylation was detected in segments perifused with medium alone (not shown). Of note, the response of both segment 1 and segment 2 to the perifusion of any one of the three growth factors appeared similar (Figure 3).

To test the hypothesis that CTS were responsible for retaining the response to growth factors within the perifused segments, segments were perifused with VEGFA in the presence of collagenase. Ninety minutes after perifusion of the VEGFA/collagenase solution into either segment 1 or 2, MAPK activation was observed not only in the perifused segment but in the adjacent segment as well [15] (Figure 4). Perifusions containing collagenase alone did not activate MAPK in either segment.

These studies were conducted using growth factors for which previous evidence suggested they or their receptors were present in the epididymis. We used epididymal segments 1 and 2 because the CTS between these two segments divide clearly different epithelia [1] with different responses to stimuli [16]. The nomenclature "segments 1 and 2" is the result of a simple numbering of all 19 segments of the rat epididymis similar to the numbering of the 10 segments of the murine epididymis [3]. Rat segments 1 and 2 correspond to zones 1A and 1B in the terminology of Reid and Cleland [1]. MAPK phosphorylation was used as a test of growth factor action because MAPK activation is the hallmark of growth factor signaling [9].

Interestingly, and consistent with our hypothesis that the CTS provide a functional barrier isolating paracrine effects within individual segments, MAPK activation induced by EGF, FGF2 and VEGFA occurred only in the perifused segments *in vivo*, whether the perifusion was in segment 1 or segment 2 (Figure 3).

Connective tissue septa are formed by eosinophilic strands with fibers, suggesting collagen as a major component. Accordingly, perifusion experiments with VEGFA plus collagenase were conducted to permit the degradation of collagen and the reduction of the intersegmental barrier's resistance to molecular movement. Under these conditions, VEGFA activated MAPK in both the perifused segment and the adjacent segment, again irrespective of which segment was perifused (Figure 4).

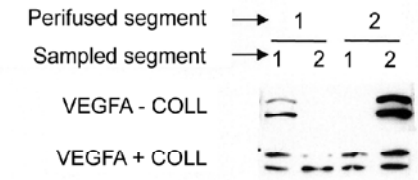

Figure 4. Western blot detection of mitogen activated protein kinase (MAPK) activation in segments 1 and 2 of the rat epididymis 90 min after microperifusion of vascular endothelial growth factor (VEFGA) alone (VEGFA – Coll) or VEGFA and collagenase (VEGFA + Coll). VEGFA alone stimulated MAPK activation only in the perifusied segments, but the addition of collagenase resulted in MAPK activation in the adjacent, non-perifused segment, as well. Other experiments showed that collagenase, alone, did not activate MAPK. Coll, collagenase. Reprinted with persmission from Tomsig *et al.* [15].

Exposure of CTS to collagenase allowed MAPK activation activity in the segment adjacent to the perifused segment. This suggests a reduction in the intersegmental barrier and a passage of VEGFA from the perifused segment into the adjacent segment. Such movement never occurred with any growth factor in the absence of collagenase, thus reinforcing the idea that under normal conditions the physical integrity of the CTS prevents substances originally appearing in one segment from freely diffusing into the next. The growth factors used in the present study (EGF [6 kDa], FGF2 [26 kDa] and VEGFA [45 kDa]) are all at or above the molecular mass of molecules previously shown to be largely retained by intraregional segments [7]; therefore, the CTS are capable of restricting the movement of a variety of paracrine molecules that may be secreted within a particular segment.

The above results can be used as proof of principle only. In nature, secreted growth factors commonly become quickly bound to interstitial binding molecules (e.g. heparan sulfate proteoglycan and FGF-binding protein 1), which might restrict diffusion, depending on local conditions [17, 18]. In the present study, molar excess concentrations of growth factors (μmol/L instead of nmol/L) were used in the perifusion medium to overcome this potential binding, thus leaving unbound growth factor available for diffusion through the CTS. The resulting data supports the possibility of intersegmental, paracrine signaling and begs the question of which signaling molecules are important in which segments of the epididymis and what specific processes they regulate. That research is underway. It is important to recall, however, that paracrine signaling within individual segments is only one aspect of cell regulation in the epididymis. Endocrine regulation of the tissue is well known, and will not be addressed here. However, non-androgenic lumicrine factors from the testis are known to be important for both epididymal development [19, 20] and maintenance [21–23]. Efferent duct ligation (EDL) obstructs the flow of testicular products into the epididymal tubule and causes the loss of testicular lumicrine factors (e.g. androgen binding protein, FGF2 and sperm membrane proteins [24]) from all points distal to the ligation. EDL also induces several alterations in the epididymal tubule [24, 25]. At present, reports of the effects of EDL on the epididymis focus on relatively few genes and give reference only to the standard epididymal

