Minireview

Is the Epididymis a Series of Organs Placed Side By Side?1

Raquel F. Domeniconi,3 Ana Cláudia Ferreira Souza,4 Bingfang Xu, Angela M. Washington, and Barry T. Hinton2

Department of Cell Biology, University of Virginia Health System, Charlottesville, Virginia

ABSTRACT

The mammalian epididymis is more than a highly convoluted tube divided into four regions: initial segment, caput, corpus and cauda. It is a highly segmented structure with each segment expressing its own and overlapping genes, proteins, and signal transduction pathways. Therefore, the epididymis may be viewed as a series of organs placed side by side. In this review we discuss the contributions of septa that divide the epididymis into segments and present hypotheses as to the mechanism by which septa form. The mechanisms of Wolffian duct segmentation are likened to the mechanisms of segmentation of the renal nephron and somites. The renal nephron may provide valuable clues as to how the Wolffian duct is patterned during development, whereas somitogenesis may provide clues as to the timing of the development of each segment. Emphasis is also placed upon how segments are differentially regulated, in support of the idea that the epididymis can be considered a series of multiple organs placed side by side. One region in particular, the initial segment, which consists of 2 or 4 segments in mice and rats, respectively, is unique with respect to its regulation and vascularity compared to other segments; loss of development of these segments leads to male infertility. Different ways of thinking about how the epididymis functions may provide new directions and ideas as to how sperm maturation takes place.

epididymal segments, epididymis, gene expression, male fertility, Wolffian duct

INTRODUCTION

It is often written that the epididymis is a highly convoluted tube divided into four regions: initial segment, caput, corpus, and cauda. It is only over the past several years that the initial segment has been included in this list, showing that this region is now viewed separately from the caput region. Furthermore, it has been established that, at least for rodents, the epididymis is subdivided into segments separated by septa [1]. In view of the repertoire of genes that are expressed in each segment, we can consider the epididymis a series of small organs placed side by side. How these segments coordinate with each other to ensure that spermatozoa come into contact with the proper luminal microenvironment is not known. This microenvironment is critical for sperm maturation. To add another level of complexity, each segment appears to have unique and common regulators of gene and protein expression and signaling events. Putting this all together, it is obvious that the description of the epididymis as a highly convoluted tubule is much simplified. Much of this review focuses on the rodent epididymis, but where possible, we have referenced what is known in other species.

CONTRIBUTION OF SEPTA TO SEGMENTATION

For clarity, the classical divisions of the epididymis, the initial segment, caput, corpus and cauda, will be referred to as regions whereas segments are defined as the points where the convoluted duct is separated by a septum. For comparison, Figure 1 illustrates two rat epididymides, one illustrates regions and the other illustrates segments. Segmentation of the duct begins as early as Embryonic Day 18 (E18) and is obvious during early postnatal stages, and segmentation progresses from anterior to posterior (also referred to as proximal to distal in some publications) in line with ductal coiling [2]. It is possible that septa originate from the walls of the capsule surrounding the duct, similar to septa formation in the heart. However, it is not clear what determines the position of each septum during development. Septa could be predetermined at precise points during development (regardless of the length of duct and its degree of coiling) and/or initiate at a certain time when ductal elongation and coiling are optimal at that particular point. Further studies of the formation of septa will be greatly improved by identification of a specific marker, although presumably, septa contain collagen [3], elastic fibers, interstitial cells and blood vessels. Whichever mechanism(s) is responsible for their formation, the epididymis is always divided into the number of segments that is dependent upon the species, except for humans (see below).

