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·*Review*·

Characterization and functions of beta defensins in the epididymis

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Abstract

The epididymal β-defensins have evolved by repeated gene duplication and divergence to encode a family of proteins that provide direct protection against pathogens and also support the male reproductive tract in its primary function. Male tract defensins also facilitate recovery from pathogen attack. The β-defensins possess ancient conserved sequence and structural features widespread in multi-cellular organisms, suggesting fundamental roles in species survival. Primate SPAG11, the functional fusion of two ancestrally independent β-defensin genes, produces a large family of alternatively spliced transcripts that are expressed according to tissue-specific and species-specific constraints. The complexity of SPAG11 varies in different branches of mammalian evolution. Interactions of human SPAG11D with host proteins indicate involvement in multiple signaling pathways. *(Asian J Androl 2007 July; 9: 453–462)*

Keywords: defensin; antibacterial; male fertility

1 Introduction

Defensins emerged from our studies on epididymis-specific proteins in which we were seeking novel male contraceptive targets. Among the candidate targets, the epididymal protease inhibitor Eppin was shown to be a successful reversible male immunocontraceptive in macaques [1]. The first defensin discovered in this program was given the clone name ESC42, and its trefoil-like motif was described [2]. Trefoil proteins are important in host defense; they maintain mucosal integrity and influence defensin and adaptive immunity gene expression [3]. After this motif was recognized as the β-defensin signature, ESC42 was named β-defensin 118 (DEFB118). DEFB118 is a member of a large family of genes clustered primarily on human chromosomes 6, 8 and 20 (Figure 1) [4–11]. Defensins have evolved by repeated gene duplication and divergence, including functional diversification [12]. Except for the 6-cysteine domain, rich in positively charged amino acids, defensins differ considerably in their amino acid sequences and target pathogen specificity [4]. A similar cysteine array is found in some lectins [13] and antibacterial protease inhibitors, including the contraceptive target Eppin [14], and secretory leukocyte protease inhibitor [15] (Figure 2). Ancient guards against pathogen invasion, lectins and protease inhibitors are also important in plant host defense [16].

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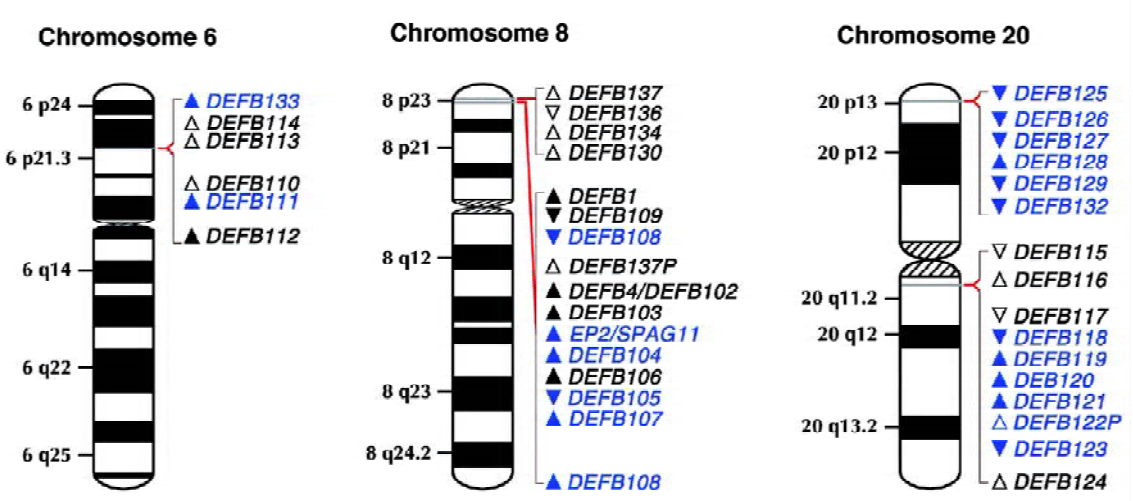

Figure 1. Major human defensin gene clusters. Gene names in black indicate widespread expression. Gene names in blue indicate expression predominantly in the male reproductive tract. Triangles point in the direction of transcription. The filled triangles indicate active genes, whereas no transcripts are known for the open triangle genes. Data are from human genome build 36.2.

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DEFB1   GNFLTGLGHRSDHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK
DEFB4/hBD2      GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP
DEFB118           AYSGEKKCWNRSGHCRKQCKDGEAVKDTCKNLRACCIPSNEDHRRVPATSPTPL
SDSTPGIIDDILTVRFTTDYFEVSSKKDMVEESEAGRGTETSLPNVHHSS
DEFB126         NWYVKKCLNDVGICKKKCKPEEMHVKNGWAMCGKQRDCCVPADRRANYPVFCVQTKTT
RISTVTATTATTTLMMTTASMSSMAPTPVSPTG
SPAG11C  RHVNHSATEALGELRERAPGQGTNGFQLLRHAVKRDLLPPRTPPYQ
EPASDLKVVDCRRSEGFCQEYCNYMETQVGYCSKKKDACCLH
hSPAG11D RHVNHSATEALGELRERAPGQGTNGFQLLRHAVKRDLLPPRTPPYQ
GDVPPGIRNTICHMQQGICRLFFCHSGEKKRDICSDPWNRCCVSNTDEEGKEKPEMDGRSGI
Eppin         PGLTDWLFPRRCPKIREECEFQERDVCTKDRQCQDNKKCCVFSCGKKCLDLKQDVCEMP
KETGPCLAYFLHWWYDKKDNTCSMFVYGGCQGNNNNFQSKANCLNTCKNKRFP
SLPI            SGKSFKAGVCPPKKSAQCLRYKKPECQSDWQCPGKKRCCPDTCGIK
CLDPVDTPNPTRRKPGKCPVTYGQCLMLNPPNFCEMDGQCKRDLKCCMGMCGKSCVSPVKA
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Figure 2. Alignment of human β-defensin signature motifs with similar arrays in antibacterial protease inhibitors. The defensin and defensin-like 6-cysteine motifs are highlighted in grey, are bold and underlined. Sequences were derived from the following accession numbers: NM_005218 DEFB1, NM_004942 DEFB4/hBD2, NM_054112 DEFB118, NM_030931 DEFB126, AF286368 Eppin, AF114471 secretory leukocyte protease inhibitor, NM_058203 SPAG11C and NM_058201 SPAG11D. Signal peptides are not shown.