regions or to the initial segment. Microarray analysis of gene expression allows quantitative detection of thousands of gene transcripts, and when performed on individual segments rather than entire regions of the epididymis increases the sensitivity of transcript detection. Because the deprivation of testicular lumicrine factors alters epithelial structure dramatically, as well as secreted proteins [22], gene expression [23] and epithelial cell apoptosis [16–26], especially in the proximal segments of the epididymis [21, 27], and because microarray analysis of individual segments allows a broadly based, yet highly specific, evaluation of epididymal gene expression, we have used this approach to examine the effect of lumicrine signaling on the segmented function of the rat epididymis.

3 Rat epididymal segments lose differential gene expression in the absence of lumicrine factors from the testis

Animals were anesthetized as above and unilateral efferent duct ligation and contralateral sham operation were performed as described previously [16, 28]. Fifteen days later, the testes and epididymides were exposed and the epididymides were removed and epididymides were immediately placed in ice-cold saline in a Petri dish under a dissecting microscope. The epididymal tunica albuginea was removed from the caput epididymidis using sharp microdissection and the first four epididymal segments (Figure 2) were isolated. As each segment was isolated it was immediately placed in RNALater (>10 × tissue volume; Ambion, Austin, TX, USA) on ice. All four segments of each epididymis were in RNALater within 15 min of epididymal extirpation, and this procedure was repeated until each epididymal segment was represented by five separate samples for RNA extraction.

Five additional animals were subjected to unilateral EDL and 15 days later both the contralateral control epididymides and the ipsilateral, EDL epididymides were extirpated, paraffin embedded, and stained with hematoxolyn-eosin for subsequent measurement of tubule diameter and epithelial height.

RNA was extracted in ice cold TRIzol by conventional procedures and purified with RNAeasy columns (Qiagen, Valencia, CA, USA). RNA quantity was determined by absorbance at 260 nm and quality was determined using an Agilent Bioanalyzer (Palo Alto, CA, USA).

3.1 Microarray processing

Five μg total RNA were used to generate biotin-labeled cRNA using an oligo T7 primer in a reverse transcription reaction followed by *in vitro* transcription reaction with biotin-labeled UTP and CTP. Ten μg cRNA were fragmented and hybridized to RAE230 2.0 arrays (Affymetrix, Santa Clara, CA, USA). Hybridized arrays were stained according to manufactures protocols on Fluidics Station 450 and scanned on Affymetrix Scanner 3000. Arrays with excessive background, low signal intensity, or major defects within the array were eliminated from further analysis. The final number of arrays used for analysis was three for all control segments and five for all EDL segments.

Signal values were determined by using Gene Chip Operating System 1.0 (GCOS, Affymetrix). For each array, all probe sets were normalized to a mean signal intensity value of 100. The default GCOS statistical values were used for all analyses. Signal values and absolute detection calls were imported into Expressionist Analysis 3.0 (Genedata; Basel, Switzerland) for analysis.

A gene transcript was considered detectable if its mean expression in any segment was greater than 50 signal units and the percentage of samples with a present call as determined by GCOS default settings was ≥ 67% in the samples within a group. Such transcripts are hereafter referred to a "qualifiers". Normalized signal values were transformed to the log base 10 and pair-wise comparative analysis of the qualifiers in each segment was performed. A qualifier was considered to be segmentally regulated if two conditions were met: (i) the qualifier had to be detected in at least 67% of the samples of at least one of the segments analyzed; and (ii) a significant difference at the level of $P \leq 0.01$ based on the Welch test had to exist between the control and EDL values. Qualifiers meeting these conditions were used for further analysis. For some computations, the expression values for each qualifier were normalized to a mean of 0 and a standard deviation of 1 (z-score normalization). This allowed direct comparison of patterns within the data without respect to absolute expression levels. Principal component analysis (PCA) was performed on the transformed data and visualized using Spotfire 7.2 (Somerville, MA, USA). The outcome of such an analysis is a set of variables visualized in a 2-D or 3-D space, a process useful for functional and biological interpretation of complex datasets [29].

3.2 Real-time reverse transcription polymerase chain reaction (qRT-PCR) analysis

cDNA samples for real-time PCR analyses were synthesized by random priming in a final volume of 20 μL using the Superscript III First Strand Synthesis System (Invitrogen; Carlsbad, CA, USA) for RT-PCR according to the manufacturer's instructions. The cDNA samples corresponding to each of the epididymal segment samples

as well as whole epididymis were diluted to a final volume of 200 μL to produce a 10 × cDNA stock.

