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Defensin in Ticks: Molecular Characterization, Expression, and Role in Innate Immunity

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Defensin in ticks: molecular characterization, expression and role in innate immunity

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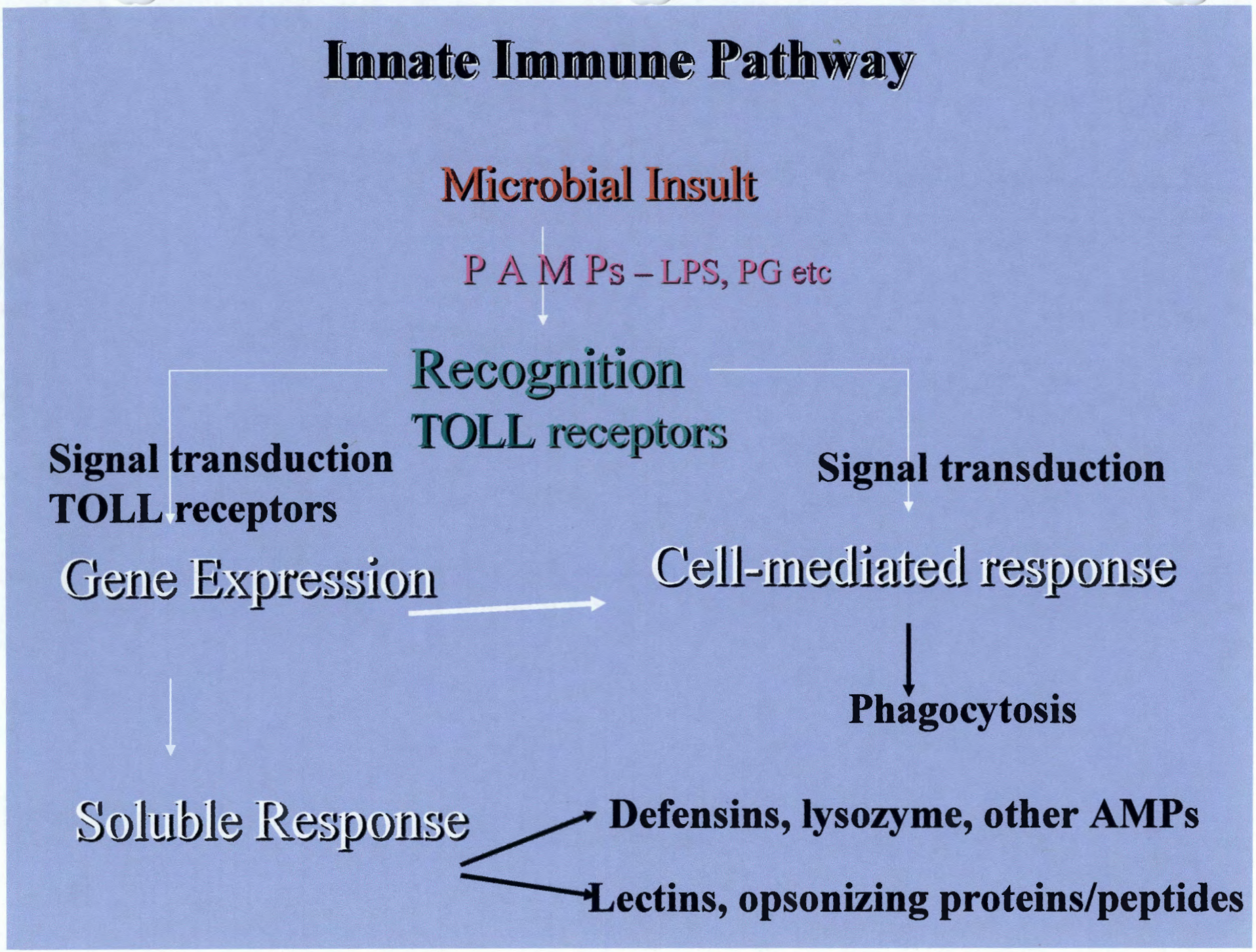
Innate Immune Pathway



MATERIALS AND METHODS

- Antimicrobial assays against *Borrelia burgdorferi,* other bacteria
- Gel electrophoresis – Western blot using anti-defensin antiserum
- mRNA isolation
- RT-PCR with defensin primer **Vsn-1** for *D. variabilis*

(F - 5' GACTGCGCTTTGAGACGACAAA 3'

(R –5' AGAAAGCATAACCATTTTTAATATGCATTT 3')

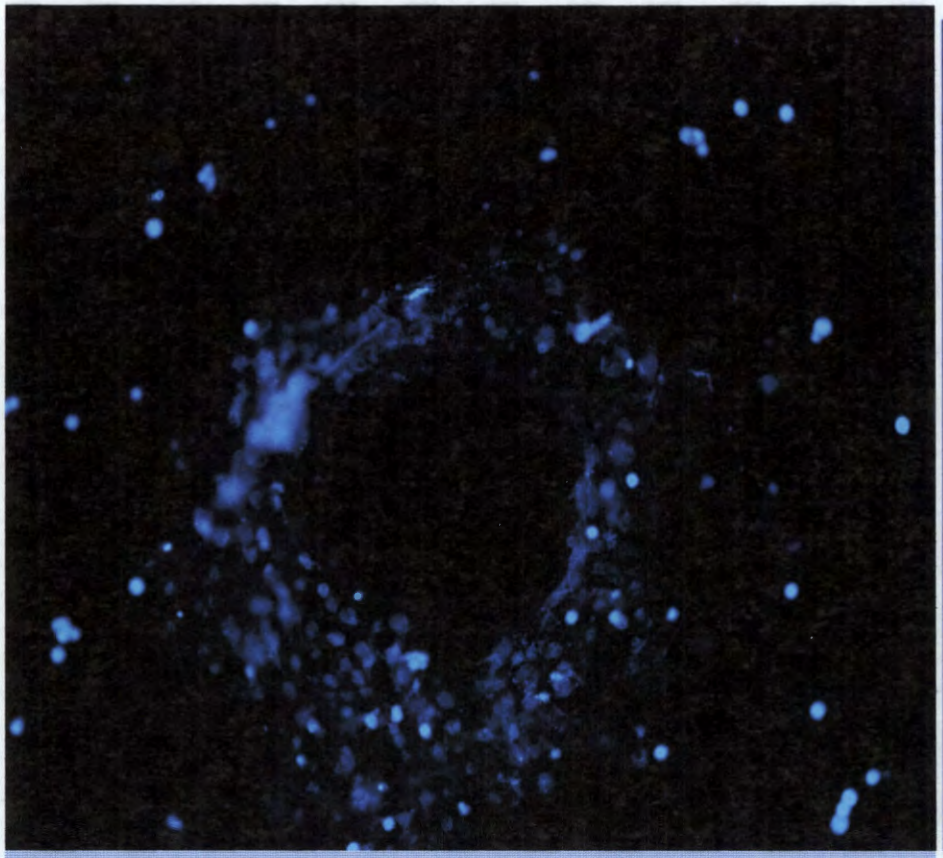
- RT-PCR with defensin primer Ix-1 for I. scapularis

(F – 5' CGAAGGCAGAACACAGGTCAAGTA 3')

(R - 5' GGAGTTTATTGAACAGCCGCCGGAAGGA 3')