2 β-defensin primary sequences and functions

Beyond the 6-cysteine signature motif, the simplest β-defensins have little additional sequence (Figure 2) and fall in the molecular weight range of 5–10 kDa. These simple defensins, such as human DEFB1 and DEFB4 (hBD2), are related to defensins in lower animals, including fish [17] and insects [18]. Similar defensins are produced in plants, particularly in the reproductive structures (flowers and seeds) [16]. Male reproductive tract defensins are known only in mammals. These defensins may be as large as 18 kDa (human DEFB129) and often have long N-terminal or C-terminal extensions, generally of unknown function. Reproductive functions are suggested by the sperm surface location of several defensins, including SPAG11 [19, 20], DEFB118 [2] and DEFB126 [21, 22]. Reproductive functions have been reported for rat SPAG11E (Bin1b) [23] and for DEFB126 [21, 22]. Bin1b promotes motility in immature spermatozoa from the caput epididymidis by a mechanism dependent on calcium uptake [23]. The long C-terminal domain of DEFB126, rich in threonine and serine, is highly O-glycosylated. A major component of the sperm glycocalyx [24], DEFB126 is shed during capacitation [22], a loss prerequisite to spermatozoa binding to the zona pellucida [21]. The highly anionic C-terminus of

DEFB118 is not thought to have a role in antibacterial action [25], which typically depends on cationic amino acids. The male reproductive tract DEFB123 has a novel function, protection against endotoxemia through restoration of normal tumor necrosis factor-α levels [26].

3 Structures of β-defensins and similar proteins

Structurally, β-defensins typically contain an N-terminal alpha helical domain joined by a disulfide bond to a 2-strand or 3-strand beta sheet stabilized by additional disulfide bridges. The similarity of this fold in human proteins hBD1 [27], SPAG11E [28], in bovine SPAG11C [29] and the human intestinal trefoil protein 3 [30] is shown in Figure 3. The fungal, insect, and plant defensins shown are strikingly similar to a scorpion neurotoxin that shows sequence homology with the male reproductive tract defensins DEFB118 and DEFB126 (identified as GenBank AA335178 and ESP13.2 in [31]). Their cysteine stabilized configuration might represent evidence of broad application of independently evolved structures to common features of host defense challenges [32], or might be evidence of ancient origins of the β-defensins conserving similar domains throughout the animal and plant kingdoms.

4 The *SPAG11* gene is a fusion of two β-defensin genes

Unique among the β-defensins, human SPAG11 represents the functional fusion of two ancestrally independent β-defensin genes [33] (Figure 4). Alternatively spliced transcripts are initiated at both promoters. Transcripts initiated at the A promoter may end after exon 3 or may continue past the poly A addition site, presumably a weak termination signal, and continue through the B promoter and the B exons. Species-specific exons are reported for human, monkey and bovine SPAG11 [29, 33–35]. There are fewer bovine mRNA splice variants (only six) than primate variants [29]. Several of the bovine splice sites are in the 3'-untranslated regions, where they may affect mRNA stability. There are three bovine-specific exons. The rat *SPAG11* gene is simpler than that in primate and bull and retains the original separate function of the A and B components. There is only one splice site and it is in the A component. No species-specific exons are found in rats [36]. Read-through transcription have to has not been reported for any other pair of defensin genes.

5 SPAG11 proteins

Translation of these alternatively spliced RNAs produces a complex protein family. Immunohistochemical staining has revealed the presence of multiple SPAG11 isoforms in the epithelial cells of the epididymis, showing that these mRNAs are actively translated [20, 29, 36]. Most primate SPAG11 proteins contain the N-terminal common region joined to C-terminal peptides encoded by different combinations of exons (Figure 5). Multiple reading frames are utilized. For human SPAG11A, exon 6 transcripts are translated in one reading frame, in a second reading frame for the D isoform and for the *Rhesus macaque* J isoform in the third reading frame. Why SPAG11 evolved these special features is not known. Perhaps it is for the same reason that