For the adult mouse 10 segments were identified and 19 for the adult rat [4–7]. However, based upon differential gene

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2Correspondence: Barry T. Hinton, Department of Cell Biology, University of Virginia Health System, PO Box 800732, Charlottesville, VA 22908. E-mail: bth7c@virginia.edu
3Current address: Department of Anatomy, Institute of Biosciences, University Estadual Paulista, Botucatu, SP, Brazil.
4Current address: Department of General Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.
expression, Zhang et al. [8] split rat epididymis segment number 12 (corpus region) into 12A and 12B (Figs. 1 and 2, respectively). In addition to separating the duct into distinct segments, the function and anatomical three-dimensional arrangement of septa are not known. What is the relationship between the septum and the point at which the duct passes through that septum? How tightly does the septum surround the duct at that point? Presumably, septa aid in supporting individual segments but also carry blood vessels and nerves deep into the tissue. Studies by Turner et al. [9] suggested septa limit paracrine signals within individual segments and that such signals do not cross the septal boundary from one segment to another. This is an intriguing hypothesis especially in light of the expression of genes, proteins, and signaling pathways in each segment. However, interstitial fluid flow, which presumably can move across or through septal boundaries, may play a role in generating gradients of paracrine molecules across segments. When the interstitium of segments in the cauda region were inoculated with bacteria, the infection stayed within that segment; the septa acted as diffusion barriers [10]. The human epididymis is more complex, and, although several reports have shown 5–10 regions [11–13], the exact number of segments is unclear. It does appear that the septa do not completely traverse the entire duct, are not as well organized as observed in the rat and mouse, and are differently placed from one human epididymis to another (R. Sullivan, personal communication). Although investigators have used regions to describe the different parts of the epididymis of other species, including boar [14, 15], ram [16, 17], echidna [18], cynomolgus monkey [19], and horse [16], upon closer inspection of the images presented by the authors of the above studies, it is apparent that distinct segments are present.

CAN AN UNDERSTANDING OF SEGMENTATION OF OTHER TISSUES HELP UNDERSTAND THE SEGMENTATION OF THE WOLFFIAN DUCT?

It may be helpful to understand the mechanisms of Wolffian duct segmentation by examining what is known about the mechanisms of segmentation of other developing structures. Somite and renal nephron segmentation are excellent examples of the development of segmented organs [20–24]. Segmentation of the renal nephron/tubule is particularly relevant given...
that both the kidney and the epididymis are derived from the same ductal system and have similar physiological functions, for example, transport of ions and organic solutes. Much of the work on segmentation of the renal nephron has been performed using several animal models including fish (e.g., zebrafish), amphibians (e.g., Xenopus spp), birds (e.g., chicks), and mice. Similar to the Wolffian duct, there are several regions along the renal tubule where each region is divided into segments: convoluted proximal tubule (segments PS1 and PS2), straight tubule (segment S3), loop of Henle (descending thin limb and ascending thin limb segments) and distal tubule (thick ascending limb and distal convoluted tubule/loop segments). The distal convoluted tubule is connected to the collecting duct system that consists of two segments, the collecting tubule and the connecting duct.

The mechanisms by which the renal nephron undergoes proximal–distal patterning are not known, but there is evidence to suggest that several genes, including the transcription factors Irx5 and Irx36 [25, 26], Notch [27–29], and Caudal [30] are necessary for proximal and distal tubule formation. WNT signaling has been implicated in the formation of the mouse renal nephron; for example, loss of Wnt9b results in the failure of nephron formation and also formation of the Wolffian duct [31]. Using chick mesonephros, Schneider et al. [32] demonstrated the importance of WNT signaling in patterning the proximal–distal nephron axis. If an ectopic WNT ligand was expressed at right angles to the axis of the endogenous WNT signal (within the coelomic epithelium), then the nephron axis was reoriented. Certainly, components of the WNT signaling pathway are expressed in the adult epididymis [33–36], but whether WNT signaling plays a role in proximal–distal patterning of the Wolffian duct remains to be examined.

In addition to the mechanisms involved in proximal–distal patterning, the mechanisms of timing of segment formation along the Wolffian are also unknown. In this instance, examining the processes of timing during somitogenesis may be of help. The total number of somites formed is constant and species-specific, which is a characteristic similar to that in the formation of epididymal segments. The time of formation for each somite along the body axis from anterior to posterior has been determined for several species. It turns out that this timing is due to a cellular oscillator/segmentation clock (a clock and wavefront model) [37]. For example, expression of the chick hairy and enhancer of split (Hes) family and the WNT signaling pathway were shown to play critical roles in the generation of oscillations (see reviews in references [20, 21, 41]). FGF8 forms a gradient (retinoic acid forms an opposing gradient) [42, 43] that, in turn, will generate a graded FGF signaling event in each putative somite; the point where a group of cells respond to FGFs would be the wavefront. This signaling event results in the arrangement of segmental boundaries [39].

