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The transcriptome of the salivary glands of the female western black-legged tick *Ixodes pacificus* (Acari: Ixodidae)

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Summary

Sequencing of an *Ixodes pacificus* salivary gland cDNA library yielded 1068 sequences with an average undetermined nucleotide of 1.9% and an average length of 487 base pairs. Assembly of the expressed sequence tags yielded 557 contigs, 138 of which appear to code for secreted peptides or proteins based on translation of a putative signal peptide. Based on the BLASTX similarity of these contigs to 66 matches of *Ixodes scapularis* peptide sequences, only 58% sequence identity was found, indicating a rapid divergence of salivary proteins as observed previously for mosquito and triatomine bug salivary proteins. Here we report 106 mostly full-length sequences that clustered in 16 different families: Basic-tail proteins rich in lysine in the carboxy-terminal, Kunitz-containing proteins (monolaris, ixolaris and penthalaris families), proline-rich peptides, 5-kDa.-, 9.4 kDa.-, and 18.7 kDa.-proteins of unknown functions, in addition to metalloproteases (class PIII-like) similar to reprolysins. We also have found a family of disintegrins, named ixodegrins that display homology to variabilin, a GPIIb/IIIa antagonist from the tick *Dermacentor variabilis*. In addition, we describe peptides (here named ixostatins) that display remarkable similarities to the cysteine-rich domain of ADAMST-4 (aggrecanase). Many molecules were assigned in the lipocalin family (histamine-binding proteins); others appear to be involved in oxidant metabolism, and still others were similar to ixodid proteins such as the anticomplement ISAC. We also identified for the first time a neuropeptide-like protein (nlp-31) with GGY repeats that may have antimicrobial activity. In addition, 16 novel proteins without significant similarities to other tick proteins and 37 housekeeping proteins that may be useful for phylogenetic studies are described. Some of these proteins may be useful for studying vascular biology or the immune system, for vaccine development, or as immunoreagents to detect prior exposure to ticks. Electronic version of the manuscript can be found at http://www.ncbi.nlm.nih.gov/projects/omes/.

Keywords

Ixodes pacificus; sialome; tick; blood-feeding; Kunitz inhibitor; Lyme disease; vascular biology; Ixolaris; vector biology; transcriptome; proteome

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Introduction

Lyme disease is the most prevalent vector-borne disease in the U.S. and is transmitted by the tick vectors *I. scapularis* and *I. pacificus* in eastern and western North America, respectively (Barbour, 1998). Humans usually acquire Lyme disease when an infected nymphal-stage *Ixodes* sp. tick attaches and transmits the spirochete *Borrelia burgdorferi* (Burgdorfer et al., 1985). *I. scapularis* and *I. pacificus* transmit other zoonotic agents besides the Lyme disease spirochete, such as *Anaplasma phagocytophilum* (both species) or *Babesia microti* (*I. scapularis* only) (Barbour, 1998). Transmission is facilitated by tick saliva that operates not only as a carrier for *Borrelia sp*. but also contains a large repertoire of molecules that counteract the host response to injury (Ribeiro and Francischetti, 2003), allowing ticks to feed for days (Sonenshine, 1985). Accordingly, many biologic activities have been described in tick saliva, including molecules that impair platelet aggregation or neutrophil function (Ribeiro et al., 1985) in addition to coagulation inhibitors such as ixolaris and penthalaris that block Factor VIIa/tissue factor complex (Francischetti et al., 2002a; Francischetti et al., 2004a) and SALP 14, which targets Factor Xa (Narasimhan et al., 2002). Enzymes such as a kininase that degrades bradykinin (Ribeiro and Mather, 1998), an apyrase that destroys ADP (Ribeiro et al., 1985), and a metalloprotease with fibrin(ogen)olytic activity (Francischetti et al., 2003) also have been reported. Tick saliva is also rich in small molecules such as prostacyclin, a potent inhibitor of platelet activation and strong inducer of vasodilation (Ribeiro et al., 1988).

As for the immune system, an inhibitor of the alternative complement pathway exists in ixodid tick saliva (Valenzuela et al., 2000). Immunomodulators affecting NK cell function (Kopecky and Kuthejlova, 1998)—in addition to inhibitors of the proliferation of T lymphocytes and an IL-2 binding activity—also are present in this secretion (Ramachandra and Wikel 1992; Gillespie et al., 2001). Finally, saliva is important in transmission of tick-borne pathogens, as it may enhance pathogen transmission (for a review, see Wikel, 1999).

The pace of discovery of tick salivary proteins has been greatly increased by novel molecular biology techniques and bioinformatics analysis (Ribeiro and Francischetti, 2003). Our goal here has been to further study the complexity of *I. pacificus* salivary glands. We report the full-length clone of 87 novel sequences and discuss their potential role in modulating host inflammatory and immune responses.

Materials and methods

Reagents

All water used was of 18 MΩ quality and was produced using a MilliQ apparatus (Millipore, Bedford, MA, USA). Organic compounds were obtained from Sigma (St. Louis, MO, USA) or as stated otherwise.

Ixodes pacificus *ticks*

Salivary gland cDNA library construction and sequencing: Ticks were collected in northern California by dragging low vegetation with a tick-drag. Salivary glands were excised and kept at −80°C until use. The mRNA from two pairs of *I. pacificus* salivary glands was obtained using a Micro-Fast Track mRNA isolation kit (Invitrogen, San Diego, CA, USA) according to the manufacturer's instructions. The PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA, USA) as described in detail in the supplemental data in Francischetti et al. (2004b). Cycle sequencing reactions using the DTCS labeling kit (Beckman Coulter, Fullerton, CA, USA) were performed as reported (Francischetti et al., 2004b) and can be found as supplemental data at http://www.ncbi.nlm.nih.gov/projects/omes/ in the section Poisonous Animals.

cDNA sequence clustering and bioinformatics: Other procedures were as reported in detail in the supplemental data described in Francischetti et al (2004b) and can be found as supplemental data at http://www.ncbi.nlm.nih.gov/projects/omes/ in the section Poisonous Animals.

Structural bioinformatics and molecular modeling: Molecular model of the histamine-binding protein-like lipocalin gi 51011604 superimposed with the crystal structure of *Rhipicephalus appendicuatus* histamine-binding protein. The 3D-PSSM web server V2.6.0, found at http://www.sbg.bio.ic.ac.uk/ server was used to generate a model of gi 51011604 based on sequence alignment using PSI Blast, secondary structure prediction and search of a fold database of known structures (Kelley et al., 2000).

Electronic version of the manuscript: The electronic version of the manuscript containing figures and table with hyperlinks can be found at http://www.ncbi.nlm.nih.gov/projects/omes/, in the section Salivary transcriptomes (sialome) of vector arthropods (*Ixodes pacificus*).

Results and Discussion

Ixodes scapularis and *I. pacificus* are the respective vectors for *B. burgdorferi* in the eastern and western U.S. (Fig. 1). After attachment to the host, infected ticks transmit *B. burgdoferi* after 1–2 days of blood-feeding (Barbour, 1998) via saliva, a secretion that contains a cocktail of bioactive molecules (Ribeiro and Francischetti, 2003). Actually, the identification of the transcripts and proteins present in the salivary gland of ticks such as *I. scapularis* (Valenzuela et al., 2002), *Boophilus microplus* (Santos et al., 2004), and *Rhipicephalus appendiculatus* (Nene et al., 2004) have been identified recently. Here we identified secretory genes from the salivary gland of *I. pacificus* by constructing a unidirectional PCR-based cDNA library (see Materials and methods). Next, 735 cDNA were randomly sequenced followed by bioinformatics analysis that included: *i)* clustering at high stringency levels, *ii)* BLAST search against the non-redundant and protein motifs databases, and *iii)* submission of the translated sequences to the Signal P server (see Materials and methods). This initial approach allowed us to obtain a fingerprint of the protein families or “clusters” present in this particular salivary gland. Several sequences were then selected based on novelty or the protein family it assigns for and extension of their corresponding cDNA were performed until the poly A was reached. Among these clusters, 87 novel full-length cDNA coding proteins or peptides were obtained, most of which appear to be secreted in the saliva.

Our results are presented in Table 1, which describes the sequence size, the presence of a putative signal peptide, the molecular weight of the mature peptide, the isoelectric point, and other parameters (each accession numbers and sequence information is hyperlinked). Fifteen large protein families of putative secreted proteins were found. Some sequences appeared to code for housekeeping proteins, whereas others without database hits but containing an open-reading frame with or without signal peptide were considered novel or unknown-function proteins. Considering the diverse roles of putative secreted proteins in blood feeding, a brief description for each protein family is presented below.

Group 1: Basic-tail proteins (BTP)

This family of proteins is highly represented in the salivary glands of both *I. pacificus* and *I. scapularis* ticks. Fig. 2A shows the alignments of the BTP of these ticks where a highly conserved signal peptide indicates their common origin from an ancestral gene. Fig. 2A also shows that the pattern of these sequences contain six cysteines (XnCX14CX3CX18CX9CX4CXn) followed by a basic tail with high content of lysines (Lys,

K). On the other hand, some proteins from this family, such as gi 22652868 and gi 22164158, display a negatively charged tail composed of six glutamic acid (Glu, E) anionic residues (Fig. 2A). The evolutionary relationships of BTP were inferred by constructing the phylogenetic tree using the NJ algorithm; a cladogram is shown in Fig. 2B. Of interest, these proteins share sequence similarities to exogenous anticoagulants such as SALP 14 (gi 15428308) from *I. scapularis*. SALP 14 is a FXa inhibitor that appears to interact with the catalytic domain of FXa and with the so-called exosite (Narasimhan et al., 2002). Exosites—regions far from the catalytic site and known to determine specificity and affinity of blood coagulation factors toward substrates—also are critical for the assembly of the prothrombinase, a multimolecular complex that leads to thrombin generation (Krishnaswamy, 2005). Targeting these domains appears to be an effective strategy evolved by blood-feeding arthropods to effectively impair blood coagulation. In fact, we recently reported that ixolaris, a FX(a) scaffold-dependent inhibitor of Factor VIIa/tissue factor complex, specifically recognizes the FXa heparin-binding exosite (Monteiro et al., 2004).