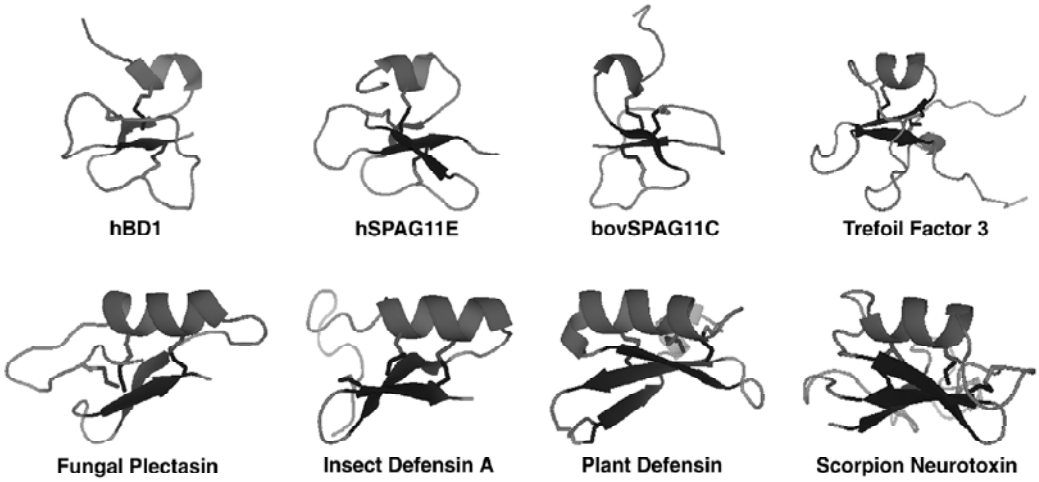


Figure 3. Similarity of β-defensin structures in different species. Models were draw in PyMol using the following Protein Data Bank files: 1E4S.pdb (hBD1), 1E9T.pdb (human intestinal trefoil protein 3), 1ZFU.pdb (fungal defensin, plectasin), 1ICA.pdb (insect defensin A), 1TI5.pdb (plant defensin VrD1), and 2SN3.pdb (scorpion toxin 3). The human SPAG11E homology model is based on 1FD3.pdb (hBD2). The bovine SPAG11C model is based on 1E4R.pdb (mouse Defb8).

families of alternative splice variants operate where discriminative protein association is crucial in immunity [37, 38], neuronal function [39, 40], hearing [41], olfactory detection [42] and fertility [43]. Families of proteins containing different combinations of peptides can have different but overlapping sets of molecular recognition