Real-time RT-PCR (qRT-PCR) analysis was carried out using FAM labeled flourogenic LUX primers from Invitrogen. Primers were chosen from published sequences for prostaglandin D_2 synthase (*Ptgds*), CD52 antigen (*Cd52*), glutathione peroxidase-3 (*Gpx3)*, cystatin 8 (*Cst8*; also known as CRES for cysteine-related, epididymal specific protein), defensin β1 (*Defb1*), 5α-reductase I (*Srd5a1)*, phosphatidylethanolamine binding protein (*Pebp1)*, lipocalin 5 (*Lcn5*; also known as ERABP or epididymal retinoic acid binding protein) and superoxide disumtase-1 (*Sod1*). Flourophore-labled primers were synthesized by Invitrogen and non-labeled primers were synthesized by Wyeth Research (Collegeville, PA, USA).

Real-time RT-PCR reactions were conducted using Platinum Quantitative PCR SuperMix-UDG (Invitrogen), according to the manufacturer's instructions. PCR reactions were run on an automated flourometer in a 96 well format. PCR conditions for all reactions was as follows: 1 cycle of 48ºC for 30 min., 95ºC for 10 min. followed by 40 cycles of 95ºC for 15 s and 60ºC for 1 min. Relative expression was determined by using the C_T method [30] using Sequence Detector software, version 1.6.3. The cDNA samples of each segment and of whole epididymis were evaluated in triplicate with the primer pair for each gene. Results were normalized to 18S ribosomal RNA expression and expressed as a ratio of expression of each gene in each segment compared to the expression of that gene in the whole epididymis.

3.3 Results of the efferent duct ligation study

The rat caput epididymidis (Figure 2A) becomes considerably reduced in size 15 days after EDL and the surface appearance of the segments is less distinct (Figure 2). A total of 2 255 qualifiers were regulated in response to EDL in segment 1 (Table 1), or approximately 18% of the total number of qualifiers detected in that EDL segment. The number of qualifiers either upregulated or downregulated after EDL declined sequentially in each more distal segment, with segment 4 showing only 420 genes (approximately 4% of total) regulated in response to EDL (Table 1). The caliber of the changes varied from gene to gene and from segment to segment, with EDL again having its largest effects in segment 1 where 1 595 genes were induced to at least a 2-fold change, 150 to at

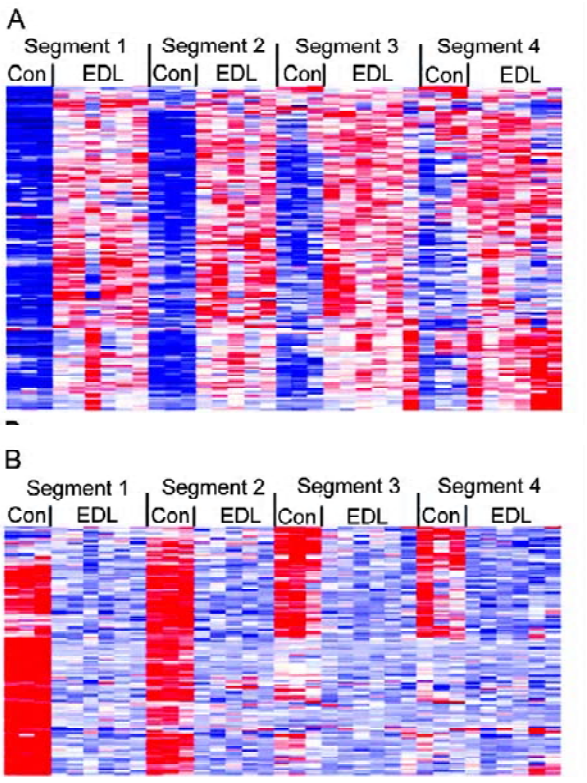

Figure 5. Heat map illustrating relative gene expression in segments 1–4 of the control rat epididymis (CON, *n* = 3/segment) and segments 1–4 15 days after efferent duct ligation (EDL, *n* = 5/segment). Relative expression of individual transcripts are displayed along the *y* axis. Blue indicates relatively low expression, white, moderate expression, and red, high expression. (A): Many genes poorly expressed in control segments, especially in segments 1–3 were moderately-to-highly upregulated after EDL making all EDL segments relatively homogenous in the expression of those genes. (B): Many other genes showed relatively high expression in segments 1 and 2, especially, but were down-regulated after EDL, again making all segments relatively homogenous. Thus, EDL induces a dedifferentiation of segments 1–4 of the rat epididymis. Modified with permissin from Turner *et al.* [12].

