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Molecular characterization of an arachnid sodium channel gene from the varroa mite (*Varroa destructor*)

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Abstract

Voltage-gated sodium channels are essential for the generation and propagation of action potentials in most excitable cells. They are the target sites of several classes of insecticides and acaricides. Isolation of full-length sodium channel cDNA is a critical and often difficult step toward an understanding of insecticide and acaricide resistance. We previously cloned and sequenced two overlapping cDNA clones covering segment 3 of domain II (IIS3) to segment 6 of domain IV (IVS6) of an arachnid sodium channel gene (named *VmNa*) from the varroa mite (*Varroa destructor*) (J. Apicultureal Res. 40 (2002) 5.). In this study, we isolated three more overlapping cDNA clones and revealed the entire coding region of *VmNa* (Genbank accession number: AY259834), thus providing the first complete cDNA sequence of an arachnid sodium channel gene. The composite *VmNa* cDNA contains 6645 nucleotides with an open reading frame encoding 2215 amino acids. The deduced amino acid sequence of VmNa shares a 51% overall identity with *Drosophila* Para and a 41% identity with the mammalian sodium channel α-subunit $Na_v1.2$. All hallmarks of sodium channel proteins are conserved in the VmNa protein. Three optional exons and one retained intron were identified in *VmNa*. The precise position and size of only one exon is conserved in three insect sodium channel genes and mammalian $Na_v1.6$ genes in human, mouse and fish, whereas the other three are novel. Interestingly, one of the novel exons is located in the C-terminus, where no alternative exons have been identified in any other sodium channel gene.

Keywords: *Para*; Voltage-gated sodium channel; Alternative splicing; Varroa mite

1. Introduction

Voltage-gated sodium channels are integral transmembrane proteins responsible for the rapidly-rising phase of action potentials in most excitable cells. Many neurotoxins and drugs act on sodium channels and disrupt membrane excitability. Pyrethroid insecticides, for example, slow the kinetics of sodium channel activation and inactivation, resulting in the prolonged opening of individual channels and leading to paralysis and death of poisoned insects (Narahashi, 2000). Intensive use of pyrethroids in insect control has led to a worldwide emergence of resistant insect populations (Mota-Sanchez et al., 2002). Many of these resistant insects carry specific point mutations in the sodium channel gene (references in Soderlund and Knipple, in press). Functional assays using *Xenopus* oocyte-expressed insect sodium channels show that these mutations reduce sodium channel sensitivity to pyrethroids and therefore represent a major mechanism of pyrethroid resistance in diverse insect pest species (references in Soderlund and Knipple, in press).

The primary structure of insect sodium channel proteins is similar to that of mammalian sodium channel α-subunits, containing four homologous domains (I–IV) and six transmembrane segments (S1–S6) in each domain. The first insect sodium channel gene, *para*, was identified in *Drosophila melanogaster* (Loughney et al., 1989). Because of their intimate involvement in insect

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resistance to pyrethroid insecticides, *para*-orthologs were isolated from several medically or agriculturally important insect species (references in Soderlund and Knipple, in press). Moreover, several insect sodium channel proteins, e.g., *Drosophila* Para, housefly Vssc1 and cockroach Para^{CSMA} sodium channels, have now been functionally expressed in *Xenopus* oocytes (Feng et al., 1995; Warmke et al., 1997; Smith et al., 1997; Vais et al., 2000; Tan et al., 2002). These functionally expressed sodium channels are used successfully to study the interactions between sodium channels and pyrethroids and other toxins at the molecular level.

Pyrethroids are also a vital management tool for controlling arachnid pest species, such as the southern cattle tick (*Boophilus microplus*) and the varroa mite (*Varroa destructor*). Unfortunately, resistance to pyrethroids has already been reported in many arachnid populations (Mota-Sanchez et al., 2002). Previous studies of more than a dozen insect species revealed the presence of an L to F (or H, or S) mutation in IIS6 (L993F in the cockroach sodium channel) in pyrethroid-resistant populations (see refs. in Soderlund and Knipple, in press). However, this mutation is not detected in several strains of pyrethroid-resistant varroa mite (*Varroa destructor*) and southern cattle tick (*Boophilus microplus*) (He et al., 1999; Wang et al., 2002). Instead, four new mutations are associated with fluvalinate resistance in the varroa mite (Wang et al., 2002), and one mutation is associated with pyrethroid resistance in the southern cattle tick (He et al., 1999). One possibility is that insects and arachnids have evolved distinct mutations because of constraints exerted by the respective sodium channel sequence backbones. To understand the selection and evolution of possibly distinct pyrethroid resistance-sodium channel mutations in insects and arachnids, it is essential to examine the effects of insect and arachnid pyrethroid-resistance-associated mutations on functional properties of both native and heterologous sodium channels. However, an in vitro functional expression system is not yet established for any arachnid sodium channel. We previously cloned and sequenced two overlapping cDNA clones covering segment 3 of domain II (IIS3) to segment 6 of domain IV (IVS6) of a sodium channel gene (named *VmNa*) from the varroa mite (Wang et al., 2002). In this study, we isolated three more overlapping cDNA clones and revealed the entire coding region of *VmNa* (Genbank accession number: AY259834), thus providing the first complete cDNA sequence of an arachnid sodium channel gene. Furthermore, we discovered several novel alternative splicing exons of *VmNa*.