The fact that BTP and SALP 14 contain a poly-Lys tail adds an additional layer of anticoagulation, as it directs the inhibitor to negatively-charged membranes (e.g., activated platelets) critical for productive blood coagulation complex assembly (Broze, 1995). As a result, the effective concentration of the inhibitor is increased at sites that are predominantly pro-coagulant. Also, we speculate that FXa—which is usually protected from physiologic inhibitors (e.g., TFPI, heparin/ATIII) when the prothrombinase is fully assembled (Mast and Broze, 1996; Rezaie, 2001)—would be more susceptible to these bifunctional molecules. Demonstration that proteins rich in positively charged residues effectively block the coagulation cascade comes from studies performed with a recombinant *Rhodnius prolixus* salivary lipocalin (nitrophorin-7, NP-7). NP-7 contains a cluster of positively charged residues in the N-terminus and specifically binds to anionic phospholipids, preventing thrombin formation by the prothrombinase (Andersen et al., 2004). Finally, a bifunctional fusion protein containing Kunitz and annexin domains was shown recently to inhibit the initiation of blood coagulation (Chen et al., 2005).

Group 2: Similar to Group 1, but without the basic tail

These sequences contain a cysteine pattern identical to Group 1 peptides except that, remarkably, the poly K tail is missing. Many other amino acids also are not conserved. Sequence alignment between the Group 1 peptides (containing poly K and poly E) and the peptides similar to Group 1 is shown in Fig. 3A. Fig. 3B shows that these proteins come from a common ancestor that appears to have evolved to display different functions. The function of the peptides of Group 2 deserves further investigation.

Group 3: Kunitz-containing proteins

Kunitz domains are about 60 residues and contain 6 specifically spaced cysteines (XnCX8CX15CX7CX12CX3CXn) that form disulfide bonds typically represented by bovine pancreatic trypsin inhibitor (BPTI). In most cases, they are reversible inhibitors of serine proteases that bind the active site (Laskowski and Kato, 1980); however, Kunitz inhibitors such as the dendrotoxins from *Dendroaspis angusticeps* snake venom block K+ channel but display negligible protease inhibitory properties (Harvey, 2001). Kunitz-containing proteins also interact with protease exosites (Monteiro et al., 2004) or platelets (Mans et al., 2002). Of note, sequencing the *I. pacificus* cDNA library yields a number of proteins containing Kunitz-like domains.

The alignment of BPTI, snake venom, and *I. scapularis* and *I. pacificus* single Kunitz-like proteins is shown in Fig. 4A. Some *I. pacificus* proteins contain one-Kunitz-like domain, here named the Monolaris-1 family (or “similar to 6.5- to 8.4-kDa proteins from *I. scapularis*”).

These molecules display the following cysteine pattern: XnCX8CX18CX5CX12CX3CXn. Other single-Kunitz sequences present in *I. scapularis* belong to the Monolaris-2 family (or "similar to 7.9- to 8.7-kDa proteins from *I. scapularis*") and display the sequence pattern XnCX8CX15CX8CX11CX3CXn. We could not, however, find members of the Monolaris-2 family sequences in our *I. pacificus* cDNA library. Fig. 4A also shows that the well-known tick anticoagulant peptide from the soft tick *Ornithodoros moubata* (Waxman et al., 1990) has Kunitz-like folding with the sequence pattern XnCX9CX17CX5CX15CX3CXn. At present, the functions of Monolaris-1 and -2 are unknown, but they may target specific proteases. The phylogenetic tree shown in Fig. 4B suggests that snake venom peptides containing Kunitz domains (non-neurotoxic or neurotoxic) and the tick families of Monolaris and basic tail peptides have diverged into two different main groups from a commom ancestor, suggesting that these proteins have evolved to perform different functions.

Additionally, cDNAs were sequenced coding for proteins containing two- or five-Kunitz domains. These proteins share sequence similarity to ixolaris (Francischetti et al., 2002b) and penthalaris (Francischetti et al., 2004a), two *I. scapularis* TFPI salivary proteins that prevent initiation of blood coagulation through specific inhibition of the Factor VIIa/tissue factor complex. It is possible that these proteins block other proteases (Ruf, 2004) or affect angiogenesis (Hembrough et al., 2004).

Fig. 4C depicts the predicted secondary folding of *I. scapularis* and *I. pacificus* Kunitz-like-containing proteins based on the crystal structure determined for BPTI (Huber et al., 1974).

Group 4: Proline-rich proteins

Group 3 cDNA sequences code for short peptides of mature molecular mass ranging from 3.5–4.8 kDa of both basic and acidic nature (Table 1). Alignments and cladograms (presented in Fig. 5A and 5B, respectively), show that all sequences are relatively glycine and proline rich in both *I. pacificus* and *I. scapularis* salivary glands. Some sequences display weak matches to proteins annotated as collagen in the NR database; these possess two conserved cysteine residues in the mature peptide and remarkable conservation of the secretory signal peptide (Fig. 5). Most amino acids of the predicted signal secretory peptide are conserved, versus few on the mature peptide, suggesting functional diversity. The possible function of these peptides remains to be characterized, but taking into account its similarity to collagen, it may somehow affect vascular biology through inhibition of cell-cell, cell-matrix, or cell-ligand interactions. These peptides may also function as adhesive molecules to cement the tick into their host's skin.

Group 5: Similar to I. scapularis 18.7-Kda protein

This group of proteins (table 1) is similar to orthologs described in *I. scapularis* and code for an acidic putative protein of unknown function. Only low e values have been found when compared with proteins in the NR database including coagulation factor X (gi 9837158, e value 0.069), venom metalloprotease acurhagin precursor (gi 4689408; e value 3.8), and proprotein convertase subtilin (gi 51771463, e value 8.4). Accordingly, this family of proteins may have evolved from a protease precursor; however, any functional assignment will be possible only after testing the recombinant protein in screening assays.

Groups 6 and 7: Similar to I. scapularis 5-kDa protein and 9.4-kDa protein

Groups 6 and 7 code for basic proteins of ~ 5 kDa and 9.4 kDa that also are present in *I. scapularis*. No protein motif was identified for either protein; accordingly, the function of these proteins is not evident.

Group 8: Metalloprotease

These enzymes are capable of hydrolyzing various components of the extracellular matrix including fibrinogen and fibronectin and reportedly affect endothelial cells, leading to apoptosis. These enzymes are organized into four classes, PI through PIV, according to size and domain composition (Bjarnason and Fox, 1995).

Our library contains a truncated cDNA that codes for a mature metalloprotease similar to one described in *I. scapularis* (gi 31322779) (Francischetti et al., 2003) and *I. ricinus* (gi 5911708). The alignment of the mature metalloproteases from *I. pacificus*, *I. scapularis*, and *I. ricinus*, where the zinc-binding motif HExxHxxGxxH common to these enzymes was identified, is shown in Fig. 6A. Fig. 6B compares the PIII class of metalloproteases from snake venom and the *I. pacificus, I. scapularis* and *I. ricinus* enzymes. It is clear that enzymes from both genera have pre-, pro-, metalloprotease, disintegrin-like, and cysteine-rich-like domains; however, the *Ixodidae* disintegrin-like and cysteine-rich like domains are significantly shorter in the number of amino acid residues when compared with the corresponding domains of metalloproteases from the reprolysin family (Bjarnasson and Fox, 1995). We suggest that this pattern of cysteines confer a different specificity for these enzymes. This family of proteins also appears to account for the α-fibrinogenase and fibrinolytic activity recently reported for *I. scapularis* saliva (Francischetti et al., 2003). Degradation of fibrinogen and fibrin are associated with inhibition of platelet aggregation and clot formation. Metalloproteases also may interact with endothelial cell integrins, leading to apoptosis and inhibition of angiogenesis (Francischetti et al, 2005).

Group 9: GPIIb/IIIa antagonists from the short neurotoxin family

Inhibitors of platelet aggregation that targets the fibrinogen receptor (GPIIbIIIa, integrin αIIbβ3) have been described in the hard tick *Dermacentor variabilis* (variabilin) and the soft ticks, *Ornithodoros moubata* (disagregin) and *O. savignyi* (savignygrin) (Karczewski et al. 1994; Wang et al. 1996; Mans et al. 2002a). Savignygrin belongs to the Kunitz-BPTI family and presents the integrin RGD-recognition motif on the substrate binding loop of the Kunitz fold (Mans et al. 2002a). In contrast, variabilin possesses an RGD-motif in its C-terminal region that is not flanked by cysteines (Wang et al. 1996). A search for possible GPIIb/IIIa antagonists with RGD-motifs and flanking cysteines, termed the Ixodegrins, identified one candidate in *I. pacificus* and several homologs in *I. scapularis* (Table 1). It is clear that the Ixodegrins are related to variabilin, but do possess flanking cysteines. Variabilin probably possesses a flanking disulphide motif too, but was missed due to the technical difficulties in identifying cysteines correctly during N-terminal sequencing. Database searches using SAM-T99 (Karplus et al. 1998), a program that utilizes hidden Markov models to find remote homologous sequences, identified dendroaspin as the highest hit. Dendroaspin, also known as mambin, is part of the short neurotoxin family found in elapid snakes (McDowell et al. 1992; Williams et al. 1992; Sutcliffe et al. 1994). Strikingly, the RGD-active site loop (loop3) is conserved between snake and tick integrin antagonists (Fig. 7A). This includes the flanking cysteines involved in a disulphide bond that constricts the RGD-loop conformation and the flanking prolines that was shown to be important for presentation of the RGD sequence (Lu et al. 2001). The tick inhibitors maintain loops 2 and 3 of the short neurotoxin fold, but do not possess the N-terminal loop 1 and the C-terminal extension (Fig. 7A). This makes them the shortest members of the short neurotoxin family described to date, with only 39 amino acids forming the core active fold. Phylogenetic analysis of the neurotoxin family indicates that dendroaspin and tick inhibitors group within one clade to the exclusion of the other short neurotoxins (Fig. 7B). This suggests either an extreme form of convergent evolution, where ticks and elapid snakes used the same protein fold to evolve the same function or raises the possibility that ticks or snakes acquired the ancestral protein via a horizontal gene transfer event or that there is a true evolutionary relationship between the ixodegrins and short neurotoxins. The fact that orthologs of the Ixodegrins are present in both *Ixodes* (prostriate) and *Dermacentor* (metastriate) ticks, suggests