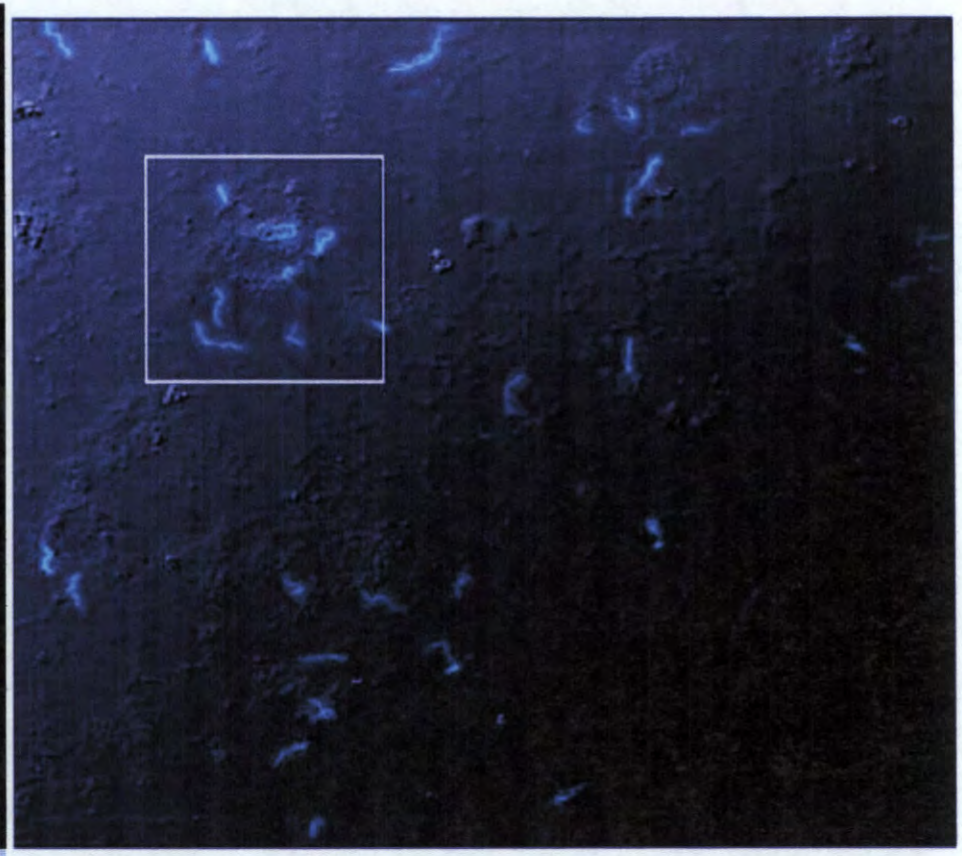
- 5' and 3' RACE with gene specific primers

- Cloning and sequencing



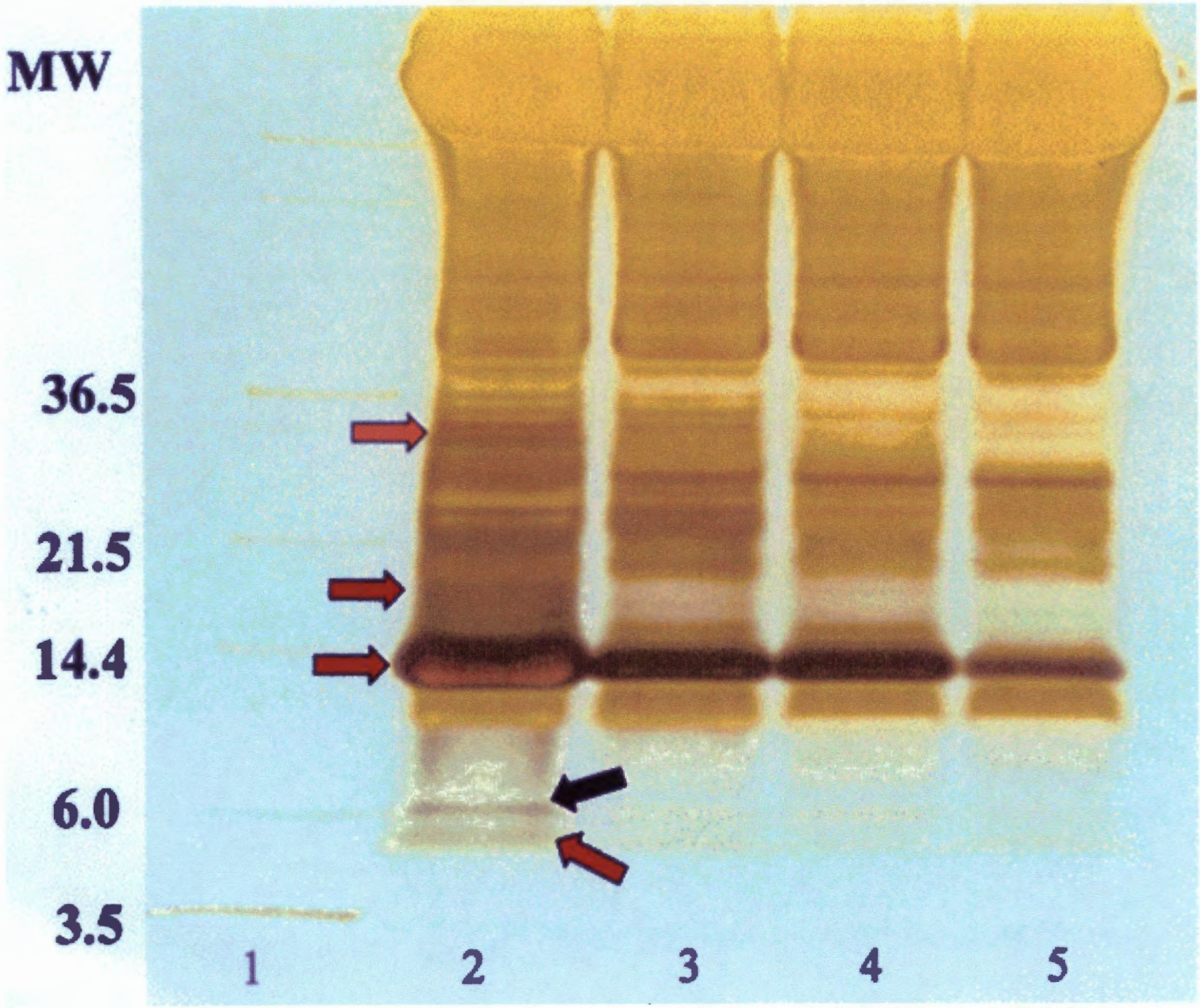
***D. variabilis* 1 hour**

Fate of *B. burgdorferi* spirochetes after injection into *D. variabilis* hemocoel