As posed at the beginning of this section, could an understanding of segmentation of another segmented structure help understand segmentation of the Wolffian duct? In comparison with somitogenesis, it is clear that 1) the number of segments of the Wolffian duct is constant and specific for each species; 2) many genes involved in the segmentation of somites are also expressed in the developing Wolffian duct [44]; 3) FGFs are expressed within each segment, although that is only shown for the adult epididymis [45]; and 4) segmentation is observed from anterior to posterior. A major difference is that the individual segments (somites) are formed and positioned in sequence, whereas the Wolffian duct is already formed, and segmentation involves elongation, coiling, and generation of a septum (segment boundary). Also, each epididymal segment is not uniform but is of different shapes, sizes, and positions [46]. However, it is still tempting to hypothesize that segmentation involves an oscillator/clock that results in the sequential generation of a segment. In further support of this hypothesis, a gradient of inhibin beta A (Inhba) has been shown to exist along the anterior–posterior axis of the Wolffian duct, with the highest expression observed at the most anterior mesenchymal cell compartment [47, 48]. Loss of Inhba resulted in loss of coiling and subsequent loss of segmentation [48]. There is some evidence to suggest that collective cell movements may synchronize locally coupled oscillators, which in turn promote global synchronization for the generation of segmented structures [49]. This is of special interest in light of recent findings that one of the major driving forces of elongation and coiling of the Wolffian duct is cell rearrangement [50]. Loss of Pdk1, a gene involved in autosomal dominant polycystic kidney disease, also results in loss of coiling of the Wolffian duct during development, thereby suggesting that the TGF-/BMP signaling pathway may also regulate coiling and possibly segment formation [51].

Homeobox (Hox) genes also play a role in segmentation of structures, and there is evidence from a microarray screen that the developing Wolffian duct expresses several Hox genes (Hoxc9, Hoxa10, Hoxa11, Hoxc4, Hoxc10, Hoxd4, Hoxd8, and Hoxd13), and other homeobox-containing genes such as meis homolog 2 (Meis2), cut-like homeobox 2 (Cux2), Lim homeobox protein 1 (Lmx1) gene, and Mohawk homeobox (Mxk) [44]. Other investigators have identified similar and additional Hox and homeobox-containing genes in the developing Wolffian duct, including Hoxa10, Hoxa13 [52–56], paired box 2 (Pax2), Pax8, Meis1, lens intrinsic membrane protein 2 (Lm2), T-cell leukemia, homeobox 2 (Tlx2), and ladybird homeobox homolog 2 (Lbx2) [57, 58]. It is not known whether one or more Hox genes are responsible for segmentation, but it is clear that during embryonic development of the Wolffian duct. 1) Hoxc4 is expressed in the efferent ducts and the entire Wolffian duct, but not the vas deferens; 2) Hoxc9 is expressed along the entire Wolffian duct but not the efferent ducts and vas deferens; and 3) Hoxa11, Hoxa10, and Hoxd13 are expressed only in the vas deferens during embryonic development. These data suggest that, from a regional perspective, Hoxc4 and Hoxc9 may play a role in boundary formation between the efferent ducts and epididymis. Interestingly, Lmx5 may be involved in boundary formation between the efferent ducts and testis or at least involved in the connection between the efferent ducts and testis [59]. Loss of Hoxa11 and Hoxa10 results in homeotic transformation of the vas deferens into cauda, which is also indicative of this gene playing a role in boundary formation between the efferent ducts and epididymis. Although it has been well established that each epididymal region of several species expresses its own repertoire and those
of overlapping genes and proteins, it was the studies by Johnston et al. [5] and Jelinski et al. [63] that showed discrete segments express their own gene and overlapping repertoire. Figure 2 shows an example of such expression. More recently, investigators have used segment terminology in identification of proteins by using immunolocalization approaches, for example, neutral endopeptidase analysis [3]. Those findings have allowed those working on the epididymis to be more precise in defining the expression of genes and proteins and, hopefully, functions at those discrete segments. The authors encourage those working on the epididymis to use segment nomenclature. It will also allow investigators to generate segment-specific conditional knockout mice and so identify those segments that are critical for sperm maturation. A number of region-specific expression studies using Cre-recombinases have been used to conditionally remove genes from that region, for example, ribonuclease, RNase A family, 10 (Rnase10), which is active in the initial segment with some proximal caput overlap [64]; lipocalin 5 (Lcn5; active in the caput) [65]; and defensin beta 41 (Defb41; active in the initial segment) [66]; but not single-segment–specific expression of Cre-recombinases.