Table 1. The number of genes transcripts detected in each segment of the rat epididymis examined and the number of gene expressions upregulated or downregulated 15 days after efferent duct ligation (EDL). †Transcripts detected in at least 67% of each control segment's samples. ‡Number of transcripts demonstrating increased expression within the segment 15 days after EDL ($P < 0.01$). §Number of transcripts demonstrating decreased expression within the segment 15 days after EDL ($P < 0.01$). Modified with permissin from Turner *et al.* [12].

Gene expression	Segment 1	Segment 2	Segment 3	Segment 4
Total gene expressions detected†	11 379	11 395	11 577	11 623
Total number of genes regulated	2 225	1 805	878	420
Expressions upregulated‡	1 232	947	481	206
Expressions downregulated§	1 023	858	397	214

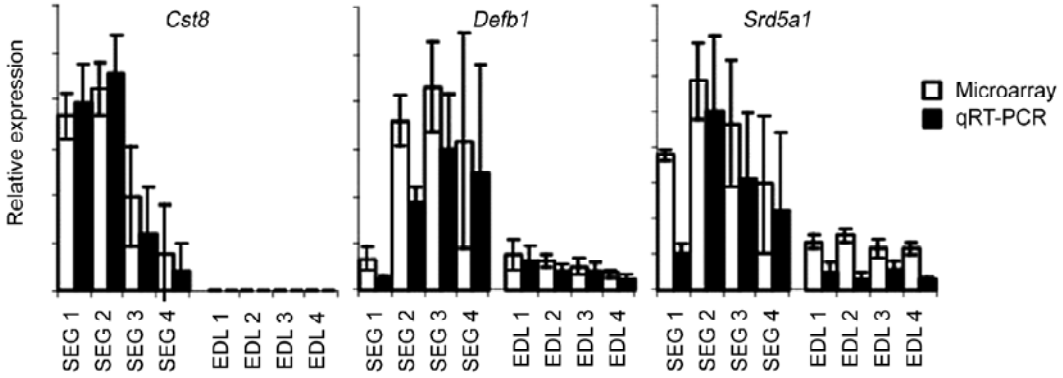

Figure 6. Confirmation of microarray results with real-time reverse transcription polymerase chain reaction (qRT-PCR) as illustrated with three different qualifiers whose expression was down-regulated by EDL as detected by both techniques. SEG, segment; EDL, efferent duct ligation. Modified with permission from Turner *et al.* [12].

least a 10-fold change and 11 to at least a 100-fold change. By segment 4, the numbers of gene expressions changing at those same levels were 420, 38, 15 and 3, respectively. The proportion of those altered gene expressions that was upregulated versus downregulated stayed roughly the same (e.g. approximately 50, 30 and 15% were upregulated at the 2-, 5-, and 10-fold levels, irrespective of segment) (not shown). All changes at the 100-fold level were downregulations.

Among the qualifiers responding to EDL, there were many with relatively low expression in control segments, but which were moderately upregulated after EDL (Figure 5A). At the same time, there were many qualifiers with relatively high expression in control segments, but which were sharply reduced in expression after EDL (Figure 5B).

The net effect of EDL was to eliminate segmental differences in gene expression among the segments studied. Microarray results were corroborated by qRT-PCR of selected genes known to be present in the epididymis (Figure 6). *Cst8*, *Defb1* and *Srd5a1*were all genes downregulated by EDL according to microarray analysis and similar results were detected by qRT-PCR. Other genes, *Ptgds*, *Cd52*, *Gpx3* and *Pebp1* among them, also showed highly similar results on the two different types of analysis.

Principal component analysis grouped samples with similar transcriptome expression patterns. When displayed in 2-D space, the analysis illustrates that rat segments 1 and 2 stand separately as unique gene-expression units of the epididymis (Figure 7). Control segments 3 and 4 span common space and are not different from each other, but EDL eliminates segmental differences and sets all 4 segments apart from their control counterparts (Figure 7).