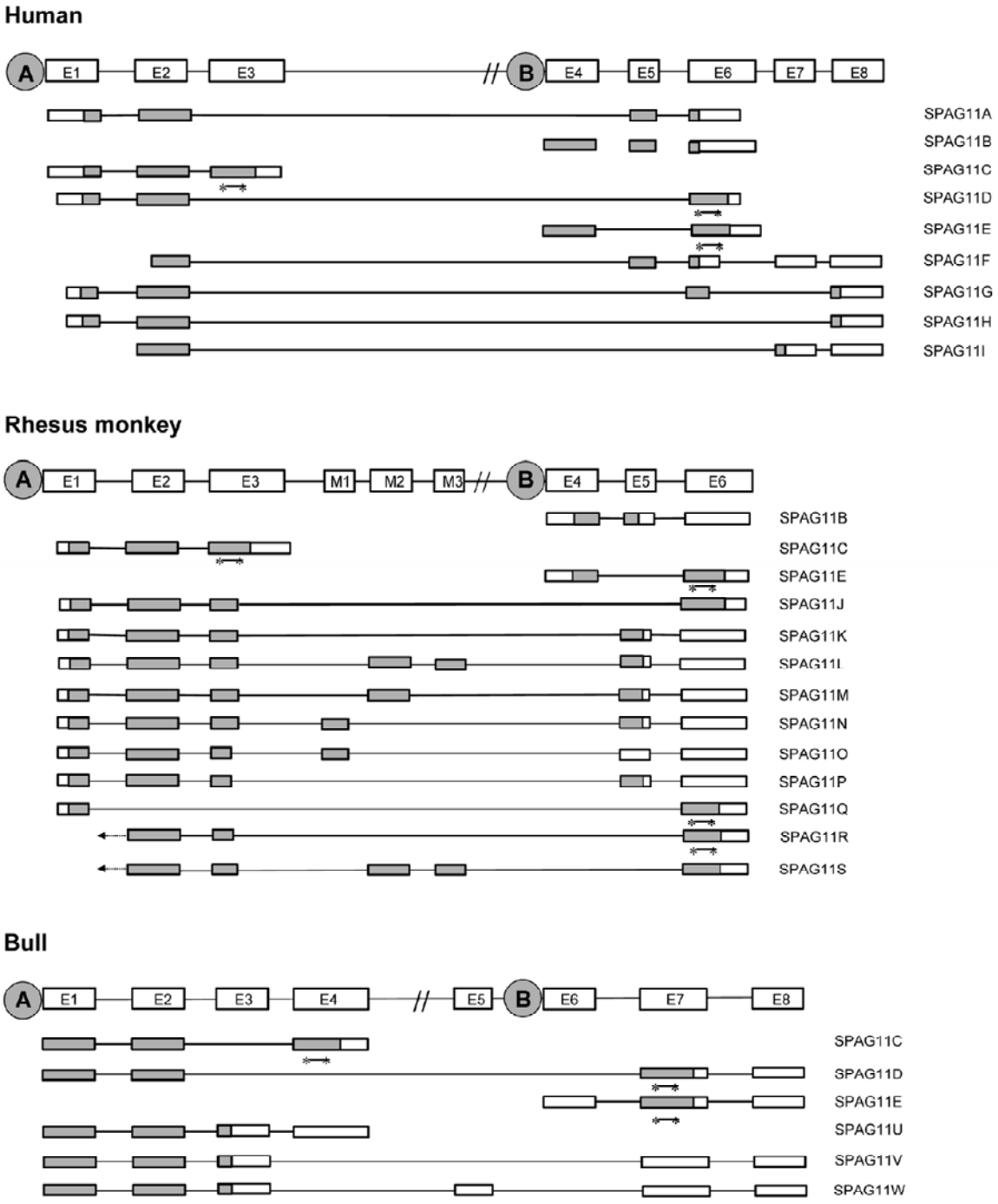


Figure 4. SPAG11 gene structure and transcripts in human, rhesus macaque and bull. Shaded circles represent promoters with adjacent exons represented by rectangles. Splice variant mRNAs are aligned with their exons of origin. Shaded portions of the transcript rectangles indicate regions encoding amino acid sequences. *–* indicates exons encoding the β-defensin signature. Portions of this figure were derived from references [29, 35].

Figure 5. Alignment of primate SPAG11 isoforms detailing exon origins of peptides. hSPAG11 indicates human isoforms, mac SPAG11 indicates macaque isoforms, rat Bin1b is rat SPAG11E. Exons are indicated above each peptide as E2–E8. M1–M3 are macaque-specific exons. Colors indicate different exon origins of the peptides.

properties and, therefore, overlapping sets of interacting partners that might be of host and/or pathogen origin. SPAG11 mRNA splicing is regulated by tissue-specific and species-specific mechanisms that have led to the suggestion that different combinations of isoforms more effectively kill the pathogens in different organs [29]. Alternatively, different combinations of isoforms might be required for specific male reproductive functions.

6 SPAG11 sequence conservation in different species

Alignment of amino acid sequences of the defensin-like SPAG11C, and E isoforms using CLUSTALW [44] reveals exon-specific rates of evolutionary divergence (Figure 6). There is strong sequence conservation indicated by the black shading in the defensin regions of SPAG11C and SPAG11E,whereas the N-terminal common region shows broad sequence diversity [29]. This region is sometimes called a propiece. The lysine-arginine cleavage site for a furin-like prohormone convertase has been identified in this propiece in humans [45] and is conserved in all species except horses. All of the SPAG11 sequences found thus far are in mammals.

7 Functions of SPAG11 isoforms

The N-terminal common region has antibacterial activity, although it lacks a defensin motif [46]. Each of the full length human, rhesus and bovine SPAG11 proteins tested as well as the C-terminal peptides of human SPAG11 A, D and G show antibacterial activity against *Escherichia coli* [28]. In addition, the C-terminal peptide of SPAG11A kills *Niesseria*, *Enterococcus* and *Staphylococcus* [47]. However, the C-terminal peptides of