that this inhibitor was present in the last common ancestor of hard ticks. Snakes evolved most of their venom properties approximately 60-80 million years ago (Fry, 2005), whereas most hard tick genera diverged at least 110 million years ago or earlier (Klompen et al. 1996). If tick and snake proteins are related, then the ancestral gene may have a platelet antagonist function and the neurotoxic properties (and the rest of the short neurotoxin fold - loop1 and the C-terminal extension) evolved later. In contrast, soft ticks in the genus *Ornithodoros* evolved integrin antagonists from the BPTI-fold which suggests that hard and soft ticks evolved different strategies to obtain a blood meal (Mans et al. 2002b; Mans and Neitz, 2004). Accordingly, ixodegrin may affect platelet or neutrophil integrin function or neutrophil function.

Group 10: Ixostatin family, or short-coding cysteine-rich peptides ("thrombospondin")

The two sequences in Group 11 match a sequence deposited in the NR database from *I. scapularis*; alignments are shown in Fig. 8A. These sequences have been annotated "thrombospondin" (gi 15428290), but thrombospondin motifs are lacking. On the contrary, these short coding region cysteine-rich peptides—here named ixostatins—are remarkably similar to the cysteine-rich domain of ADAMTS (Fig.8B). Of note, ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs), also known as aggrecanase, are enzymes involved in cartilage cleavage (Flannery et al., 2002). The role of the cysteine-rich domain of ADAMTS proteases is unknown, but it is postulated to interact with integrins and/or other attachment motifs of cells and matrix proteins (Porter et al., 2005). Accordingly, the ixostatin family of peptides could be involved in disruption of platelet aggregation or neutrophil function, cell-matrix interactions, or inhibition of angiogenesis (Porter et al., 2005). The protein modules of ixostatin and of ADAMST-4 are compared in Fig 8C.

Group 11: Histamine-binding proteins (lipocalins)

Group 11 contains sequences with similarities to histamine-binding proteins discovered in the saliva of *Rhipicephalus appendiculatus* ticks (Paesen et al., 1999). The alignments of these sequences (Fig. 9A) reveal that they do not display a highly conserved signal peptide which suggest that they may not share a common ancestor. In addition, the mature proteins contain few consensus sequences indicating that they may have diverged to perform distinct functions (Fig. 9B). This contention is also supported by the cladogram presented in Fig. 9B. It is likely that these proteins function by binding small ligands such as histamine, serotonine, and adrenaline (Andersen et al., 2005). Fig. 9C shows a predicted 3-D model for sequence gi 51011604 that has an e value of -768 for HBP from *R. appendiculatus*. The figure shows amino acid side chains of the histamine-binding protein from *R. appendiculatus* (red) surrounding the bound histamine ligand with the corresponding residues for gi 51011604 shown in cyan. In the histamine-binding protein, the imidazole ring of the ligand is stabilized by surrounding aromatic residues, while in the *I. scapularis* protein the binding pocket remains hydrophobic and fewer aromatic residues are present, suggesting different ligand specificity. Polar residues (Tyr 36 and Glu 135) forming electrostatic interactions with the aliphatic amino group of histamine in the histamine-binding protein are conserved in gi 51011604 suggesting the possibility of a similar role in this protein.

Group 12: Neuropeptide-like (npl-31) protein with GGY repeat

A cDNA coding for a protein that shows remarkable sequence homology to a neuropeptide-like protein (npl-21) described in *Caenorhabditis elegans* (Nathoo et al., 2001). This family of peptides displays a potent antimicrobial activity toward *Drechmeria coniospora*, *Neurospora crassa*, and *Aspergillus fumigatus* (Couillault et al., 2004). Identification of these peptides in ticks reinforces the notion that saliva contains a cocktail of antimicrobial peptides. These peptides may prevent growth of yeast and bacteria that, *per se*, can elicit an

inflammatory/immune response that may be detrimental to the feeding behavior of the attached ticks. Expression of these molecules is particularly important *vis-à-vis* the remarkably immunosupressive property of the saliva (Wikel, 1999) that helps ticks to feed for days but otherwise creates an appropriate environment for pathogen overgrowth. The sequence alignments for *C. elegans* npl-21 and *I. pacificus* npl-21-like proteins are presented in Fig. 10A and the cladogram in Fig. 10B. This is the first time that this family of antimicrobial peptides has been identified in the salivary gland of a blood-sucking arthropod.

Group 13: Oxidant metabolism

Proteins with similarity to glutathione peroxidase and a putative secreted superoxide dismutase were found (Table 1). These sequences categorize the prominent salivary gland proteins in *I. pacificus* and demonstrate the presence of a potent antioxidant in tick saliva. Of interest, cluster F12_IPL_P23 has sequence similarity to SALP 25, a protein that catalyzes the reduction of hydrogen peroxide in the presence of reduced glutathione and glutathione reductase (Das et al., 2001). The functions of these proteins are likely related to maintenance of the physiologic redox of cellular intracellular milieu or to modulation of the extracellular levels of pro-oxidants often associated with inflammatory events.

Group 14: Similar to other ixodid proteins

A number of sequences show sequence homology to proteins from *Ixodidae* described before. We have found sequences similar to SALP 15, a immunodominat protein in *I. scapularis* (Das et al. 2001), and to ISAC, the anti-complement from *I. scapularis* (Valenzuela et al., 2002). We also have found sequences similar to domain 8 of human ADAMS and Factor VII.

Group 15: Novel, unknown

Some sequences containing a signal peptide and a stop codon and with a clear open reading frame were without database hits and were characterized as unknown-function proteins. Assignment of function for these proteins will only be possible after expressing and screening for testable biologic activities.

Group 16: Housekeeping cDNA

Thirty-seven sequences with homology to housekeeping protein are given in Table 1. They assign to ribosomal, glutathione S-transferase, vacuolar aasorting proteins, cytochrome, ATP synthase subunit, and NADH-ubiquinone oxidoreductase, among other molecules. In addition, housekeeping proteins may be useful in phylogenetic studies (Black and Piesman, 1994).

I. pacificus salivary gland protein diversity: modulators of vascular biology and candidates for an anti-saliva experimental vaccine—We describe the set of cDNA present in the salivary glands of *I. pacificus* salivary gland. Our library contains a remarkably large degree of redundancy, as shown by the many related mRNAs. It appears that the long evolutionary history of ticks may be responsible for the complexity of transcripts reported here. Also, many protein families we have identified were found previously in *I. scapularis* salivary glands (Valenzuela et al., 2002) which confirms the diverse nature of these secretions compared with the salivary composition of fast feeders such as sand flies (Charlab et al., 1999) and mosquitoes (Francischetti et al., 2002a). This variability in the tick salivary gland is consistent with the high polymorphism of salivary proteins among individual ticks analyzed by SDS-PAGE (Wang et al., 1999). Also, the diversity across and within species could reflect the range of host species and the need to have modulators of specific pathways that differ in distinct host species. The adaptive role of this diversity appears to be explained at least in part by a gene-duplication phenomenon. This contention is supported by the diversity of sequences containing Kunitz-like domains in addition to a weak similarity observed among members of the lipocalin

family of proteins reported here. It may be that these inhibitors have evolved to inhibit different proteases or to bind to different ligands (Andersen et al., 2005). It is also plausible that gene duplication may help ixodid ticks to evade the immune system. If so, this may help to explain why hard ticks can remain attached to many hosts for days without apparent detrimental effects (Ribeiro and Francischetti, 2003). Finally, the possible closer association of *I. persulcatus* with *I. pacificus* makes the former an interesting species for future salivary gland transcriptome analysis and phylogenetic studies.

The functions of many tick sequences described in this paper are unknown. Cloning and expressing select cDNAs will help in the identification of molecule specificity and to find potential targets for gene silencing (Sanchez-Vargas et al., 2004), and accordingly, our understanding of how ticks successfully feed on blood. It also may provide tools to understand vascular biology and the immune system. A diagram with the putative targets of salivary proteins and how they may affect vascular biology is shown in Fig. 11. Finally, defining the most abundant antigens or those that may effectively help ticks to feed or transmit *Borrelia* could be an effective approach to develop a protective vaccine directed toward tick salivary molecules (Lane et al., 1999).