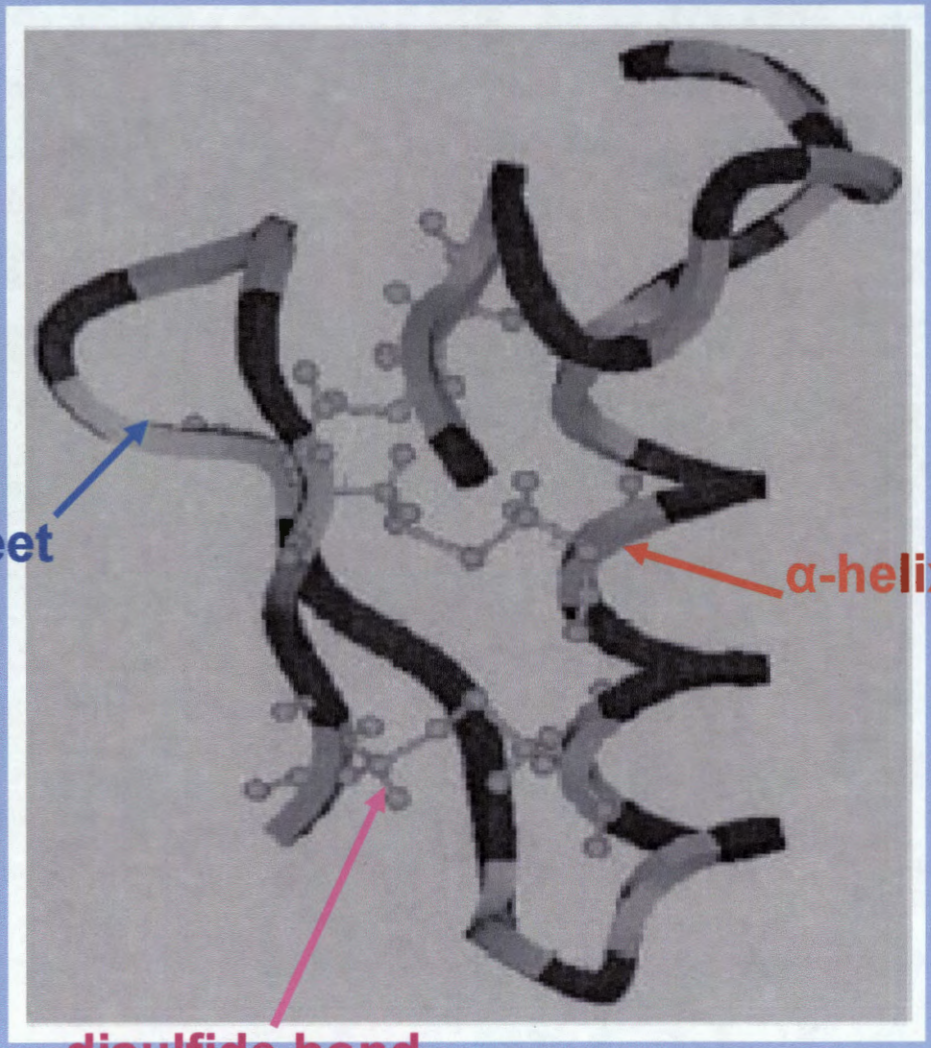


***I. scapularis* 1 hour**

Fate of *B. burgdorferi* spirochetes after injection into *I. scapularis* hemocoel


MW
36.5
21.5
14.4
6.0
3.5
1
2
3
4
5
MW
1 h
6 h
24 h
Solvent

Defensin: Structure

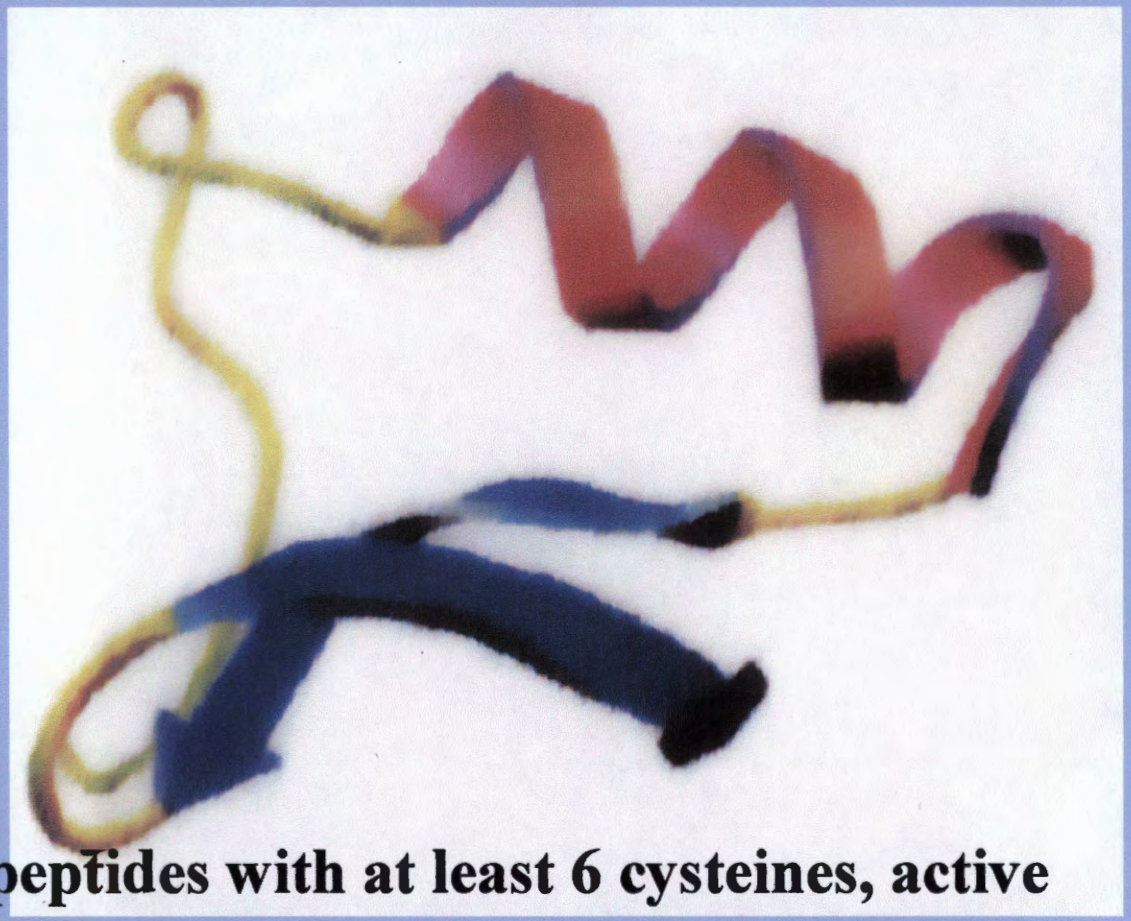


- Primary structure contains 6 conserved cysteine residues
- Tertiary structure- CSαβ motif
  - –Central amphipathic alpha helix
  - –Anti-parallel double stranded beta sheet
  - –Disulfide bridges link alpha helix and beta sheet
  - –Motif stabilized by 3 disulfide bonds