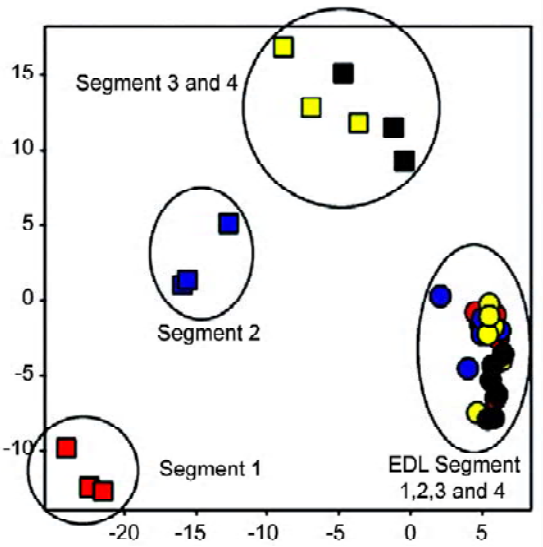

Figure 7. Principal component analysis (PCA) of qualifier expression profiles in segments 1–4 of the control rat epididymis and segments 1–4 15 d after efferent duct ligation (EDL). PCA identified four different data sets based on overall gene expression profiles: control segment 1, control segment 2, control segments 3 and 4, and EDL segments 1–4, together. Colors indicate the different segments, as noted in the figure. Squares indicate control segments, circles indicate EDL segments. Modified with permission from Turner *et al.* [12].

4 Summary

It is well known that interruption of luminal contribution from the testis alters epididymal gene and protein expression [24, 25]; however, the breadth of the changes have been difficult to assess because only a score or so of specific genes or proteins have been studied in the epididymis after EDL or after orchidectomy with andro-

gen supplementation. Microarray analysis of gene expression has previously been used in rats [31] and mice [32, 33] to evaluate more broadly the changes in epididymal gene expression after orchidectomy with androgen replacement, and these studies have documented widespread change in gene expression throughout the epididymis. Unfortunately, direct comparisons among these studies have been difficult because of the use of different species, different epididymal dissection patterns, and different micro-array platforms. Direct comparison of those studies with the present study is difficult, as well, but the aim was to use a segment-by-segment evaluation of gene expression changes in the proximal segments after an EDL of sufficient time to allow the initial wave of epithelial apoptosis [16, 27] to diminish and for the epithelium to come to a post-EDL steady state.

Unilateral EDL was used, which allowed preservation of circulating androgens while eliminating lumicrine factors from the ipsilateral epididymis. The results make it clear that thousands of gene expressions are altered by loss of lumicrine factors, and that those factors can be suppressive (> 1 200 qualifiers upregulated after loss of lumicrine factors) or stimulatory (> 1 000 qualifiers downregulated after loss of lumicrine factors; Table 1). It has been demonstrated previously that loss of testicular factors can upregulate or downregulate individual gene expressions in select regions of the rat [31] and murine [32, 33] epididymis, but the present study focused specifically on the first four segments of the rat caput epididymidis because those segments receive intraluminal factors most directly from the testis. It seemed likely these segments of the epididymis would be most sensitive to lumicrine factors [24].

The first three segments of the rat epididymis make up what has historically been referred to in the rat as the "initial segment", initially identified by Reid and Cleland [1] as zones 1a, 1b and 1c. The initial segment nomenclature presents difficulties described elsewhere [4], not the least of which is that under that single heading all three or even four segments of the rat "initial segment" have been assayed together as if they are a single unit. They are not. The segments are transcriptionally different (Figures 2 and 3), and the more proximal the segment, the more profoundly its gene transcription is affected by EDL (Table 1, Figure 5). Nearly 20% of the segment 1 transcriptome was altered after 15 days of EDL, and this proportion declined to approximately 4% by segment 4 (Table 1).

Segments of the epididymis that are highly differentiated in control animals become transcriptionally undifferentiated after EDL (Figures 5 and 7). Fifteen days after EDL, none of the segments studied were transcriptionally different from each other in an overall statistical sense, but they were all different from their respective control tissues (Figure 7). In other words, EDL induces a loss of differentiation of the proximal segments of the rat epididymis. The implications of this are that even in the patent male tract, testicular secretions in addition to testosterone are required for epididymal function in a way that is far more broad than previously appreciated. A more complete determination of important lumicrine molecules is called for, as well as a elucidation of their role both in direct signaling to the tubule epithelium and in indirect signaling to cells in the epididymal interstitium. Those latter cells might play an important role in tubule regulation through the paracrine signaling mechanisms discussed previously.

Therefore, at least two different signaling processes are important in regulating the rat epididymis in addition to endocrine regulation: (i) a paracrine signaling process wherein growth factors or other signaling molecules traffic between interstitial and epithelial cells within a given segment, signaling limited to individual segments by the CTS forming the segment borders; and (ii) a lumicrine signaling process wherein intra-luminal molecules can pass from segment to segment, yet are still required for the direct or indirect regulation of thousands of gene expressions in a segment-specific manner. These processes speak of the complexity of the epididymal tubule and call for further investigations into the specific molecules involved in both the lumicrine and the paracrine signals that regulate the epididymal epithelium.

Acknowledgment

This work was supported by National Institutes of Health grant DK45179 (T. T. Turner) and Wyeth Research.

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