It has been technically difficult to identify the expression of genes along different regions of the epididymal duct during embryonic development, although studies have revealed differences in gene expression as early as E12.5 between the mesonephric tubules (which will form the efferent ducts, the entire Wolffian/mesonephric duct, and the vas deferens; GenitoUrinary Development Molecular Anatomy Project [GUDMAP]; http://www.gudmap.org/index.html) [44]. For example, the GUDMAP database shows in situ hybridization localization of the collagen triple helix repeat containing 1 (Cthrc1) gene in both mouse mesonephric tubules and Wolffian/mesonephric ducts at E10.5. However, at E12.5, Cthrc1 was only localized to mesonephric tubules. The proprotein convertase subtilisin/kexin type 9 (Pcsk9) gene was localized to the mesonephric tubules at both E10.5 and E12.5. It would also be of great interest to identify gradients of gene expression along the entire duct. More studies are required to identify specific gene expression and putative signaling pathways during and following the development of a segment. The findings will also help in identification of putative segmentation clock genes as described above.

More recently, investigators have performed micro-RNA (miRNA) and Piwi-interacting RNA (piRNA) profiling along adult mouse, rat, and human epididymal ducts [8, 67–69], but because the profiling was performed in regions and not segments, it is not clear if miRNAs play a role in the development of segments and/or their function in the adult.

LACK OF INITIAL SEGMENT RESULTS IN MALE INFERTILITY

In the mouse, there are 2 segments associated with the initial segment. The rat has 4, and the human is more complex. Human epididymis has a discontinuous initial segment-like epithelium (epithelium VII), and the initial segment does not form a gross anatomical appearance similar to that seen in other species [70]. The earliest indication that the initial segment was important for male fertility came from a study using mice that had a null mutation in the Ros1 proto-oncogene (Ros1), an orphan tyrosine kinase receptor [71]. Both the extracellular domain and the kinase domain were found to play a role in the male infertility phenotype. Follow-up studies revealed an interesting sperm phenotype [72–75], in which the sperm were unable to volume regulate once in the female reproductive tract and displayed hairpin bends at the mid-piece, resulting in failure to fertilize an egg. The function of Ros1 playing a role in the initial segment has been confirmed [76]. A very similar phenotype was observed in mice in which the large T antigen was targeted to the initial segment/caput regions by using the glutathione peroxidase 5 (Gpx5) promoter, although the mechanism by which the hairpin bends formed differed from that in the Ros1 knockout animal [77, 78]. In addition, a similar sperm phenotype was observed in mice in which the phosphatase and tensin homolog (Pten) gene was conditionally removed from the initial segment. In that instance, hairpin bends were observed in spermatozoa as they progressed along the epididymal duct. In addition to all three animal mutations having similar sperm phenotypes, it was suggested that a common underlying mechanism of loss of function of the initial segment was a defect in cellular differentiation [79]. In the case of the Ros1 null mutation, the initial segment failed to undergo differentiation during the prepubertal period, whereas in the conditional Pten knockout mice, the initial segment underwent dedifferentiation [79]. It remains to be seen whether any initial segment genes that regulate male fertility are potential targets for the development of a human male contraceptive. Ros1 is expressed in human epididymis but is not confined to the proximal region [80].