2. Materials and methods

Varroa mites (*Varroa destructor*) collected from the Michigan State University apiary were used to isolate total RNA and genomic DNA. Total RNA was isolated from about 50 varroa mites using RNA isolation kits (Gibco-BRL, Bethesda, MD), according to the manufacturer's instructions. For isolation of genomic DNA, 50 mites were homogenized in an extraction buffer (10 mM of Tris–Cl (pH 8.0), 0.1 M EDTA, 20 μg/ml RNAase, 0.5%SDS) using a glass–glass homogenizer. Proteinase K (100 μg/ml) was added to the homogenate. The mixture was then incubated in a water bath for 3 h at 50 °C. After phenol extraction twice, 0.2 volume of 3 M sodium acetate and 2 volumes of ethanol were added, followed by centrifugation at 5000 ***g*** for 5 min. Genomic DNA was resuspended in 500 μl of water.

First-strand cDNA was synthesized from 5 μg of total RNA using Superscript II RNase H$^-$ reverse transcriptase (Gibco-BRL, Bethesda, MD) in the presence of oligo(dT)$_{12\text{-}18}$ at 42 °C, or from 5 μg of total RNA using ThermoScript reverse transcriptase (Gibco-BRL, Bethesda, MD) in the presence of gene-specific primer 3 or 6 (Table 1) at 50 °C. Amplification of cDNA fragments by polymerase chain reaction (PCR) was performed in a 50 μl mixture, which contained 1 μl cDNA, 5 μl 10 × PCR buffer, 0.2 μM of each gene-specific primer or 0.4 μM of each degenerate primer, 200 μM each dNTP, 1.5 mM $MgCl_2$, and 2.5 U *Taq* polymerase. PCR was started in a thermocycler (GeneAmp® 2400, Applied Biosystems) with an initial denaturation at 94 °C for 2 min, followed by 35 cycles (30 s at 94 °C, 30 s at 58 °C for gene-specific primers or 53 °C for degenerate primers, and 2.5 min at 72 °C) of amplification, and a final cycle of 7 min at 72°C.

PCR products were separated by agarose gel electrophoresis, and desired DNA fragments were purified using the Prep-A-Gene kit (Bio-Rad, Hercules, CA) and cloned into pCR-TOPO using the TOPO® TA PCR cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Competent Top 10 Cells (Invitrogen) were used for transformation. Plasmid DNA was isolated using the Wizard® Plus Minipreps DNA Purification System (Promega, Madison, WI). Insert-positive clones were identified by restriction enzyme digestion. cDNA inserts were sequenced by primer walking at the W. M. Keck Laboratory at Yale University. Sequence data were analyzed using DNAStar (DNAStar Inc.).

For RACE (rapid amplification of cDNA ends) of 5′ and 3′ ends of the VmNa cDNA, first strand cDNA was synthesized from 5 μg total RNA using ThermoSeript™ reverse transcriptase at 50 °C in the presence of the Gibco-BRL adapter primer (AP) for 3′ RACE, or gene-specific antisence primer 9 (Table 1) at 55 °C for 5′RACE. In 5′ RACE, a poly-dC tail was added to the first-strand cDNA according to the manufacturer's instructions. In both 5′ and 3′ RACE, 2 μl of cDNA was used as a template in the PCR. Gibco-BRL's adapter-specific AP primer and gene-specific primer 12 (Table

Table 1
Oligonucleotide primers[a]