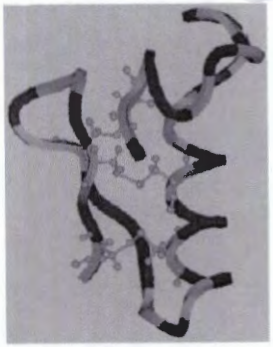
DEFENSIN: basics

Cationic

PI ~ 8.5 – 9.3

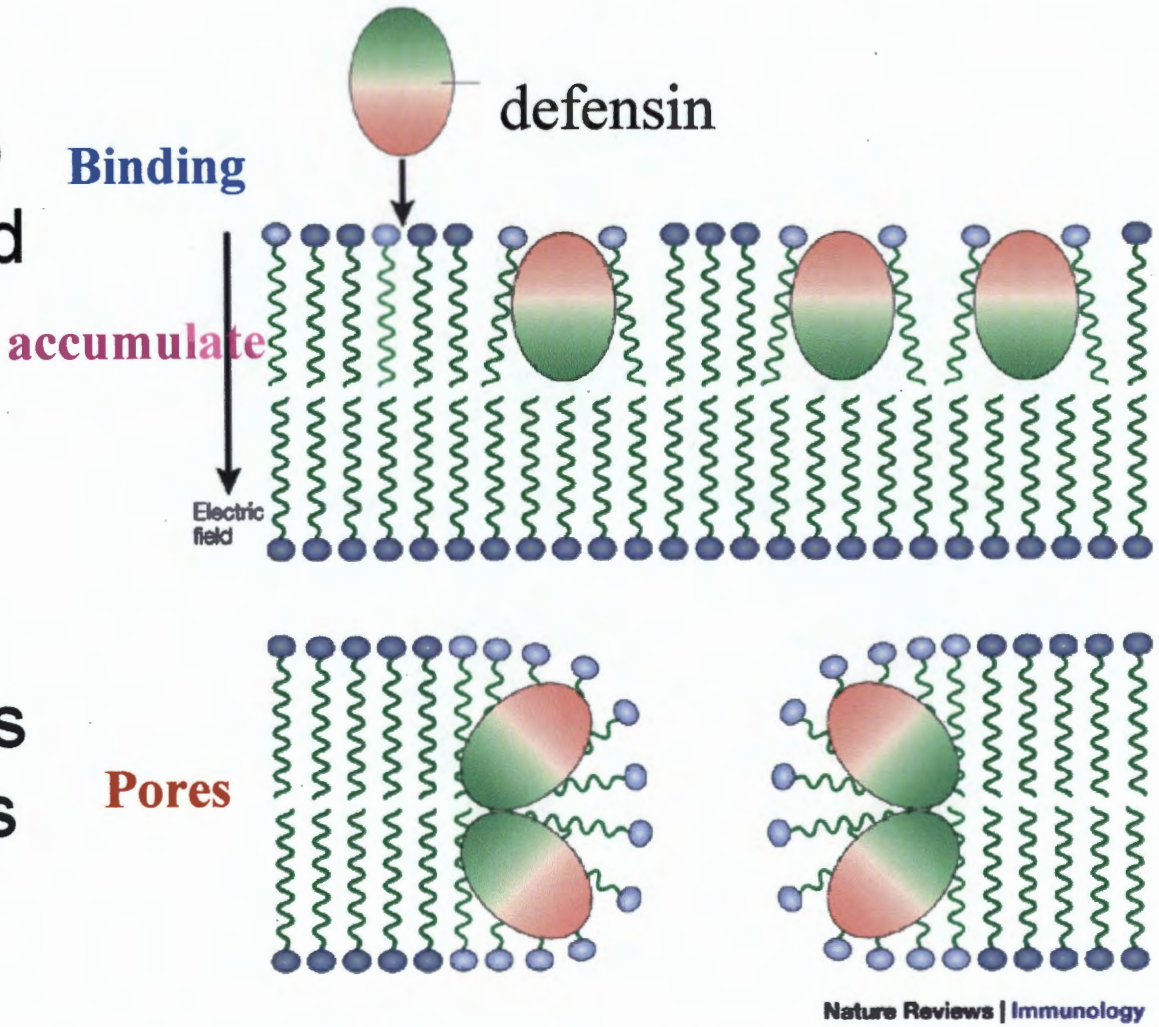


- **Most defensins 4 kDa peptides with at least 6 cysteines, active primarily against Gram + bacteria – also *B. burgdorferi***
- **Defensins ubiquitous plant and animal kingdoms**
- **In insects, found fat body, hemocytes, midgut**
- **In ticks – primarily in hemocytes.**



Defensin: Mode of action

- Defensin binds to negatively charged membrane
- Defensin molecules accumulate
- Membrane strains and result in pores permeabilizes membrane



Ganz. 2003. Nature Rev. Immunol. 3:710-720

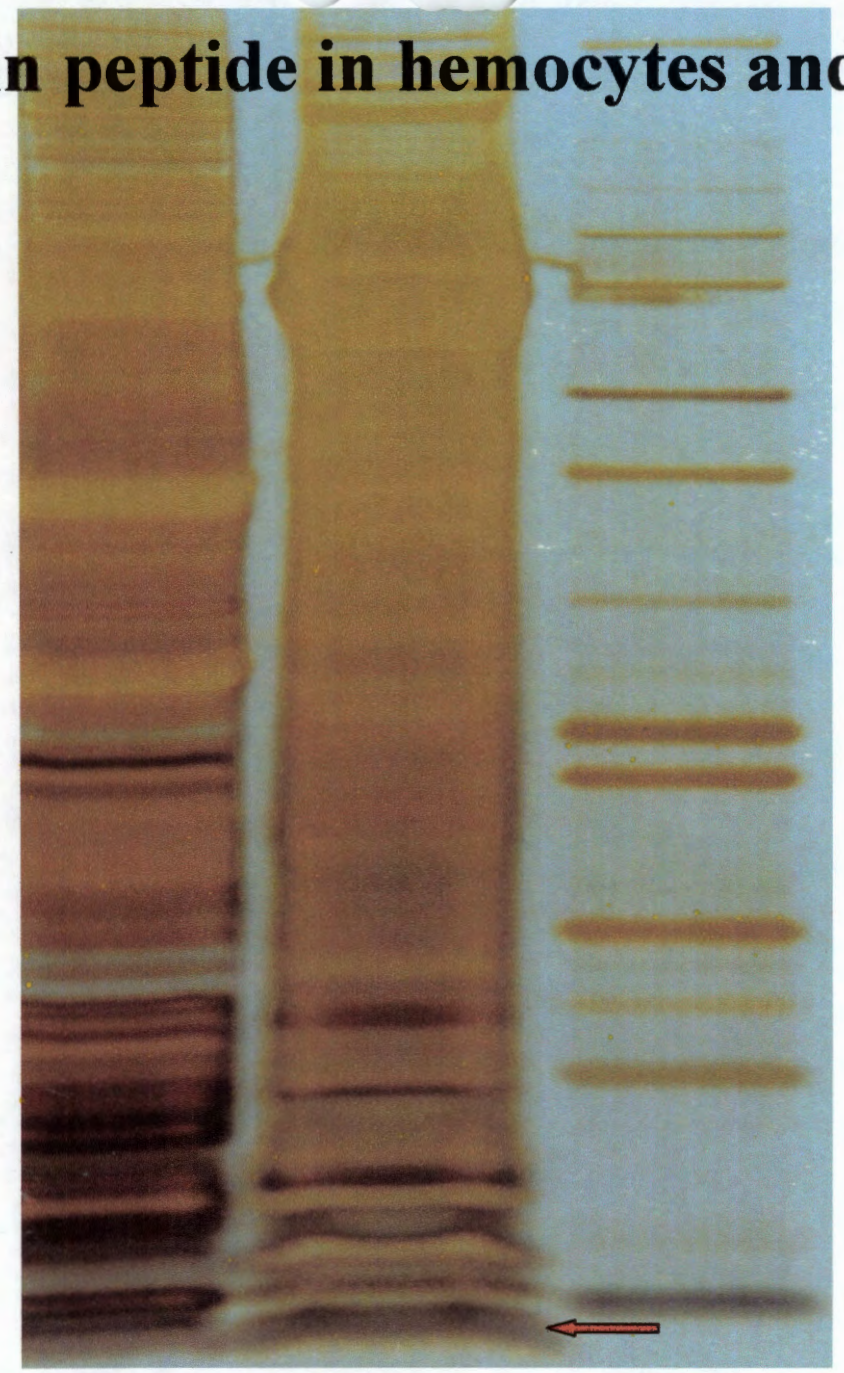
Defensin: Mode of action (continued)

- Gram negative LPS layer
  - –Competitive displacement of divalent cations
  - –Rapid permeabilization of outer membrane
- Defensins facilitate entrance of other proteins
  - lysozyme