Another unique feature of the initial segment region is its intense vascularity, which is very obvious in the epididymis of several species [46, 81, 82] and is reflected in its blood flow compared to the remainder of the epididymis [83]. Suzuki [84] defined two types of anatomical arrangements of capillaries surrounding the semiferous tubules and different regions along the epididymis of the mouse: testicular type and deferential type. Initial segment and cauda region capillaries are differentially arranged, which is characterized by having arterioles and venules running parallel to the duct for a considerable length before (or after) communicating with the capillary network. There is much interconnectivity between the capillaries surrounding the initial segment duct. The vascular arrangement surrounding the initial segment duct is absent in Ros1 knockout and XX sex reversed (XXXsx) mice [85], presumably due to the failure of the initial segment to develop. Further studies showed that the loss of the initial segment in XXXsx males was androgen-independent [85], maybe pointing to the role of testicular factors. These studies also demonstrated the intimate relationship between development of the initial segment and concomitant development of its vasculature. In addition, Gpx5-Tag2 mice, in which the SV40 tag is under the control of the Gpx5 promoter [77], which is highly expressed in the proximal region of the epididymis, show a developed initial segment that lacks the degree of vasculature found in control initial segments. This initial segment epithelium did show an increase in apoptosis compared to that in control and was dysplastic. Both of the phenotypes could certainly alter the function of the initial segment, resulting in the failure of the vasculature to develop. The capillaries within the mouse initial segment, segment 1 only, are fenestrated [86], whereas the more distal segments are nonfenestrated. The function of fenestrated capillaries within segment 1 are not known, although it is often speculated that fenestration allows for rapid interchange of fluid and epididymal luminal fluid components between the epididymis and blood. However, more than 90% of testicular fluid is absorbed along the efferent ducts [87, 88], so it is not clear as to the reason why the capillaries in this region are fenestrated. Although there is a network of capillaries within the initial segment, it has the lowest percentage of lymphatic capillaries per epididymal interstitium (0.69%) compared to the more distal regions; the
cauda showing the most (4.92%) [89]. More studies are required that examine the development of the initial segment vasculature and the influence played by the initial segment epithelium on its development (and/or vice versa).

INITIAL SEGMENT IS UNIQUELY REGULATED COMPARED TO OTHER REGIONS

A clue to the uniqueness of the regulation of this epididymal region originated from early studies in which the efferent ducts were ligated and cellular apoptosis was observed throughout the initial segment epithelium [90–93]. Apoptosis could not be reversed by exogenous administration of testosterone or dihydrotestosterone. Further studies clarified the degree and rate of apoptosis [94, 95]. These and other studies hypothesized that luminal fluid components or lumicrine factors were responsible for cell survival in the initial segment, and lumicrine regulation was a unique regulation feature of the initial segment [96]. Examining the roles of lumicrine factors in the course of development of the initial segment revealed that lumicrine factors regulated initial segment differentiation [9, 97]. During the differentiation stage, lumicrine factors were required for both cell proliferation and cell survival of differentiating epithelial cells in the initial segment. During the postdifferentiation stage, lumicrine factors were required for cell survival of differentiated cells in the initial segment epithelium [9, 97]. Among the multiple signal pathways, the ERK pathway was in the center of lumicrine regulation [79, 98, 99], which rapidly responded to stimulation and loss of lumicrine factors. A high activity level of the ERK pathway was a feature of the differentiated initial segment that was distinct compared to other regions [79, 98, 99]. Although the identity of lumicrine factors was unknown, BMPs [100, 101], fibroblast growth factors [102], and luminal fluid androgen [58] have been suggested to be putative lumicrine factors. It was hypothesized that growth factors regulated permissive signal pathways, including the ERK pathway, while non-genomic actions of androgen receptor (AR) enhanced signal transduction regulation. Interestingly, earlier studies by Brooks [103] clearly showed that lumicrine factors are also negative regulators of protein synthesis and secretion by the initial segment epithelium. Upon closer inspection of the initial segment epithelium following efferent duct ligation, it was clear that not all cells underwent apoptosis. Could there be two distinct cell populations of cells within the initial segment? Different lineages? Stem cell-like population?