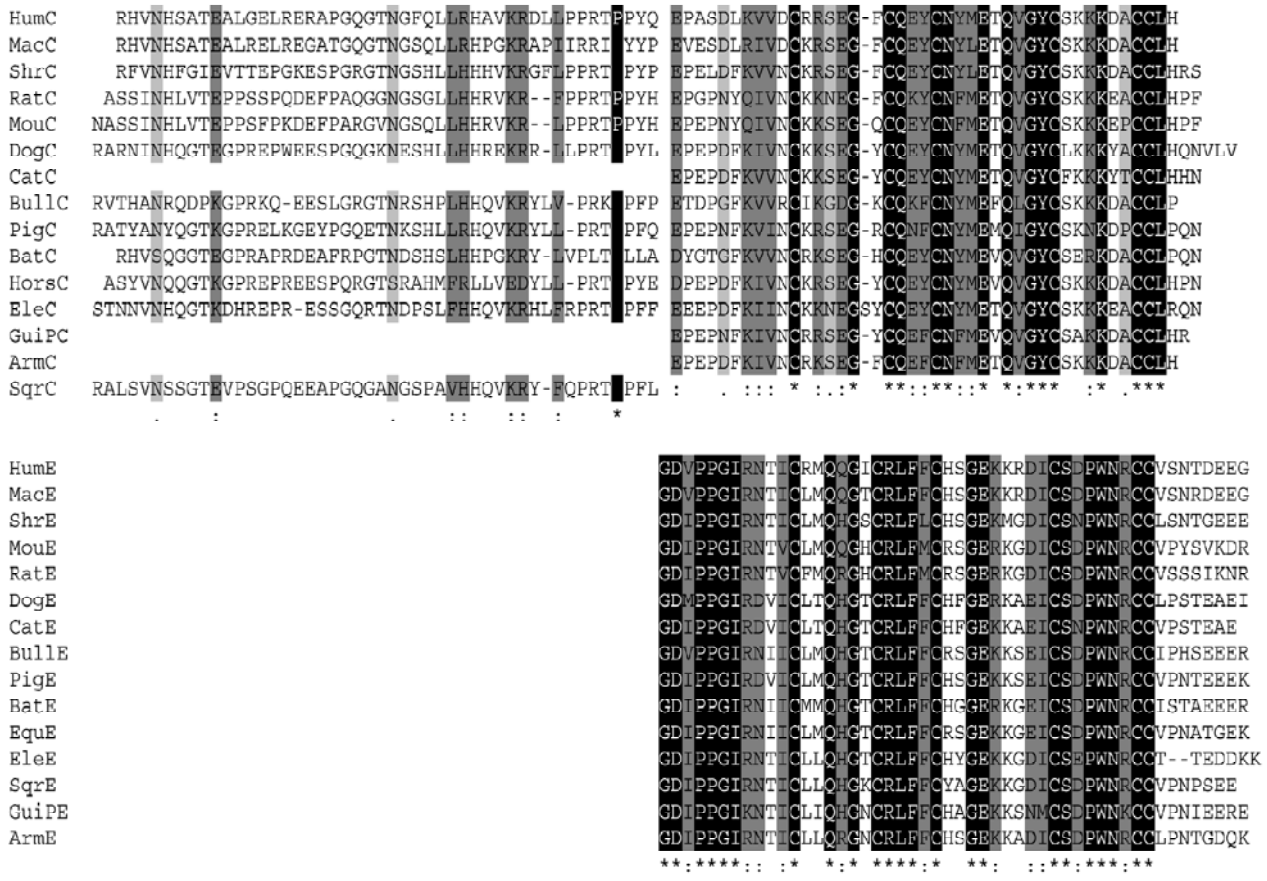


Figure 6. Alignment of SPAG11 C and SPAG11E proteins from different mammalian species. Amino acid sequences translated from human exons 2, 3 and 6 and their orthologs in other species were aligned using CLUSTALW at http://www.ebi.ac.uk/clustaw. Highlighting is based on conservation symbols (* . :) determined by CLUSTALW and indicated at the bottom of each alignment. Black highlighting indicates 100% conservation, dark grey indicates highly similar substitutions and light grey indicates lower similarity substitutions. GenBank accession numbers are given in Table 1, where "Not found" indicates sequences not yet found in GenBank by Blast searching.

Table 1. GenBank accession numbers for the SPAG11 sequences aligned in Figure 6. "Not found" indicates sequences not yet found in GenBank by Blast searching.

Names			GenBank accession numbers		
Abbrevation	Common name	Latin name	Exon 2	Exon 3	Exon 6
Hum	Human	*Homo sapiens*	NM_058203		NM_058201
Mac	Rhesus monkey	*Macaca mulatta*	AY528234		AY528235
Shr	Tree shrew	*Tupaia belangeri*	AAPY01597056		AAPY01099460
Rat	Rat	*Rattus norvegicus*	DQ012093		NM_145087
Mou	Mouse	*Mus domesticus*	AY882530		NM_153115
Dog	Dog	*Canis familiaris*	DQ012011		DQ012012
			AACN010495936		AACN010006967
Cat	Cat	*Felis catus*	Not found	AANG01403694	AANG01403689
Bull	Bull	*Bos taurus*	DQ838981		DQ838982
					AAFC03115069
Pig	Pig	*Sus scrofa*	BX925543		BK005523
Bat	Bat	*Myotis lucifugus*	AAPE01640175		AAPE01607778
Hors	Horse	*Equus caballus*	AM039964 and AAWR01019888		
Ele	Elephant	*Loxodonta africana*	AAGU0155657	AAGU01556569	AAGU01684534
GuiP	Guinea pig	*Cavia porcellus*	Not found	AAKN01209773	AAKN01390451
Arm	Armadillo	*Dasypus novemcinctus*	Not found	AAGV01229326	AAGV01738043
Sqr	Thirteen-lined ground squirrel	*Spermophilus tridecemlineatus*	AAQQ01500990	Not found	AAQQ01421830

human and rhesus SPAG11C, and rhesus SPAG11K and SPAG11L lack antibacterial activity [46]. SPAG11 isoforms and other defensin-like proteins of the male tract kill *E. coli* by a membrane disrupting mechanism that has been measured within minutes of contact with the recombinant SPAG11 proteins using fluorescent probes specific for the outer and inner bacterial membranes [14, 25, 28, 48]. SPAG11 and other proteins also inhibit bacterial macromolecular synthesis [46, 48]. Damage to the bacteria can be visualized by scanning electron microscopy [14, 25, 46, 48]. *E. coli* exposed to different SPAG11 peptides shows a range of responses, including shrinkage, loss of cell contents, especially at the division septa, and knob-like distortions (Figure 7).

The rapid mechanism of β-defensin bacterial killing is illustrated in Figure 8. Defensin proteins might be initially randomly distributed around a bacterium, but rapidly begin to bind the negatively charged bacterial surface. Membrane disruption assays have shown that within 30 s, the outer membrane is damaged and within a few minutes, the inner membrane is also disrupted [25, 48]. Defensins interfere with macromolecular synthesis by destroying the outer and inner membrane barriers and/or by entering the cell [25, 48]. Scanning electron microscopy shows that 30 min of treatment results in the release of cell contents. Bacteria that are unable to seal these pores are not likely to survive.

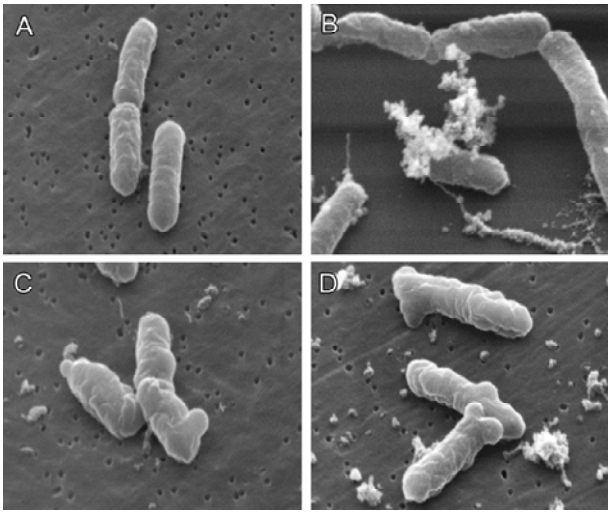


Figure 7. *Escherichia coli* treated with recombinant defensin proteins 50 μg/mL for 30 min. (A): Untreated, and (B): treated with amino acids 1–46 of mature human SPAG11. (C): Full length macaque SPAG11K. (D): Full length macaque SPAG11L.