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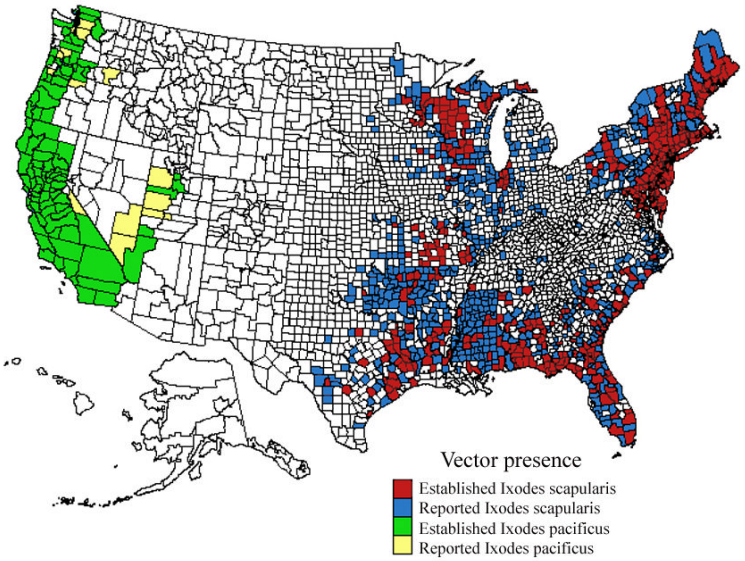


Fig. 1.

Established and reported distribution of the Lyme disease vectors *Ixodes scapularis* (*I. dammini*) and *Ixodes pacificus* by county, United States, 1907–1996. Distribution was reported by the Centers for Disease Control and Prevention and can be found at http://www.cdc.gov/ncidod/dvbid/lyme/tickmap.htm.

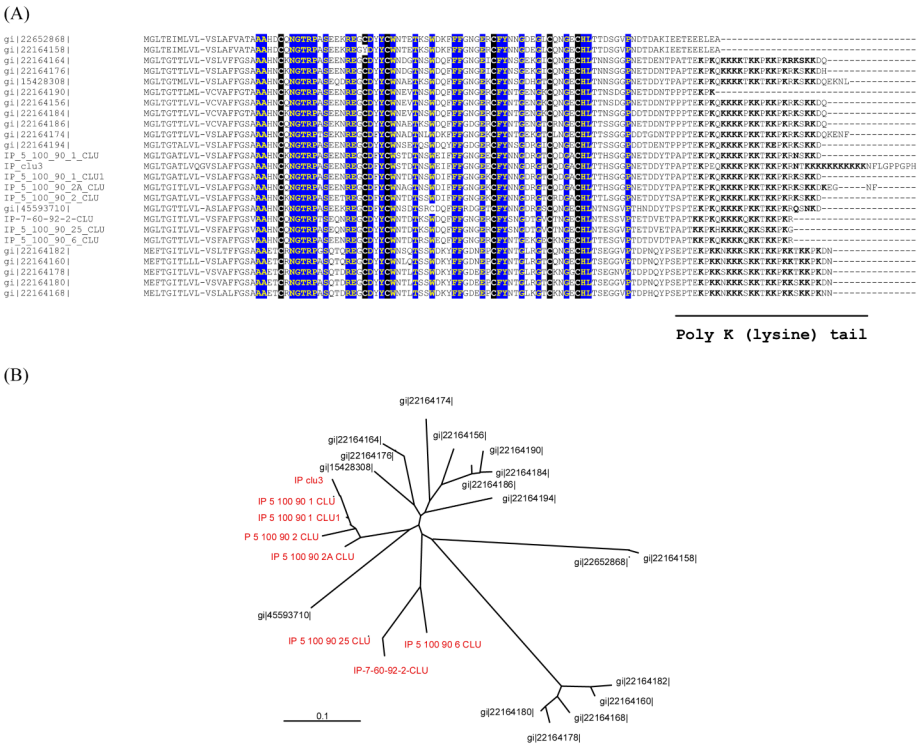
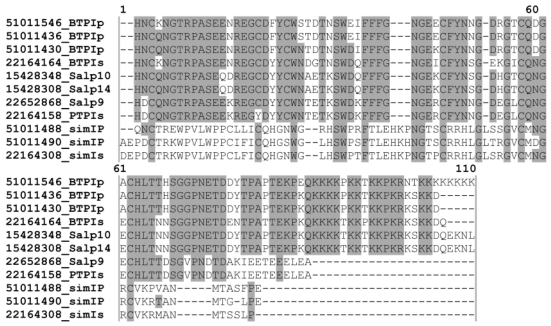


Fig. 2.

Group 1: Basic tail proteins (BTP). (A) Alignments of peptides from *I. pacificus* (Table 1) and *I. scapularis* BTP deduced from cDNA libraries. Conserved amino acid residues are shown in black background. Lysine residues (K) are shown in bold (Poly K, lysine tail). (B) The bar represents the degree of divergence among sequences.

(A)



(B)

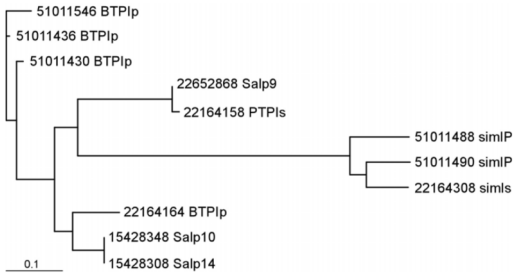


Fig. 3.

Group 2: Similar to Group 1, without basic tail. (A) Alignment of Group 2 peptides (Table 1). Conserved amino acid residues are shown in gray background. (B) The unrooted cladogram of all sequences. The bar represents the degree of divergence among sequences.

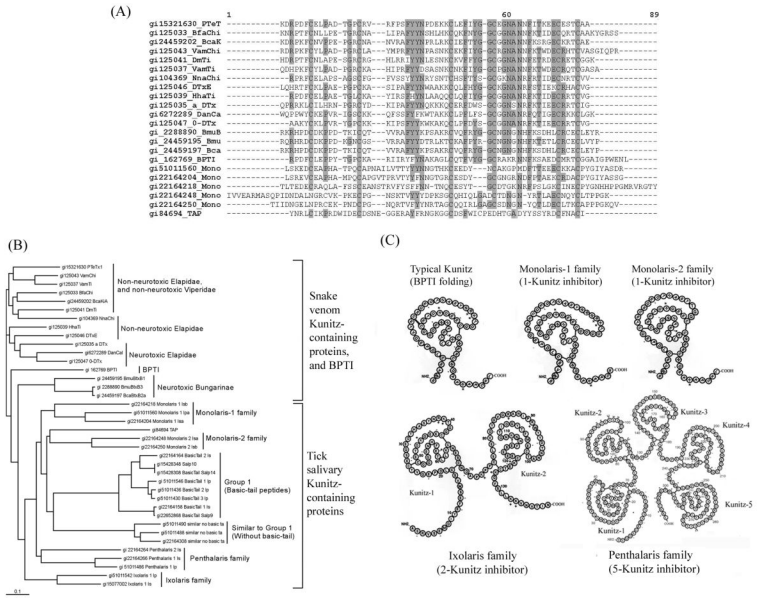


Fig. 4.

Group 3: Kunitz-containing proteins. (A) Alignment of Group 3 peptides (Table 1) with single Kunitz-containing protein from snake venoms. Conserved amino acid residues are shown in gray background. (B) The phylogram was constructed using protein from snake venom single-kunitz (neurotoxic or non-neurotoxic from Elapidae and Viperidae families) and tick salivary gland, plus BPTI (all accession numbers are depicted). The bar represents the degree of divergence among sequences. (C) Predicted secondary folding of Kunitz containing proteins from Ixodidae sp. based on BPTI folding (Huber et al., 1974).

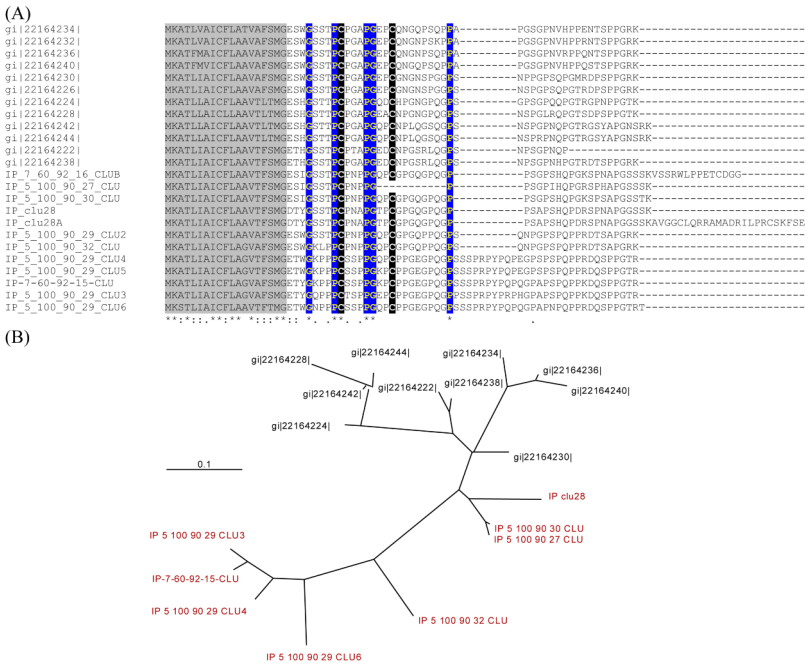
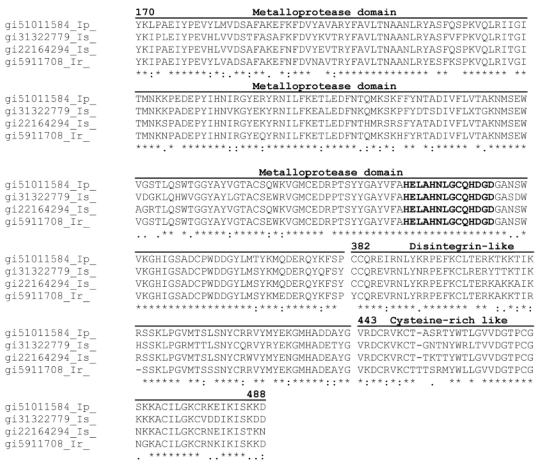


Fig. 5.