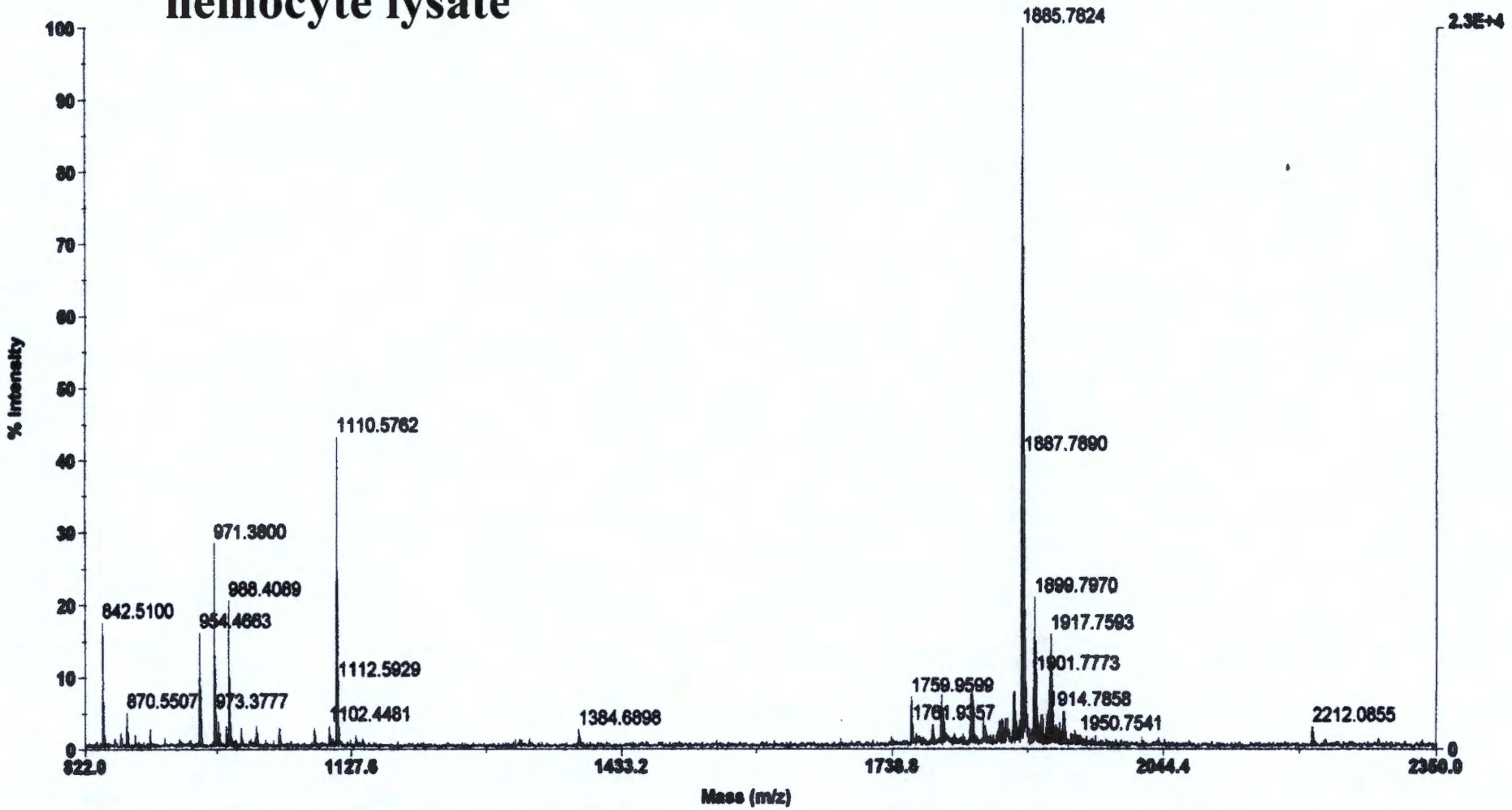
Defensin peptide in hemocytes and fat body

Hemocyte and Fat body Lysates

Peptide absent in midgut



MALDI-TOF of tryptic digest putative defensin band from hemocyte lysate



Submitted	Peptide sequence	NCBI accession no.	ID
1110.58	R)RGGYCSGIIK(Q)	"	"
1884.79	R)GFGCPLNQGACHNHCR(S)	"	"

RESULTS – *D. variabilis*

32

- Obtained 624 bp cDNA from hemocytes
- Same from fat body and midgut
- Sequencing 624 bp cDNA fragment revealed 225 bp ORF corresponding to a 74 amino acid **preprodefensin**
- MRGLCICLVFLLVCGLVSATAAAPAESEVAHLRVRR
  GFGCPLNQGACHNHCRSIR-RRGGYCSGIIKQTCTCYRN

1 – 32: Prepro region;

32 – 36: signal peptide

37 – 74: mature peptide (38 AA)

RESULTS *I. scapularis*

33

Nucleotide and derived amino acid sequence of *scapularisin*, a defensin-like peptide in the tissues of the black-legged tick *Ixodes scapularis*

```
AACAAGCTTTTTNNNCCGGCACACTGCGAAGGCAGAACACAGGTCAAGTAGAACACTGCATCATC
CCTTGAAATC
  1 M  R  V  I  A  V  T  L  I  A  L  L  V  A  G  A  F  M  T  S
 1 ATGAGGGTCATTGCTGTTACCTTGATCGCCCTTCTGGTTGCTGGAGCGTTTATGACTTCC
 21 S  A  Q  E  E  E  N  Q  V  A  H  V  R  V  R  R  G  F  G  C
61 AGCGCACAAGAGGAAGAAAACCAAGTGGCTCACGTTCGAGTTCGACGTGGTTTTGGATGT
 41 P  F  D  Q  G  A  C  H  R  H  C  Q  S  I  G  R  R  G  G  Y
121 CCCTTCGACCAAGGGGCGTGTCACAGGCACTGCCAGAGCATCGGACGTCGCGGAGGTTAC
 61 C  A  G  F  I  K  Q  T  C  T  C  Y  H  N  *
181 TGCGCGGGATTTATCAAGCAGACGTGCACATGCTACCACAACTAG
```

1 – 32 = Prepro region; Pink arrow = 33 – 36 signal sequence; red arrow = 37 – 74 mature peptide (38 AA).

Consensus alignment

```
Is   MRVIAVTLIALLVAGAFMTSSAQEEEN-QVAHVRVRRG---------YGCPFNQGACHRHCQSIG-RRGGYCAGFIKQTCTCYHN-
Bm   MRGIYICLXFVLXCGLVSGLADVPAES-EMAHLRVRRG---------FGCPFNQGACHRHCRSIR-RRGGYCAGLIKQTCTCYRN-  87%
Dv   MRGLCICLVFLLVCGLVSATAAAPAES-EVAHLRVRRG---------FGCPLNQGACHNHCRSIR-RRGGYCSGIIKQTCTCYRN-  79%
OmA  MNKLFIVALVVALAVATMAQEVHNDVE-EQSVPRVRRG---------YGCPFNQYQCHSHCSGIRGYKGGYCKGTFKQTCKCY---  65%
OmB  MNKLFIVALVVALAVATMAQEVHDDVE-EQSVPRVRRG---------YGCPFNQYQCHSHCRGIRGYKGGYCTGRFKQTCKCY---  68%
OmC  MNKLFIVALVLALAVATMAHEVYDDVE-EPSVPRVRRG---------YGCPFNQYQCHSHCSGIRGYKGGYCKGLFKQTCNCY---  65%
OmD  MNKLFIVALVLALAVATMAHEVHDDIE-EPSVPRVRRG---------FGCPFNQYECHAHCSGVPGYKGGYCKGLFKQTCNCY---  62%
Ir   MKVLAVSLAFLLIAGLISTSLAQNEEGGEKELVRVRRGG--------YYCPFFQDKCHRHCRSFG-RKAGYCGGFLKKTCICVMK-  60%
Ah   MATVRNSRPEAAGEPSGVSSTEGDWRHIEKRDVSYQGEGNTRRFDNPFGCPADEGKCFDHCNNKA-YDIGYCGGSYRATCVCYRK-  39%
MG   MKAAFVLLVVGLCIMTDTA------------------G---------FGCPNNYACHQHCKSIRGYCGGYCGWFRLRCTCYRCG -  60%
```

Is = *I. scapularis;* Bm = *B. microplus;* Dv = *D. variabilis;* Om = *Ornithodoros moubata;* Ir = *I. ricinus;* Ah = *Amblyomma hebraeum;* MG = *Mytilus galloprovincialis* (mussel).