It is possible that lumicrine factors are secreted by epididymal regions, which in turn regulate the function of downstream regions [104]. Ligation of the duct between the distal caput and proximal corpus (corresponding to segments 6 and 7, respectively [3]) resulted in microscopic cellular changes in the epithelium of the corpus (segment 7). However, these data could also be interpreted as the result of the absence of testicular luminal fluid factors. Further studies are needed to test the hypothesis that lumicrine factors are secreted by the epididymal epithelium, which in turn regulate the functions of downstream epithelial cells.

ARE OTHER SEGMENTS DIFFERENTIALLY REGULATED?

Extensive genomic and proteomic studies have clearly shown that testosterone and 5α-testosterone up- and down-regulate the expression of genes and proteins along different regions of the epididymis [58]. For example, γ-glutamyl transpeptidase (Ggt) mRNA IV is down-regulated in the rat initial segment following orchietomy, but the same mRNA is up-regulated in the cauda region [105]. The fucosyltransferase (Fut) family of genes is upregulated (Fut1 and Fut 4) and downregulated (Fut2 and Fut9) in the caput region following loss of androgens [106]. There are many more examples showing differential regulation of expression of genes and proteins at the regional level. Presumably, the cells in each region (segment?) express a unique repertoire of transcription factors that respond to androgens by enhancing or repressing gene expression.

LUMINAL FLUID COMPOSITION CHANGES ALONG THE EPIDIDYMAL DUCT SEGMENT BY SEGMENT

If we consider each segment as an individual “organ,” then presumably the luminal fluid milieu changes segment by segment. Each segment will contribute in unique ways to that luminal fluid microenvironment via secretion, absorption, sperm modifications contributing to changes of that fluid at that segment and epididymosome secretion. Examination of the composition of the luminal fluid at each region clearly shows it rapidly changes from one region to the next (see reviews in [58, 107]). An example of changes of luminal fluid composition from one segment to the next is the sudden appearance of l-carnitine in the fluid of the rat distal caput region (region 2a) [108]; it was not present in the luminal fluid of the proximal caput region (region 2) [108]. Comparing the numbered epididymal micropuncture sites with the numbering of each segment in the rat epididymis [4], region 2 corresponds to segment 3, and region 2a corresponds to segment 4, the same location in which the specific l-carnitine transporter OCTN2 was identified [109]. Micropuncture studies can be used to collect fluid from different regions, but it will be technically challenging to collect fluid from only one discrete segment for analysis. However, an approach, the split-drop stopped-flow micropuncture technique, was used to measure the secretion of amino acids at discrete segments [110]. It is the combination of cell types within that region/segment that is responsible for generating a unique luminal fluid milieu within that segment. We are beginning to understand the functions of each cell type. However, cells do not function in isolation but interact with neighboring and/or downstream cells via paracrine mechanisms. Therefore, it is important to uncover those mechanisms by which the different cell types, within the same region/segment, communicate or cross-talk with each other. This is well illustrated in the studies by Breton et al. [111] and Shum et al. [112], in which communication between clear, principal, and basal cells is essential in generating an optimal luminal fluid pH and bicarbonate concentration for sperm maturation.

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“The real voyage of discovery does not consist in seeing new landscapes but having new eyes.”—Marcel Proust

It is time to think in a different light about how the epididymis functions, if we are to uncover new and unique processes that are critical to sperm maturation. Thinking about the epididymis as a series of organs side-by-side is one way, but other ways of thinking will contribute. For example, examining the development of the Wolfian duct can be thought of in terms of the development of a biological tube. How do tubes form? How do tubes coil? How do tubes segment? Let us see how other organs tackle this issue and then see if we observe similar or dissimilar morphogenetic events or mechanisms between other biological tubes and the Wolfian/epididymal duct. Examining the functions of other segmented structures, for example, the formation of the renal nephron and
somites presented in this review may provide clues as to how epididymal segmentation is regulated. Although this may be considered blasphemy, we could think about the epididymis as an organ first, forgetting about sperm maturation. One mentor to one of the authors (BTH) once said: “The testis would be a wonderful organ to study if it were not for those darn sperm.” Perhaps “the epididymis would be a wonderful organ to study if it were not for those darn sperm.” It is just another way of thinking and the paths that open up with this way of thinking may well uncover how sperm maturation takes place.

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