Group 4: Proline-rich peptides. (A) Alignment of Group 4 peptides (Table 1). Signal peptide is shown in gray background, and conserved amino acid residues are shown in black background. (B) The unrooted cladogram of all sequences. The bar represents the degree of divergence among sequences.

(A)



(B)

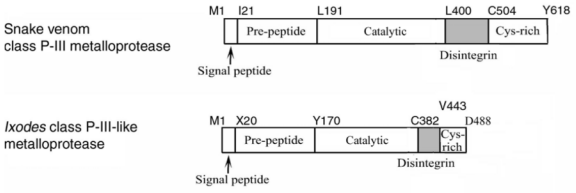


Fig. 6.

Group 8: Metalloproteases. (A) Alignment of metalloproteases from *I. pacificus* (Ip) (Table 1), *I. scapularis* (Is), and *I. ricinus* (Ir). The characters in bold represent the conserved Zn binding motif present in the catalytic domain. Asterisks, colons, and stops below the sequences indicate identity, high conservation, and conservation of the amino acids, respectively. (B) Diagram comparing the protein motifs (pre, pro, catalytic, disintegrin-like, and cysteine rich-like domains) of class III metalloproteases from snake venoms and tick class III-like metalloproteases.

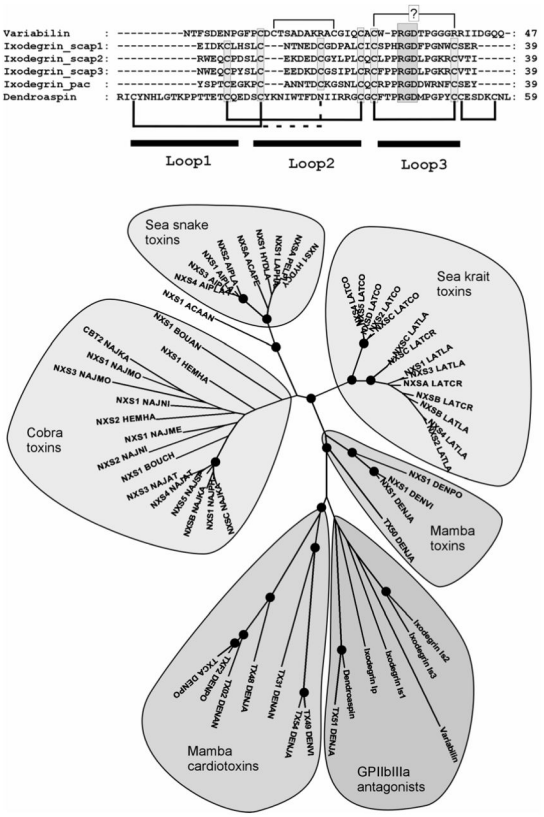


Fig. 7.

Group 9: Ixodegrin: disintegrins. (A) Alignment of the ixodegrins from *Ixodes pacificus* (Ixodegrin_Ip), *I. scapularis* (Ixodegrin_Sc1/2/3), variabilin and dendroaspin. Shadowed in gray are conserved cysteine regions and the RGD motif. Also indicated are the loops and disulphide bond pattern of the short neurotoxin fold and the inferred disulphide bond patterns of the ixodegrins. (B) A neighbor-joining tree of the short neurotoxin family. Indicated are different functional clades found for the family. Snake proteins are referred to by their Swiss-Prot name. Black circles indicate confidence levels >70% from 10 000 bootstraps.

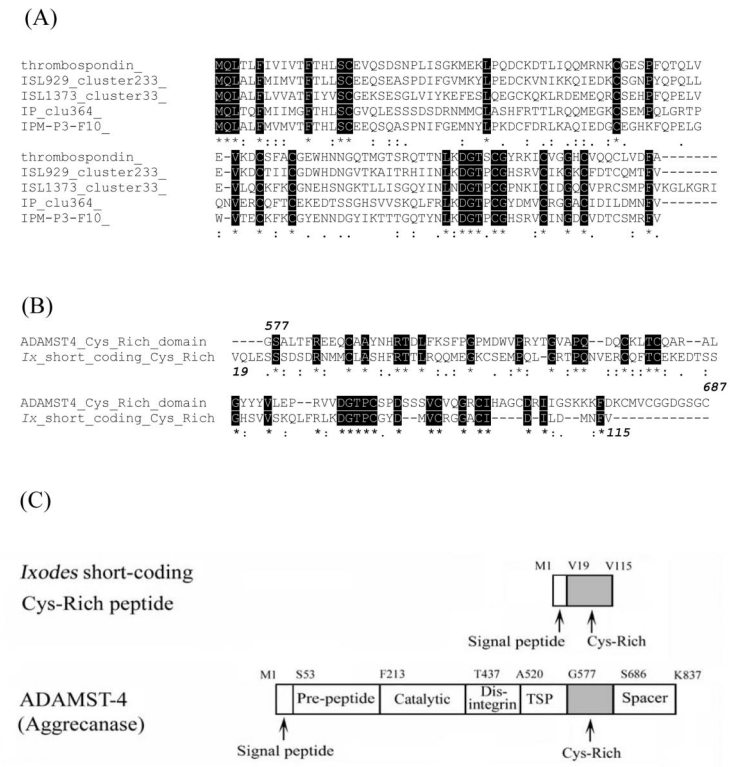


Fig. 8.

Group 10: Ixostatin: short coding cysteine-rich peptides. (A) Alignment of Group 9 peptides from *I. pacificus* (Table 1) and *I. scapularis*. Conserved amino acid residues are shown in black background. (B) Alignment between ixostatin and the cysteine rich-domain of ADAMST-4 (aggrecanase). (C) Diagram comparing the protein motifs (pre, pro, catalytic, disintegrin-like, cysteine rich-like, and spacer domains) of ADAMST-4 (Flannery et al., 2002) and ixostatin.

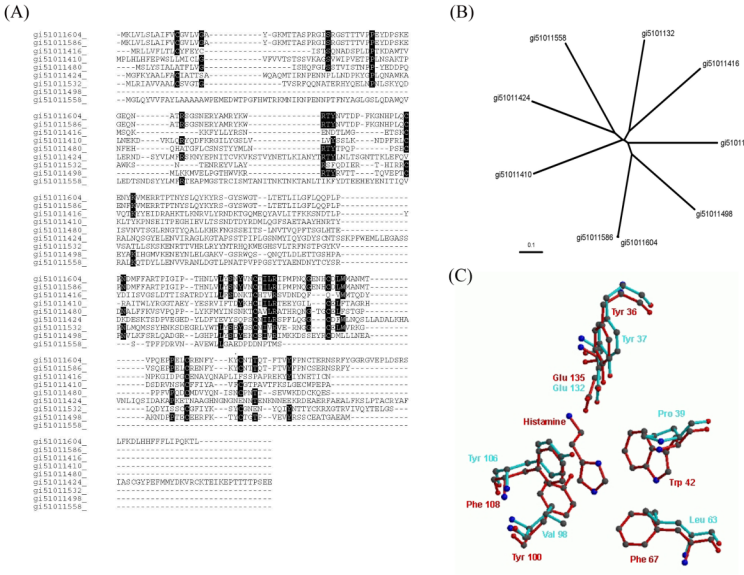


Fig. 9.

Group 11: Histamine-binding proteins (lipocalins). (A) Alignment of Group 10 peptides from *I. pacificus* (Table 1). Conserved amino acid residues are shown in black background. (B) The unrooted cladogram of all sequences. The bar represents the degree of divergence among sequences. (C) The figure shows amino acid side chains of the histamine-binding protein from *R. appendicuatus* (red) surrounding the bound histamine ligand. The corresponding residues for gi 51011604 are shown in cyan. In the histamine-binding protein, the imidazole ring of the ligand is stabilized by surrounding aromatic residues. In the *I. scapularis* protein the binding pocket remains hydrophobic, fewer aromatic residues are present, suggesting a different ligand specificity.

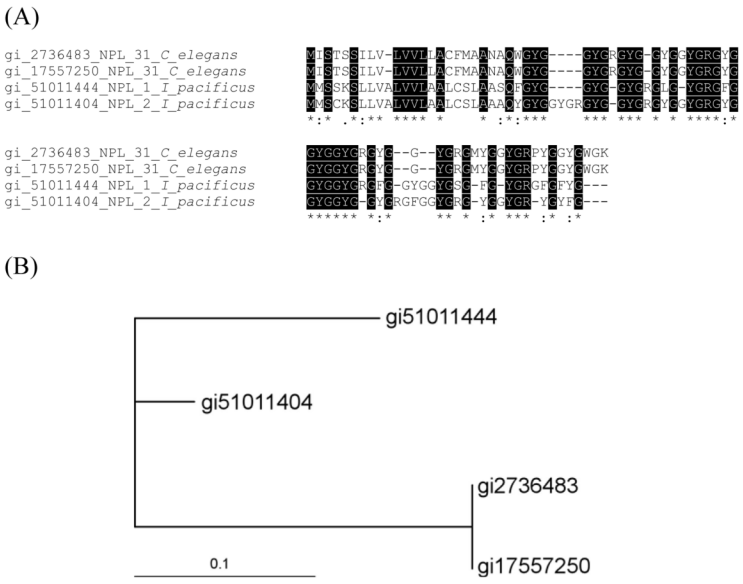


Fig. 10.

Group 12: Neuropeptide-like (npl-31) peptides. (A) Alignment of Group 11 peptides from *I. pacificus* (Table 1) and *I. scapularis*. Conserved amino acid residues are shown in gray background. (B) The unrooted cladogram of all sequences. The bar represents the degree of divergence among sequences.