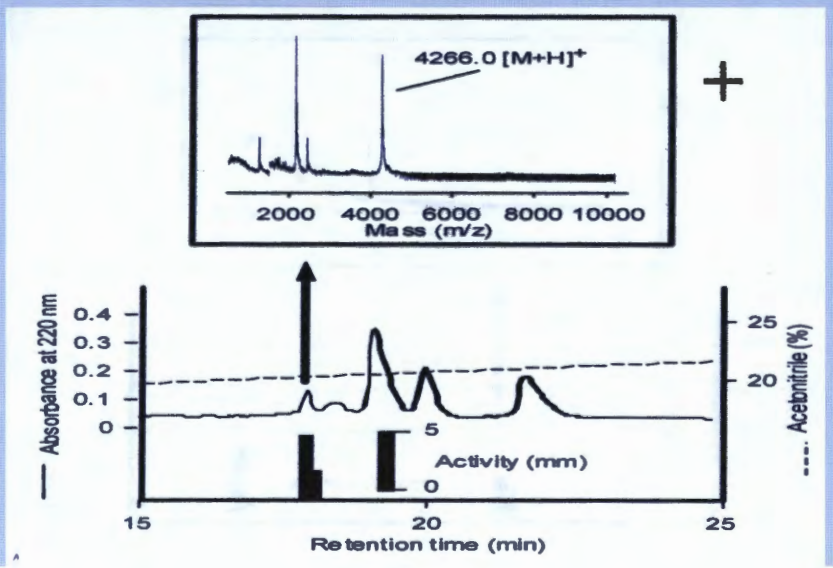
- High degree variability among tick defensins
- Phylogenetic analysis *I. ricinus* complex, *I. scapularis* and *I. ricinus* are not sister taxa (Xu et al., 2003).

Control of invading microbes
MIDGUT

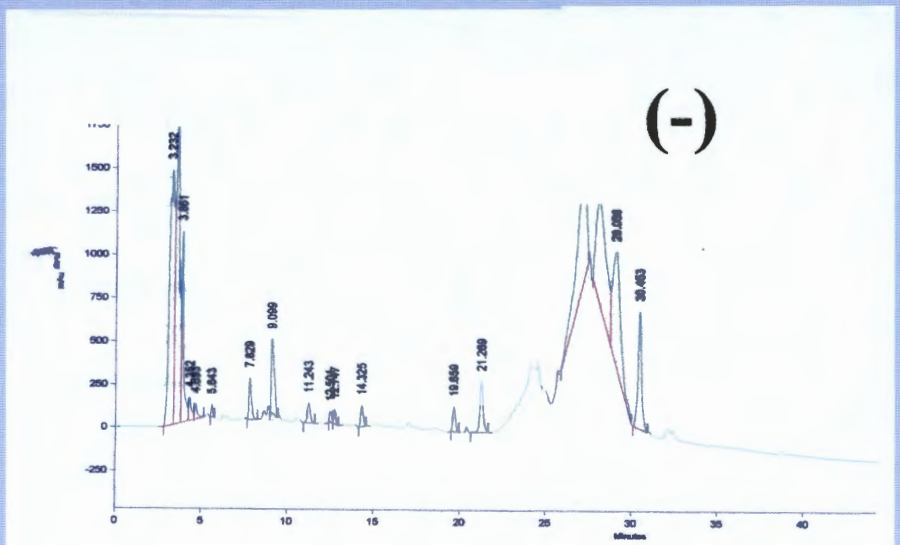
Novel Antimicrobial peptides

Is defensin present?