In the homology model of the SPAG11D defensin domain, conserved residues (light grey) [49] and additional basic residues (dark grey) form a potential protein interaction domain (Figure 9). The possibility that a pro-

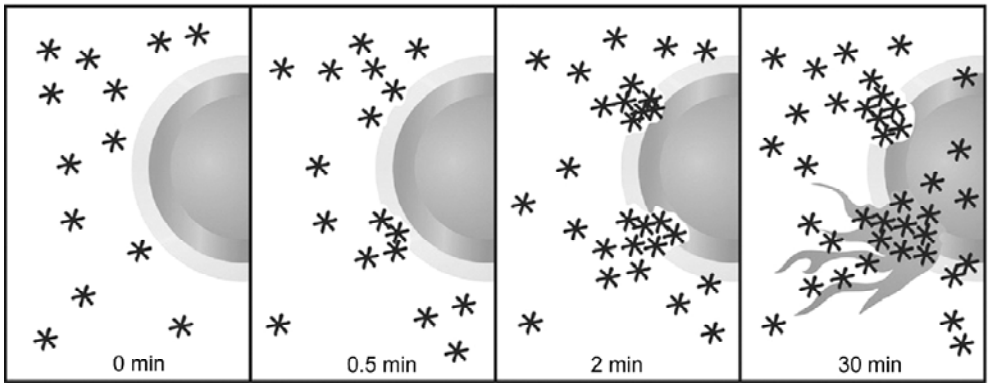
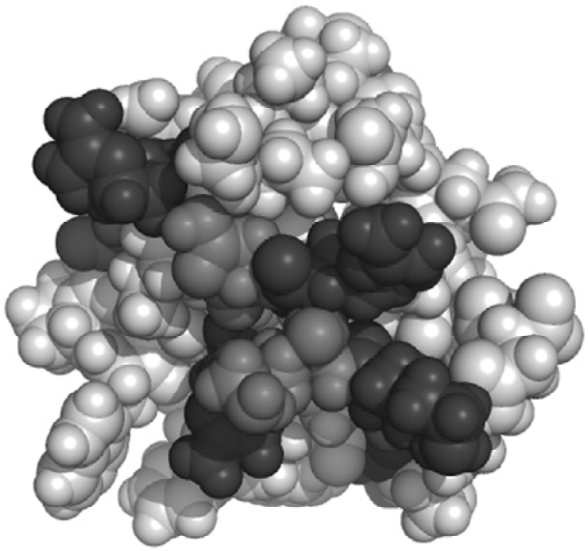


Figure 8. Membrane disrupting mechanism of killing bacteria by β-defensins. The asterisks represent individual β-defensin molecules. The rounded structure represents an *Escherichia coli* with its two compositionally distinct membranes. At the bottom of each panel is given the number of minutes after exposure to recombinant proteins that events were observed.

A



B

GI**R**NTIC**R**MQQGICRLFFC**H**SGE**K**K**R**DICSDPWN**R**CCVSN

Figure 9. (A): Potential protein interaction domain of human SPAG11D defensin domain. Space-filled homology model of SPAG11D peptide. Lighter shading indicates conserved residues identified using ConSeq (http://conseq.bioinfo.tau.ac.il) based on an alignment of SPAG11C and SPAG11D from different species. Darker shading indicates additional basic residues. The model represents the peptide (B) where conserved residues are lightly shaded and additional basic residues are bold and underlined.

tein receptor for SPAG11D on sperm might bind this region prompted us to look for interacting partners. In recent studies, using yeast 2 hybrid screening, we identified a number of epididymal proteins that interact with the full length mature human SPAG11D protein in yeast, but not with the amino terminal common region alone (Radhakrishnan *et al*., unpublished data). Each of these proteins has a role in male fertility that potentially could be modulated by interaction with SPAG11D. Further studies on the interactions of SPAG11 isoforms with epididymis and sperm surface proteins should lead to a better understanding of the full range of male reproductive functions of these antibacterial proteins.

8 Conclusion

The β-defensin proteins are involved in innate immunity and male reproductive functions. Evolutionary conservation of the β-defensin fold in animal and plant kingdoms attests to the broad success of this paradigmatic structure in promoting species survival. Multiple interacting partners of SPAG11D suggest involvement in host signaling pathways.