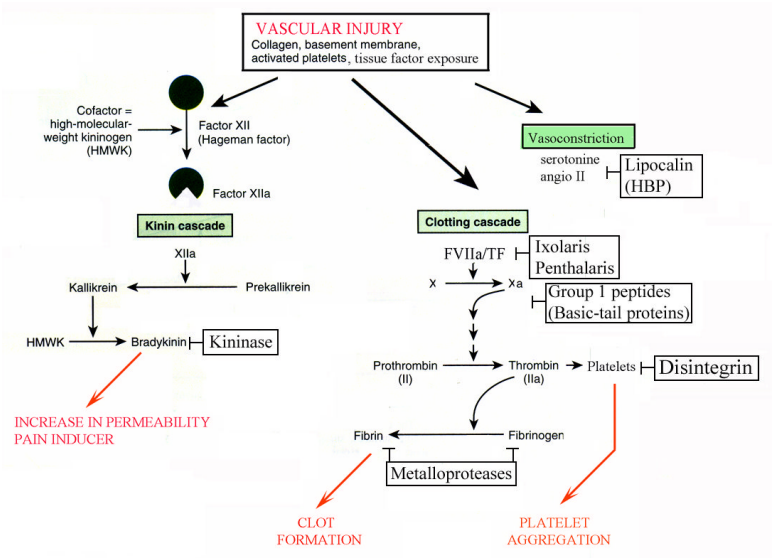


Fig. 11.

Negative modulators of vascular biology are present in *I. pacificus and I. scaularis* saliva. Vascular injury is accompanied by vasoconstriction and activation of the extrinsic and intrinsic pathways of blood coagulation (Broze et al., 1995). Vasoconstriction is mediated by molecules such as serotonine that may be removed by salivary protein with a lipocalin folding (Andersen et al., 2005). The extrinsic pathway is initiated by tissue factor/factor VIIa complex and effectively blocked by ixolaris (Francischetti et al., 2002b) and penthalaris (Francischetti et al., 2004a). FXa generated by the intrinsic or extrinsic Xnase may be inhibited by Group 1 peptides containing a basic tail that may prevent productive prothrombinase complex assemble (Rezaie, 2000; Narasimhan et al., 2002; Andersen et al., 2004, Monteiro et al., 2004). Platelet, neutrophil, and endothelial cell function may be affected by Ixodegrins (disintegrins) or Ixostatins (short-coding cysteine-rich peptides). Metalloproteases appear to cleave fibrinogen and fibrin, therefore inhibiting platelet aggregation and clot formation (Francischetti et al., 2003). Metalloproteases also may affect endothelial cell function and angiogenesis (Francischetti et al, 2005). The intrinsic pathway that is activated by contact (Broze et al., 1995) leads to bradykinin formation, a peptide that increases vascular permeability and induces pain. Bradykinin is degraded by a salivary kinininase, thus preventing its pro-inflammatory effects (Ribeiro and Francischetti, 2003).

Seq name	Seq size	Link to nucleotide sequence	SigP Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
Group 1 - sequences with basic tail similar to Group I peptides of *I. scapularis* - Putative anti-hemostatic											
IP_5_100_90_1A_CLU	120	IP_5_100_90_1A_CLU	SIG	21-22	13.27	8.52	11.042	8.53	14 kDa salivary gland protein [Ixodes	1E-059	Group 1 basic tail salivary peptide
IP_5_100_90_1_CLU1	120	IP_5_100_90_1_CLU1	SIG	21-22	13.282	8.91	11.054	8.91	14 kDa salivary gland protein [Ixodes	3E-061	Group 1 basic tail salivary peptide
IP-7-60-92-14-CLU	120	IP-7-60-92-14-CLU	SIG	21-22	13.284	9.04	11.054	9.04	14 kDa salivary gland protein [Ixodes	1E-060	Group 1 basic tail salivary peptide
IP_5_100_90_1_CLU	120	IP_5_100_90_1_CLU	SIG	21-22	13.257	8.52	11.028	8.53	putative secreted protein [Ixodes sca	9E-059	Group 1 basic tail salivary peptide
IP_5_100_90_25_CLU	115	IP-7-60-92-1-CLU	SIG	21-22	12.728	8.75	10.395	8.76	putative secreted protein [Ixodes sca	2E-052	Group 1 basic tail salivary peptide
IP-7-60-92-2-CLU	115	IP-7-60-92-2-CLU	SIG	21-22	12.892	8.93	10.559	8.93	putative secreted protein [Ixodes sca	6E-053	Group 1 basic tail salivary peptide
IP_5_100_90_2A_CLU	125	IP_5_100_90_2A_CLU	SIG	21-22	13.806	9.04	11.542	9.04	putative secreted salivary protein [I	1E-062	Group 1 basic tail salivary peptide
IP_5_100_90_2_CLU	121	IP_5_100_90_2_CLU	SIG	22-23	13.353	9.17	11.024	9.17	putative secreted salivary protein [I	1E-059	Group 1 basic tail salivary peptide
IP_5_100_90_2_CLU2	120	IP_5_100_90_2_CLU	SIG	21-22	13.252	9.17	11.024	9.17	putative secreted salivary protein [I	1E-059	Group 1 basic tail salivary peptide
IP_clu3	138	IP_clu3	SIG	22-23	15.299	9.46	13	9.46	putative secreted salivary protein [I	2E-058	Group 1 basic tail salivary peptide
IP_5_100_90_6_CLU	115	IP_5_100_90_6_CLU	SIG	21-22	12.933	8.93	10.613	8.92	salivary secreted protein [Ixodes sca	4E-055	Group 1 basic tail salivary peptide
IP_5_100_90_146_CLU	118	IP_5_100_90_146_CLU	SIG	21-22	13.212	4.71	10.678	4.58	salivary secreted protein [Ixodes sca	3E-007	Group 1 basic tail salivary peptide
Group 2 - Similar to Group 1 proteins from I. scapularis and *I. pacificus*, without basic tail - Putative anti-hemostatic											
IP_5_100_90_55_CLU	91	IP_5_100_90_55_CLU	SIG	20-21	10.145	9.16	7.855	9.22	putative 8.4 kDa secreted protein [Ix	6E-043	
IP_5_100_90_55A_CLU	90	IP_5_100_90_55A_CLU	SIG	18-19	10.071	9.12	8.011	9.21	putative 8.4 kDa secreted protein [Ix	3E-044	
Group 3 - Sequences with Kunitz domains											
Monolaris family (1-Kunitz domain) - Putative anti-coagulant											
IP_clu491	85	IP_clu491	SIG	21-22	9.341	4.95	6.953	4.67	putative secreted protein [Ixodes sca	4E-029	Group 2 of Ixodes scapularis - single Kunitz proteins
Ixolaris family (2-Kunitz domains) - Anti-coagulant											
IP_clu258	167	IP_clu258	SIG	31-32	18.56	4.79	14.94	5.03	ixolaris [Ixodes scapularis] 205 3e-052	3E-052	Ixolaris homologue
mys5	186	mys5	SIG	20-21	21.477	9.09	19.074	9.2	CG33103-PB [Drosophila melanogaster	1E-009	Kunitz and thrombospondin similarity

Seq name	Seq size	Link to nucleotide sequence	Sig P Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
F04_IPM_P22_JIN	142	F04_IPM_P22_JIN	SIG	20-21	16.479	9.08	13.999	9.08	tissue factor pathway inhibitor 2 [	4E-022	similarity to tissue factor pathway inhibitor 2
F02_IPL_P19	167	F02_IPL_P19	SIG	21-22	18.93	6.16	16.453	7.44	putative secreted protein [Ixodes sca	2E-014	Kunitz protease inhibitor domain
Penthalaris (5-Kunitz domains) - Anti-coagulant											
IP_5_100_90_547_CLU	330	IP_5_100_90_547_CLU	SIG	22-23	38.13	8.46	35.37	8.43	putative secreted protein [Ixodes sca	1E-167	Kunitz protease inhibitor domain
Group 4 - of related sequences rich in proline found in other *Ixodidae* - Unknown function											
IP_5_100_90_29_CLU2	65	IP_5_100_90_29_CLU2	SIG	19-20	6.556	8.66	4.406	8.96	putative secreted protein [Ixodes sca	3E-023	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_30_CLU	65	IP-7-60-92-16-CLU	SIG	19-20	6.294	8.63	4.11	8.9	putative secreted protein [Ixodes sca	9E-018	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_32_CLU	65	IP_5_100_90_32_CLU	SIG	19-20	6.609	9.1	4.469	9.5	putative secreted protein [Ixodes sca	5E-021	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_27_CLU	54	IP_clu27	SIG	19-20	5.377	8.89	3.192	9.51	putative secreted protein [Ixodes sca	7E-013	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_29_CLU3	74	IP_5_100_90_32_CLU	SIG	19-20	7.574	7.73	5.422	8.04	putative secreted protein [Ixodes sca	1E-014	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_29_CLU4	74	IP_5_100_90_29_CLU4	SIG	19-20	7.613	7.71	5.428	8.05	putative secreted protein [Ixodes sca	7E-016	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_29_CLU5	74	IP_5_100_90_29_CLU4	SIG	19-20	7.613	8.61	5.428	8.86	putative secreted protein [Ixodes sca	9E-016	Group 3 collagen-like salivary secreted peptides
IP_5_100_90_29_CLU6	75	IP_5_100_90_29_CLU6	SIG	19-20	7.754	7.72	5.525	8.06	putative secreted protein [Ixodes sca	2E-015	Group 3 collagen-like salivary secreted peptides
IP_clu28	65	IP_7_60_92_16_CLUA	SIG	19-20	6.377	7.74	4.19	8.05	putative secreted protein [Ixodes sca	9E-018	Group 3 collagen-like salivary secreted peptides
IP-7-60-92-15-CLU	74	IP-7-60-92-15-CLU	SIG	19-20	7.513	8.99	5.36	9.24	putative secreted protein [Ixodes sca	7E-015	Group 3 collagen-like salivary secreted peptides
IP_7_60_92_16_CLUB	79	IP_7_60_92_16_CLUB	SIG	19-20	7.794	7.68	5.61	7.96	putative secreted protein [Ixodes sca	2E-018	Group 3 collagen-like salivary secreted peptides
Group 5 - Sequences similar to I. scapularis 18.7 kDa protein - Unknown function											
IP_5_100_90_33_CLU	189	IP_5_100_90_33_CLU	SIG	20-21	21.271	4.78	18.945	4.92	putative 18.7 kDa secreted protein [I	4E-042	putative 18.9 kDa secreted protein