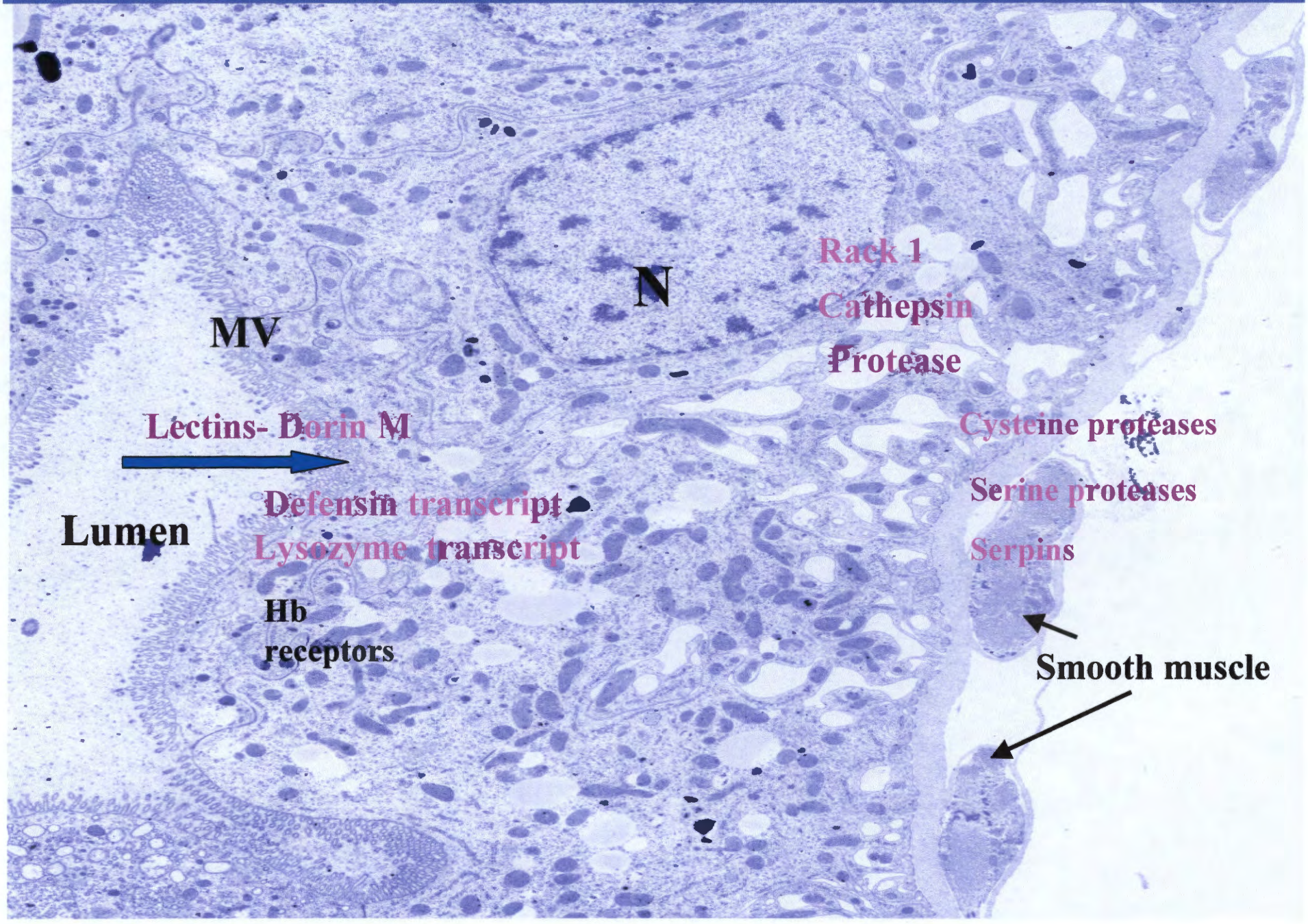
Soft tick (*O. moubata*) +



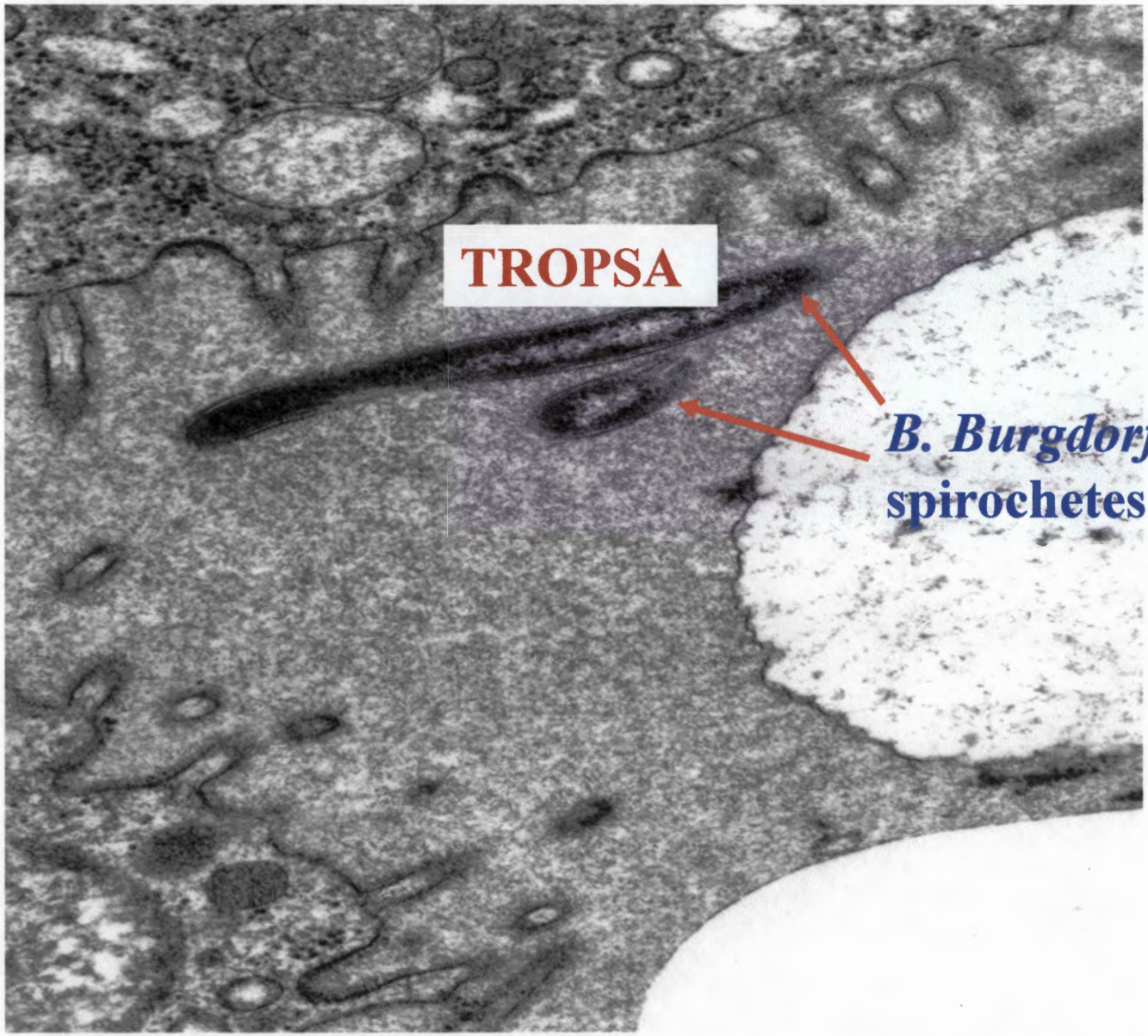
Hard tick (*D. variabilis*) --



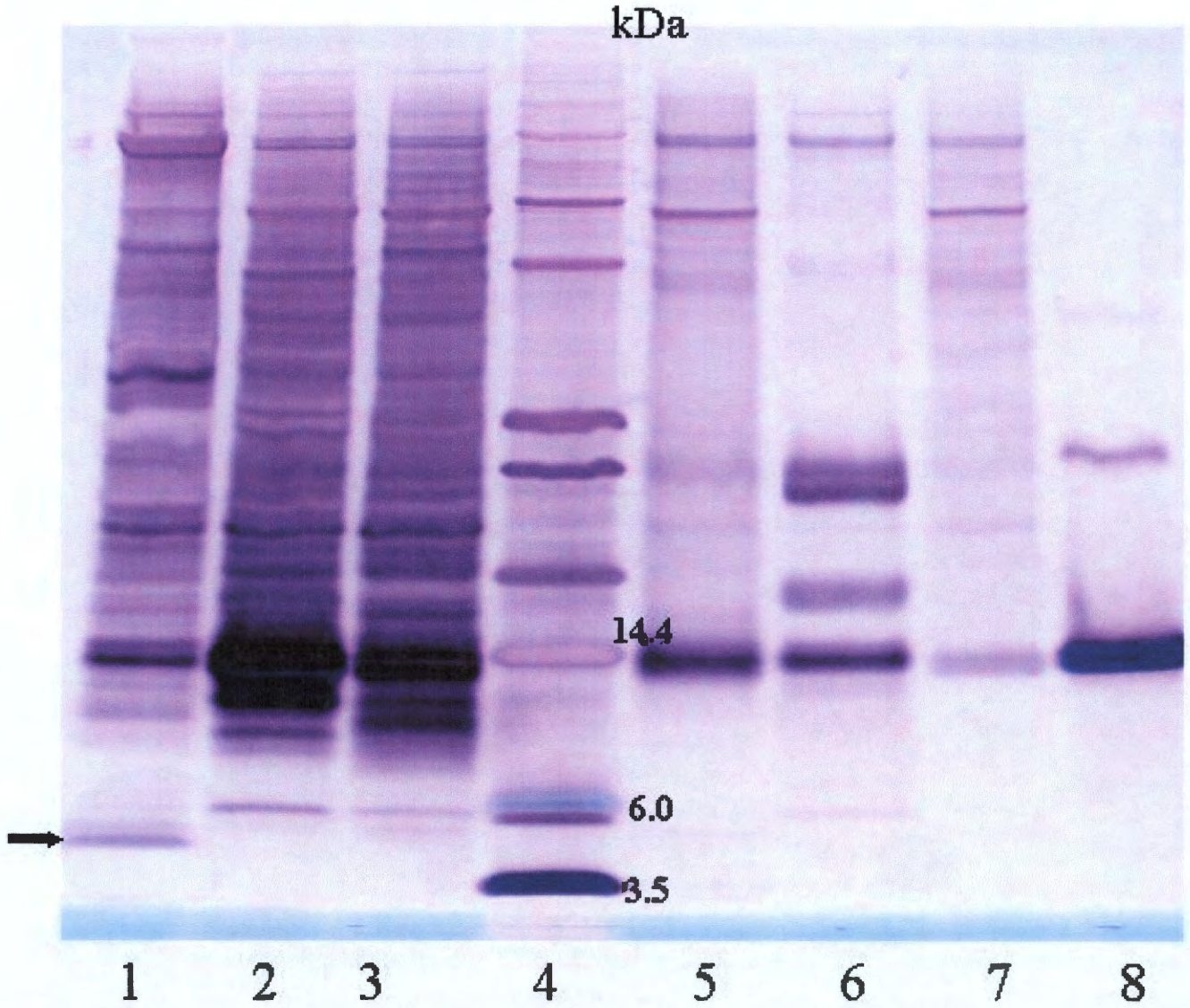
D. variabilis fed female midgut cDNA library proteins/peptides