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References

1 O'Rand M G, Widgren EE, Sivashanmugam P, Richardson RT, Hall SH, French FS, *et al*. Reversible immunocontraception in male monkeys immunized with eppin. Science 2004; 306: 1189–90.
2 Liu Q, Hamil KG, Sivashanmugam P, Grossman G, Soundararajan R, Rao AJ, *et al*. Primate epididymis-specific proteins: characterization of ESC42, a novel protein containing a trefoil-like motif in monkey and human. Endocrinology 2001; 142: 4529–39.
3 Baus-Loncar M, Schmid J, Lalani el N, Rosewell I, Goodlad RA, Stamp GW, *et al*. Trefoil factor 2 deficiency in murine digestive tract influences the immune system. Cell Physiol Biochem 2005; 16: 31–42.
4 Semple CA, Rolfe M, Dorin JR. Duplication and selection in the evolution of primate beta-defensin genes. Genome Biol 2003; 4: R31.
5 Patil AA, Cai Y, Sang Y, Blecha F, Zhang G. Cross-species analysis of the mammalian β-defensin gene family: presence of syntenic gene clusters and preferential expression in the male reproductive tract. Physiol Genomics 2005; 23: 5–17.
6 Kao CY, Chen Y, Zhao YH, Wu R. ORFeome-based search of airway epithelial cell-specific novel human β-defensin genes. Am J Respir Cell Mol Biol 2003; 29: 71–80.
7 Jia HP, Schutte BC, Schudy A, Linzmeier R, Guthmiller JM, Johnson GK, *et al*. Discovery of new human beta-defensins using a genomics-based approach. Gene 2001; 263: 211–8.
8 Garcia JR, Krause A, Schulz S, Rodriguez-Jimenez FJ, Kluver E, Adermann K, *et al*. Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. Faseb J 2001; 15: 1819–21.
9 Premratanachai P, Joly S, Johnson GK, McCray PB Jr, Jia HP, Guthmiller JM. Expression and regulation of novel human beta-defensins in gingival keratinocytes. Oral Microbiol Immunol 2004; 19: 111–7.
10 Rodriguez-Jimenez FJ, Krause A, Schulz S, Forssmann WG, Conejo-Garcia JR, Schreeb R, *et al*. Distribution of new human beta-defensin genes clustered on chromosome 20 in functionally different segments of epididymis. Genomics 2003; 81: 175–83.
11 Schutte BC, Mitros JP, Bartlett JA, Walters JD, Jia HP, Welsh MJ, *et al*. Discovery of five conserved beta-defensin gene clusters using a computational search strategy. Proc Natl Acad Sci U S A 2002; 99: 2129–33.
12 Semple CA, Gautier P, Taylor K, Dorin JR. The changing of the guard: molecular diversity and rapid evolution of beta-defensins. Mol Divers 2006; 10: 575–84.
13 Rao J, Herr JC, Reddi PP, Wolkowicz MJ, Bush LA, Sherman NE, *et al*. Cloning and characterization of a novel sperm-associated isoantigen (E-3) with defensin- and lectin-like motifs expressed in rat epididymis. Biol Reprod 2003; 68: 290–301.
14 Yenugu S, Richardson RT, Sivashanmugam P, Wang Z, O'Rand MG, French FS, *et al*. Antimicrobial activity of human EPPIN, an androgen-regulated, sperm-bound protein with a whey acidic protein motif. Biol Reprod 2004; 71: 1484–90.
15 Singh PK, Tack BF, McCray PB Jr, Welsh MJ. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. Am J Physiol Lung Cell Mol Physiol 2000; 279: L799–805.
16 Lay FT, Anderson MA. Defensins–components of the innate immune system in plants. Curr Protein Pept Sci 2005; 6: 85–101.
17 Radhakrishnan Y, Hamil KG, Yenugu S, Young SL, French FS, Hall SH. Identification, characterization, and evolution of a primate beta-defensin gene cluster. Genes Immun 2005; 6: 203–10.
18 Bulet P, Stocklin R. Insect antimicrobial peptides: structures, properties and gene regulation. Protein Pept Lett 2005; 12: 3–11.
19 Osterhoff C, Kirchhoff C, Krull N, Ivell R. Molecular cloning and characterization of a novel human sperm antigen (HE2) specifically expressed in the proximal epididymis. Biol Reprod 1994; 50: 516–25.
20 Hamil KG, Sivashanmugam P, Richardson RT, Grossman G, Ruben SM, Mohler JL, *et al*. HE2beta and HE2gamma, new members of an epididymis-specific family of androgen-regulated proteins in the human. Endocrinology 2000; 141: 1245–53.
21 Tollner TL, Yudin AI, Treece CA, Overstreet JW, Cherr GN. Macaque sperm release ESP13.2 and PSP94 during capacitation: The absence of ESP13.2 is linked to sperm-zona recognition and binding. Mol Reprod Dev 2004; 69: 325–37.
22 Yudin AI, Tollner TL, Li MW, Treece CA, Overstreet JW, Cherr GN. ESP13.2, a member of the beta-defensin family, is a macaque sperm surface-coating protein involved in the capacitation process. Biol Reprod 2003; 69: 1118–28.
23 Zhou CX, Zhang YL, Xiao L, Zheng M, Leung KM, Chan MY, *et al*. An epididymis-specific beta-defensin is important for the initiation of sperm maturation. Nat Cell Biol 2004; 6: 458–64.
24 Yudin AI, Treece CA, Tollner TL, Overstreet JW, Cherr GN. The carbohydrate structure of DEFB126, the major component of the cynomolgus Macaque sperm plasma membrane glycocalyx. J Membr Biol 2005; 207: 119–29.
25 Yenugu S, Hamil KG, Radhakrishnan Y, French FS, Hall SH. The androgen-regulated epididymal sperm-binding protein, human beta-defensin 118 (DEFB118) (formerly ESC42), is an antimicrobial beta-defensin. Endocrinology 2004; 145: 3165–73.
26 Motzkus D, Schulz-Maronde S, Heitland A, Schulz A, Forssmann WG, Jubner M, *et al*. The novel beta-defensin DEFB123 prevents lipopolysaccharide-mediated effects *in vitro* and *in vivo*. FASEB J 2006; 20: 1701–2.
27 Bauer F, Schweimer K, Kluver E, Conejo-Garcia JR, Forssmann WG, Rosch P, *et al*. Structure determination of human and murine beta-defensins reveals structural conservation in the absence of significant sequence similarity. Protein Sci 2001; 10: 2470–9.
28 Yenugu S, Hamil KG, Birse CE, Ruben SM, French FS, Hall SH. Antibacterial properties of the sperm-binding proteins and peptides of human epididymis 2 (HE2) family; salt sensitivity, structural dependence and their interaction with outer and cytoplasmic membranes of Escherichia coli. Biochem J 2003; 372: 473–83.
29 Avellar MC, Honda L, Hamil KG, Radhakrishnan Y, Yenugu S, Grossman G, *et al*. Novel aspects of the sperm-associated antigen 11 (*SPAG11*) gene organization and expression in cattle (*Bos taurus*). Biol Reprod 2007; 76: 1103-16.
30 Lemercinier X, Muskett FW, Cheeseman B, McIntosh PB, Thim L, Carr MD. High-resolution solution structure of human intestinal trefoil factor and functional insights from detailed structural comparisons with the other members of the trefoil family of mammalian cell motility factors. Biochemistry 2001; 40: 9552–9.
31 Perry AC, Jones R, Moisyadi S, Coadwell J, Hall L. The novel epididymal secretory protein ESP13.2 in *Macaca fascicularis*. Biol Reprod 1999; 61: 965–72.
32 Shiau YS, Horng SB, Chen CS, Huang PT, Lin C, Hsueh YC, *et al*. Structural analysis of the unique insecticidal activity of novel mungbean defensin VrD1 reveals possibility of homoplasy evolution between plant defensins and scorpion neurotoxins. J Mol Recognit 2006; 19: 441–50.
33 Frohlich O, Po C, Young LG. Organization of the human gene encoding the epididymis-specific EP2 protein variants and its relationship to defensin genes. Biol Reprod 2001; 64: 1072–9.
34 Frohlich O, Ibrahim NM, Young LG. EP2 splicing variants in