Seq name	Seq size	Link to nucleotide sequence	Sig P Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
IP_5_100_90_34_CLU	189	IP_5_100_90_34_CLU	SIG	20-21	21.338	4.82	19.004	4.95	putative 18.7 kDa secreted protein [I	4E-040	putative 19 kDa secreted protein [Ixodes pacificus]
IP-7-60-92-12-CLU	189	IP-7-60-92-12-CLU	SIG	20-21	21.362	4.79	19.028	4.92	putative 18.7 kDa secreted protein [I	3E-043	putative 19 kDa secreted protein [Ixodes pacificus]
Group 6 - Sequences similar to *I. scapularis* 5.3 kDa peptide - Unknown function											
IP_clu163A	61	IPM_P18_D1	SIG	18-19	6.519	8.82	4.283	9.38	putative 5.3 kDa secreted protein [Ix	0.015	similar to I. scapularis 5.3 kDa peptide
IPM_P18_D1	72	IPM_P18_D1	SIG	29-30	7.762	9.18	4.284	9.38	putative 5.3 kDa secreted protein [Ix	0.014	similar to I. scapularis 5.3 kDa peptide
IP_clu448	66	IP_clu448	SIG	22-23	7.757	9.32	4.991	9.59	putative 5.3 kDa secreted protein [Ix	0.033	similar to I. scapularis 5.3 kDa peptide
IP_clu526	79	IP_clu526	SIG	22-23	9.038	9.14	6.314	9.57	ENSANGP00000017973 [Anopheles gambi	0.34	similar to I. scapularis 5.3 kDa peptide
Group 7 -Sequences similar to I. scapularis 9.4 kDa peptide - Unknown function											
IPL-P23-C2	103	IPL-P23-C2	SIG	20-21	11.79	8.19	9.57	8.23	putative 9.4 kDa secreted protein [Ix	3E-019	similar to I. scapularis 9.4 kDa peptide
C02_IPL_P23	101	C02_IPL_P23	SIG	20-21	11.639	8.44	9.416	8.46	putative 9.4 kDa secreted protein [Ix	6E-035	similar to I. scapularis 9.4 kDa peptide
IP_5_100_90_152_CLU	79	IP_5_100_90_152_CLU	SIG	18-19	8.849	5.19	6.779	5.19	putative 7 kDa secreted protein [Ixod	2E-034	similar to I. scapularis 9.4 kDa peptide
Group 8 - Metalloprotease family - Putative anti-hemostatic											
IPM_P3_A1	344	IPM_P3_A1	CYT		39.565	9.18			truncated secreted metalloprotease [I	0.0	truncated secreted metalloprotease
Group 9 - Ixodegrin family: disintegrins - Putative anti-hemostatic											
IP_5_100_90_505_CLU	60	IP_5_100_90_505_CLU	SIG	21-22	6.545	4.93	4.173	6.47	HL01481p [Drosophila melanogaster] 33 1.8	1.8	Cys-rich
Group 10 - Ixostatin family: short-coding cysteine-rich peptides similarly found in ADAMST-4 (“Thrombospondins”) - Putative anti-hemostatic											
IP_clu364A	115	IP_clu364A	SIG	18-19	12.952	5.85	10.688	5.6	thrombospondin [Ixodes scapularis] 80 1e-014	1E-014	Kunitz protease inhibitor domain
IPM-P3-E1-JIN	114	IPM-P3-E1-JIN	SIG	17-18	12.987	5.26	10.821	5.6	putative secreted protein [Ixodes sca	1E-036	Kunitz protease inhibitor domain

Seq name	Seq size	Link to nucleotide sequence	Sig P Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
Group 11 - Histamine binding proteins - Putative anti-hemostatic											
G07_IPL_P19	313	G07_IPL_P19	SIG	17-18	35.209	5.16	33.149	5.08	putative secreted histamine binding p	1E-101	putative secreted histamine binding protein
IP_clu479	191	IP_clu479	SIG	18-19	21.704	4.45	19.53	4.49	putative secreted histamine binding p	5E-030	putative secreted histamine binding protein
IP_5_100_90_515_CLU	196	IP_5_100_90_515_CLU	SIG	18-19	21.832	5.8	19.708	5.66	putative 22.7 kDa secreted protein [I	1E-093	putative secreted histamine binding protein
G05_IPM_P18_JIN	265	G05_IPM_P18_JIN	CYT		31.348	5.5			putative secreted histamine binding p	1E-142	truncated histamine binding protein
mys4	244	mys4	SIG	20-21	28.177	9.01	25.901	8.85	putative protein [Ixodes scapularis] 331 7e-090	7E-090	putative secreted histamine binding protein
IPM_P3_D9	215	IPM_P3_D9	SIG	20-21	24.816	8.93	22.52	8.71	putative protein [Ixodes scapularis] 333 2e-090	2E-090	putative secreted histamine binding protein
IP_7_60_92_102_CLU	160	IP_7_60_92_102_CLU	CYT		18.265	6.89			histamine binding protein [Ixodes sca	4E-017	truncated histamine binding protein
IP_7_60_92_97_CLU	210	IP_7_60_92_97_CLU	SIG	16-17	24.498	9.37	22.767	9.35	serotonin and histamine binding prote	2E-005	putative secreted histamine binding protein
E10_IPL_P23_JIN	214	E10_IPL_P23_JIN	SIG	27-28	24.515	6.3	21.262	6.12	putative 22.5 kDa secreted protein [I	0.79	putative secreted histamine binding protein
F05_IPL_P19	193	F05_IPL_P19	SIG	19-20	22.536	6.12	20.018	6.16	putative secreted protein [Ixodes sca	4E-027	putative secreted histamine binding protein
Group 12 - Neuropeptide-like protein with GGY repeats - Putative anti-microbial											
IP_5_100_90_226_CLU	73		SIG	23-24	7.306	9.61	4.758	9.63	Neuropeptide-Like Protein (nlp-31)	6E-017	Reference
D12_IPL_P19	78		SIG	23-24	7.965	9.55	5.416	9.7	Neuropeptide-Like Protein (nlp-31)	6E-020	Reference
Group 13 - Oxidant metabolism											
F12_IPL_P23	116	F12_IPL_P23	CYT		13.643	9.48			plasma glutathione peroxidase [Homo sa	5E-027	Truncated glutathione peroxidase
IPM_P3_F10	189	IPM_P3_F10	SIG	23-24	20.168	9.17	17.439	7.15	Mn superoxide dismutase [Melopsittacu	8E-051	Mn superoxide dismutase
Group 14 - Similar to other Ixodid proteins											
IP_5_100_90_518_CLU	119	IP_5_100_90_518_CLU	SIG	16-17	13.222	8.79	11.293	9.01	15 kDa salivary gland protein [Ixodes	5E-026	Salp15 family