39

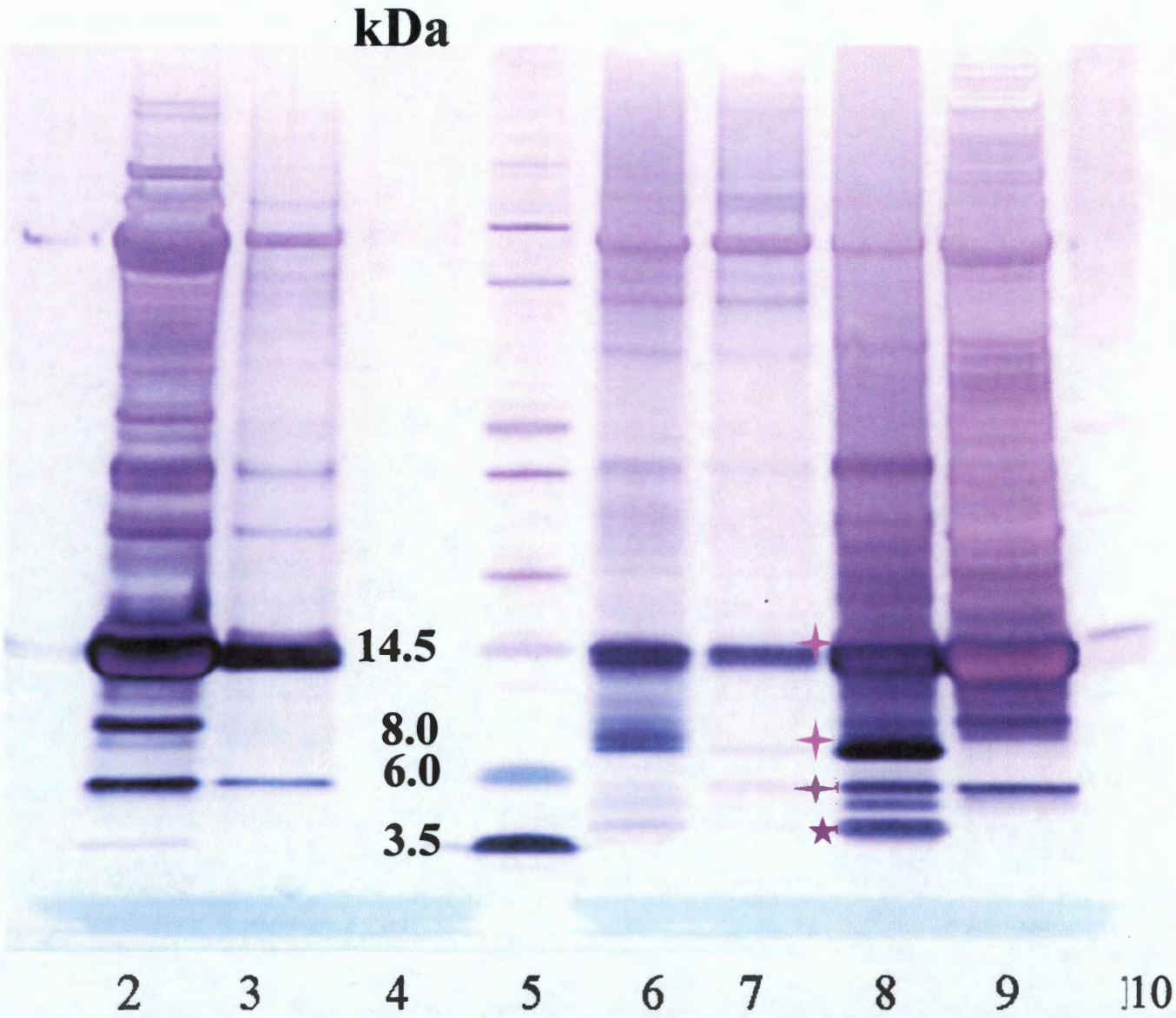


TROPSA – midgut receptor for OspA *B. burgdorferi* spirochetes



1 = Hemocyte lysate; 2 = Midgut no challenge; 3 = midgut Bb; 4 = MW markers; 5 = Midgut peptidoglycan; 6 = midgut 5% hemoglobin; 7 = midgut rabbit serum; 8 = hemoglobin standard

Arrow = defensin confirmed by tryptic digestion/mass spectrometry.



C18 Sep Paks. 2, 30% ACN; 3, 40% ACN; 4, 70% ACN; 5, MW stds; 6, 30% ACN; 7, 40% ACN; 8, 80% ACN; 9, crude midgut extract; 10, rabbit serum; sample loading 10 µg/lane. Red bands submitted for analysis.

Table 1. Results of tryptic digestion/mass spectrometry analysis for identification of peptides recovered from gel slices of protein gels.

A. **Hemocytes**

rMW band	Representative partial amino acid sequences found	Mass (D)	Identification	Native MW (D)
Hemocyte lysate 4.95 band	**GFGCPLNQGACHNHCRS**	1884.8	Tick defensin (varisin)	4229.0

B. Midgut

5.01-5.62 kDa band	**AVGHLDDLPGALSTLSDHAHK**	2267.2	α -chain hemoglobin	15482.5
"	**VLAAFSEGLSHLDNLK**	1713.9	β-chain hemoglobin	16141.5
"	**TITLEVEPSDTIENVK**	1787.9	Ubiquitin	17959.7
14 – 16 kDa band	**FLANVSTVLTSK**	1279.7	α -chain hemoglobin	15482.5
"	**VVAGVANALAHK**	1149.7	β-chain hemoglobin	16141.5
"	**AADETWEPFASGK**	1408.6	Transthyretin	14000.0

Abbreviations: D = Daltons; kDa = kilodaltons

SUMMARY

D. variabilis

- Preprodefensin produced/stored in hemocytes; mature peptide secreted in response to microbial challenge, e.g., *B. burgdorferi*.
- Sequencing generated 624 bp amplicon; 225 bp translates 74 AA preprodefensin, 38 AA mature peptide; only 1 isoform.
- Midgut – transcript found but no peptide; defensins (multiple isoforms) found in soft ticks.
- Small peptides (from 5 – 11 kDa) are digestive fragments of α-chain, ß-chain hemoglobin, ubiquitin and transtherytin.

SUMMARY (continued)
I. scapularis

- Sequencing generated 410 bp amplicon; 225 bp translates 74 AA preprodefensin, 38 AA mature peptide, 1 isoform
- *I. scapularis* similarity to *D. variabilis* 79%; to *I. ricinus* 60%.
- Transcript present in different tissues
- No evidence peptide – not upregulated in response to microbial challenge

FUTURE RESEARCH

- *D. variabilis:*
  - How do hemocytes recognize - regulate expression of defensin. Role of TOLL /IMD (other?) regulatory pathways.
  - How does tick midgut control invading microbes?
  - How do tick-borne pathogenic microbes escape destruction and colonize tick tissues?
- *I. scapularis:*
  - Defensin transcript expressed; why no peptide? Role in innate immunity?
- Other tick species?
  - Hebraein (*Amblyomma hebraeum)* and other novel antimicrobial peptides