rhesus monkey (Macaca mulatta) epididymis. Biol Reprod 2003; 69: 294–300.
35 Avellar MC, Honda L, Hamil KG, Yenugu S, Grossman G, Petrusz P, *et al*. Differential expression and antibacterial activity of epididymis protein 2 isoforms in the male reproductive tract of human and rhesus monkey (*Macaca mulatta*). Biol Reprod 2004; 71: 1453–60.
36 Yenugu S, Hamil KG, Grossman G, Petrusz P, French FS, Hall SH. Identification, cloning and functional characterization of novel sperm associated antigen 11 (SPAG11) isoforms in the rat. Reprod Biol Endocrinol 2006; 4: 23.
37 Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. Semin Immunol 2006; 18: 263–75.
38 Kurata S. Recognition and elimination of diversified pathogens in insect defense systems. Mol Divers 2006; 10: 599–605.
39 Lipscombe D, Pan JQ, Gray AC. Functional diversity in neuronal voltage-gated calcium channels by alternative splicing of Ca(v) alpha1. Mol Neurobiol 2002; 26: 21–44.
40 Rougon G, Hobert O. New insights into the diversity and function of neuronal immunoglobulin superfamily molecules. Annu Rev Neurosci 2003; 26: 207–38.
41 Beisel KW, Rocha-Sanchez SM, Ziegenbein SJ, Morris KA, Kai C, Kawai J, *et al*. Diversity of Ca^{2+}-activated K^+ channel transcripts in inner ear hair cells. Gene 2007; 386: 11–23.
42 Young JM, Shykind BM, Lane RP, Tonnes-Priddy L, Ross JA, Walker M, *et al*. Odorant receptor expressed sequence tags demonstrate olfactory expression of over 400 genes, extensive alternate splicing and unequal expression levels. Genome Biol 2003; 4: R71.
43 Ziegler A, Dohr G, Uchanska-Ziegler B. Possible roles for products of polymorphic MHC and linked olfactory receptor genes during selection processes in reproduction. Am J Reprod Immunol 2002; 48: 34–42.
44 Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, *et al*. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 2003; 31: 3497–500.
45 von Horsten HH, Derr P, Kirchhoff C. Novel antimicrobial peptide of human epididymal duct origin. Biol Reprod 2002; 67: 804–13.
46 Yenugu S, Hamil KG, French FS, Hall SH. Antimicrobial actions of human and macaque sperm associated antigen (SPAG) 11 isoforms: influence of the N-terminal peptide. Mol Cell Biochem 2006; 284: 25–37.
47 Liao M, Ruddock PS, Rizvi AS, Hall SH, French FS, Dillon JR. Cationic peptide of the male reproductive tract, HE2alpha, displays antimicrobial activity against *Neisseria gonorrhoeae, Staphylococcus aureus* and *Enterococcus faecalis*. J Antimicrob Chemother 2005; 56: 957–61.
48 Yenugu S, Hamil KG, French FS, Hall SH. Antimicrobial actions of the human epididymis 2 (HE2) protein isoforms, HE2alpha, HE2beta1 and HE2beta2. Reprod Biol Endocrinol 2004; 2: 61.
49 Berezin C, Glaser F, Rosenberg J, Paz I, Pupko T, Fariselli P, *et al*. ConSeq: the identification of functionally and structurally important residues in protein sequences. Bioinformatics 2004; 20: 1322–4.