Seq name	Seq size	Link to nucleotide sequence	Sig P Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
B06_IPL_P23_JIN	124	B06_IPL_P23_JIN	SIG	20-21	13.857	5.19	11.314	4.66	15 kDa salivary gland protein [Ixodes	0.001	Salp15 family
IPM-P22-C4	154	IPM-P22-C4	SIG	18-19	16.574	5.31	14.408	5.14	salivary gland 16 kD protein [Ixodes	1E-031	Salp15 family
B12-IPL-P20	123	B12-IPL-P20	SIG	18-19	13.493	7.54	11.19	6.26	salivary gland 16 kD protein [Ixodes	4E-010	Domain 8 of human ADAMS
IP_clu537	108	IPM-P3-F7	SIG	24-25	11.943	8.18	9.089	8	16 kDa salivary gland protein A [Ixod	4E-004	some similarity with factor VII
IPM-P2-G12-JIN	178	IPM-P2-G12-JIN	SIG	22-23	19.773	4.37	17.328	4.38	20 kDa salivary gland protein [Ixodes	7E-065	anti-complement, ISAC
C08_IPL_P20	119	C08_IPL_P20	SIG	26-27	12.827	8.22	9.778	7.76	Is3 [Ixodes scapularis] 36 0.13	0.13	similar to is3 protein
D11_IPM_P17	240	D11_IPM_P17	SIG	18-19	23.194	8.94	21.295	9.03	hypothetical protein [Ixodes ricinus] 267 2e-070	2E-070	possible cuticle or salivary duct protein
Group 15 - Novel - unknown											
IP_5_100_90_39_CLU	87	IP_5_100_90_39_CLU	SIG	23-24	8.888	4.13	6.298	4.13	Insecticidal neurotoxin Tx4(5-5	0.75	Cys-rich, weak similarity to neurotoxin
IP_5_100_90_516_CLU	120	IP_5_100_90_516_CLU	SIG	20-21	13.696	4.51	11.203	4.36	nematocyst outer wall antigen precurs	0.077	
B07-IPL-P20	163	B07-IPL-P20	SIG	30-31	18.147	8.57	14.98	8.58	probable K5 antigen synthesis [Vibrio	3.4	
mys3	117	mys3	SIG	19-20	13.361	9.65	11.113	9.59	AGR_C_2052p [Agrobacterium tumefaci	0.13	
mys1	78	mys1	SIG	23-24	7.928	4.69	5.358	4.9	OSJNBa0086B14.5 [Oryza sativa (japon	0.35	polyGly tail - glue?
IP_5_100_90_411_CLU	45	IP_5_100_90_411_CLU	SIG	19-20	4.768	4.72	2.496	4.84	COG3451: Type IV secretory pathwa	2.5	HEAHEAHEA protein
IP_clu193A	59	IP_clu193A	SIG	22-23	7.007	9.56	4.117	7.92			
IP_clu62A	122	IP_clu62A	BL	19-20	13.805	9.65	11.717	9.83	predicted protein [Ustilago maydis 521] 31 4.2	4.2	Unknown, possible Dopachrome tautomerase precursor
A08_IPS_P16	124	A08_IPS_P16	SIG	23-24	13.221	5.78	10.597	5.28	keratin associated protein 18-7; ke	6E-005	very cys-rich - glue?
D03_IPM_P18	168	D03_IPM_P18	SIG	21-22	18.08	7.57	15.407	6.54	putative protein (4I100) [Caenorhab	0.003	
IPM-P3-B7	47	IPM-P3-B7	SIG	37-38	5.718	5.88	0.976	11	hypothetical protein Tb927.2.4250 [	1.5	
IP_5_100_90_511_CLU	99	IP_5_100_90_511_CLU	SIG	19-20	10.949	9.06	8.723	9.06	hypothetical protein MGC63561 [Dani	4E-025	unknown conserved protein
H04_IPM_P17	54	H04_IPM_P17	SIG	20-21	6.319	7.95	4.02	7.01	Hypothetical protein CBG15127 [Caeno	0.099	
E02_IPL_Pl_P20	225	E02_IPL_Pl_P20	SIG	23-24	25.358	5.02	22.54	4.92	cytotoxin [Bacteriophage phi CTX] >	6E-004	
mys2	205	mys2	SIG	17-18	23.744	8.33	21.659	8.65	similar to tenascin-N [Rattus norve	5E-020	
F07-IPL-P20	196	F07-IPL-P20	SIG	26-27	21.704	8.78	18.516	8.71	CG17035-PA [Drosophila melanogaster	2E-025	group XIV secreted phospholipase A2
Group 16 - Probably housekeeping proteins											

Seq name	Seq size	Link to nucleotide sequence	Sig P Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
Other possible housekeeping proteins											
IP_7_60_92_101_CLU	74	IP_7_60_92_101_CLU	CYT		7.937	6.71			CG32446-PA [Drosophila melanogaster	3E-014	Copper transport protein
IP_7_60_92_132_CLU	174	IP_7_60_92_132_CLU	CYT		20.028	4.71			CG3595-PA [Drosophila melanogaster]	5E-080	Myosin regulatory light chain
IPL_P4_H10	147	IPL_P4_H10	CYT		16.754	6.81			CG7013-PA [Drosophila melanogaster]	7E-047	ARMET-like protein precursor, truncated
IP_5_100_90_203_CLU	93	IP_5_100_90_203_CLU	CYT		10.641	9.45			CG7630-PA [Drosophila melanogaster]	0.030	unknown
IP_clu406	101	IP_clu406	CYT		10.876	8.96			heat shock protein 10 [Gallus gallu	8E-029	heat shock protein 10
IP_CLU3A	80	IP_CLU3A	CYT		8.731	9.03			hypothetical protein Magn027998 [	0.34	unknown
A10_IPL_P19_JIN	265	A10_IPL_P19_JIN	CYT		30.229	8.31			Isopentenyl-diphosphate delta-is	3E-063	Isopentenyl-diphosphate delta-isomerase
IP_7_60_92_136_CLU	232	IP_7_60_92_136_CLU	CYT		26.212	8.53			similar to Shwachman-Bodian-Diamond	9E-084	Shwachman-Bodian-Diamond syndrome homolog
IP_5_100_90_24_CLU	66	IP_5_100_90_24_CLU	CYT		7.315	11.8			unnamed protein product [Tetraodon n	1.8	
IP_7_60_92_79_CLU	66	IP_7_60_92_79_CLU	CYT		7.286	11.64			unnamed protein product [Tetraodon n	1.4	
Kunitz-containing intracellular proteins											
B05-IPL-P19	179	B05-IPL-P19	CYT		20.474	5.81			putative secreted protein [Ixodes sca	4E-072	Truncated peptide with Kunitz protease inhibitor domain
A06-IPM-P17	205	A06-IPM-P17	CYT		23.844	8.22			putative secreted protein [Ixodes sca	9E-028	Truncated peptide with Kunitz protease inhibitor domain
Ribosomal proteins											
IP_7_60_92_87_CLU	165	IP_7_60_92_87_CLU	CYT		17.812	9.3			CG3195-PA [Drosophila melanogaster]	1E-068	ribosomal protein
IP_clu78	123	IP_clu78	CYT		14.42	11.46			ribosomal protein L35 [Mus musculus	7E-046	ribosomal protein
IP_7_60_92_114_CLU	100	IP_7_60_92_114_CLU	CYT		11.44	11.96			60S ribosomal protein L37 >gnll	1E-037	ribosomal protein
IP_7_60_92_138_CLU	105	IP_7_60_92_138_CLU	CYT		12.515	10.55			ribosomal protein L44 [Chlamys farreri] 194 3e-049	3E-049	ribosomal protein
IP_7_60_92_78_CLU	268	IP_7_60_92_78_CLU	CYT		30.534	10.77			ribosomal protein L6 [Gallus gallus	7E-057	ribosomal protein
IP_clu307	90	IP_clu307	CYT		10.086	10.29			ribosomal protein L7a [Argopecten irr	8E-035	ribosomal protein
IP_7_60_92_88_CLU	112	IP_7_60_92_88_CLU	CYT		12.486	9.71			ribosomal protein L30 [Argopecten irr	1E-052	ribosomal protein
IP_7_60_92_98_CLU	269	IP_7_60_92_98_CLU	CYT		30.703	10.69			Unknown (protein for MGC:73183); wu	1E-113	ribosomal protein

Seq name	Seq size	Link to nucleotide sequence	SigP Result	Cleavage Position	MW	pI	Mature MW	pI	Best match to NR protein database	E value	Identification
Probably secreted proteins											
IP_7_60_92_83_CLU	151	IP_7_60_92_83_CLU	CYT		16.134	10.31			ribosomal protein S14; wu:fa92e08 [	8E-072	ribosomal protein
IP_7_60_92_142_CLU	149	IP_7_60_92_142_CLU	CYT		17.294	10.04			ribosomal protein S15 [Argopecten irr	2E-068	ribosomal protein
IP_7_60_92_475_CLU	25		CYT		3.483	12.61			ribosomal protein L41 [Mus musculus]	2E-006	ribosomal protein
IP_7_60_92_551_CLU	81	IP_7_60_92_551_CLU	CYT		9.027	10.69			ribosomal protein S4 [Argopecten irra	2E-032	ribosomal protein
IP_7_60_92_569_CLU	114	IP_7_60_92_569_CLU	BL		11.508	4.96			ribosomal protein, large P2 [Mus mu	3E-037	ribosomal protein
IP_7_60_92_99_CLU	133	IP_7_60_92_99_CLU	CYT		14.562	9.27			Finkel-Biskis-Reilly murine sarcoma	3E-036	ribosomal protein
IP_7_60_92_100_CLU	209	IP_7_60_92_100_CLU	CYT		23.295	9.76			40S ribosomal protein S5 [Dermacentor	1E-110	ribosomal protein
Oxidant metabolism											
IP_7_60_92_147_CLU	230	IP_7_60_92_147_CLU	CYT		26.173	5.17			putative glutathione S-transferase [D	1E-036	glutathione S-transferase
IP_7_60_92_113_CLU	220	IP_7_60_92_113_CLU	CYT		25.716	7.86			glutathione S-transferase [Boophilus	1E-080	glutathione S-transferase
Vacuolar sorting protein											
E05-IPL-P23	222	E05-IPL-P23	CYT		20.055	4.99			neuroendocrine differentiation factor	1E-073	Vacuolar sorting protein VPS24
Energy metabolism											
IP_7_60_92_95_CLU	109	IP_7_60_92_95_CLU	CYT		12.109	9.63			Cytochrome c >gnl|BL_ORD_ID|145934	8E-050	cytochrome c
IP_7_60_92_68_CYTOCHROME	139	IP_7_60_92_68_CYTOCHROME	CYT		17.498	5.5			cytochrome c oxidase subunit Va [Rhyz	5E-050	cytochrome c oxidase subunit Va
IP_7_60_92_109_CLU	73	IP_7_60_92_109_CLU	CYT		8.19	11.88			cytochrome oxidase subunit VIIc [Mac	5E-013	cytochrome oxidase subunit VIIc
IP_7_60_92_82_CLU	152	IP_7_60_92_82_CLU	CYT		15.585	10.03			ATP synthase c-subunit [Dermacentor v	1E-065	ATP synthase c-subunit
IP_7_60_92_128_CLU	134	IP_7_60_92_128_CLU	CYT		15.157	5.21			CG2140-PB [Drosophila melanogaster]	6E-036	cytochrome b5
IP_7_60_92_77_CLU	69	IP_7_60_92_77_CLU	CYT		7.494	10.29			ENSANGP00000013087 [Anopheles gambi	0.002	cytochrome c oxidase polypeptide VIII
H11-IPM-P18	182	H11-IPM-P18	CYT		19.869	9.57			CG9350-PA [Drosophila melanogaster]	2E-010	Probable NADH-ubiquinone oxidoreductase subunit