Primer#	Primer sequence (5′→ 3′)		Location in the VmNa protein (degenerate amino acid sequence or amino acid positions)
	Primers used to amplify cDNAs		
1	GTIATDATDGAYAAYTTAA	S	IIIS6 (VIIDNFN)
2	ATNACIGCDATRTACATRTT	AS	IVS6 (NMYIAVI)
3	GATCCCATTTTCTTCATTGCG	AS	IIIS6-IVS1(1749–1755)
4	GARGGNTGGAAYATHTTYGA	S	IIS3 (GWNIFDF)
5	CCTCCAGCTTTCTTCTTTTGTTC	AS	IIIS6-IVS1 (1727–1734)
6	GTTCTTGCCGAAAAGTTGTATGCC	AS	IIS5-IIS6 (1020–1027)
7	TGGAAYTGGYTIGAYTTYGT	S	IS3 (WNWLDFI)
8	TCGAAATGAACGCAACACAGAC	AS	IIS3-IIS4 (968–974)
	Primers used in RACE		
9	TCCGAGAAAGATAATGCGACAAAG	AS	IS6 (440–448)
10	GAAGGTGTCGAAGTTGGTGAAGC	AS	IS5-IS6 (398–405)
11	TCGATAACAGCGCCTACAATGGTC	AS	IS4-IS5 (295–303)
12	GTTTGCCCTCGCCATGTCTCTACC	S	IVS4-IVS5 (1900–1908)
13	ACGTCCGCCGGCTGGAGTGATGTC	S	IVS5-IVS6 (1962–1969)
	Primers used to amplify genomic DNA		
14	GAAGAAGATGTCGAAGCCAAATG	S	IS6-IIS1 (835–842)
15	ATTGTCGTTACTCGAGTTGGGTC	S	IIS3-IIS4 (954–961)
16	GTTCTTGCCGAAAAGTTGCATGCC	AS	IIS5-IIS6 (1020–1027)
17	AACGCGTGGTGTTGGCTCGAC	S	IIIS2-IIIS3 (1526–1533)
18	AAGCGTTCAAGACGATGAGAAC	S	IIIS4 (1555–1561)
19	GTTCTCATCGTCTTGAACGCT	AS	IIIS4 (1555–1561)
20	TGGCAGGAATGGCTTGCACTAGAG	AS	IIIS4-IIIS5 (1584–1592)
21	ACAAGTGATTCACATCGACCGGC	S	3′-terminus (2130–2137)
22	AGCGGGCGAGGGACGGATAACCAC	AS	3′-terminus (2196–2203)

[a] Designation of oligonucleotide mixtures: R = A+G; Y = C+T; S = G+C; H = A+C + T; N = A+G + C+T. S: sense primer; AS: antisense primer.

1) was used for 3′-RACE, and adapter-specific AAp primer and gene-specific primer 10 (Table 1) was used for 5′-RACE. cDNA fragments (Fig. 1) of the 5′ and 3′ ends were amplified in a second round of PCR using nested primers 11 and 13 (Table 1), respectively. The PCR was performed with the standard conditions described above.

Amplification of genomic DNA by PCR was performed using eLONGase (Invitrogen), which is suitable for amplification of long PCR fragments (up to 12 kb). The PCR mix contained 0.2 μg genomic DNA, 0.4 μM of each primer, 200 μM of each dNTP, and 1 U eLONGase. The PCR conditions were 30 cycles of 30 s at 94 °C, 30 s at 58 °C, and 10 min at 68 °C. The PCR products were isolated from agarose gel using the Prep-A-Gene kit for direct sequencing.

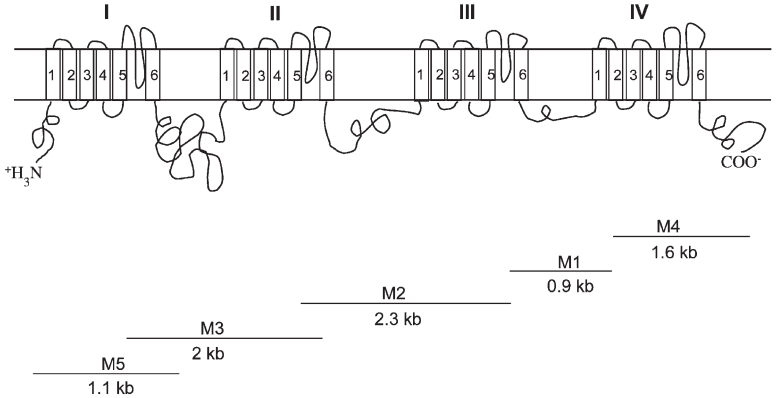


Fig. 1. Cloning of *VmNa* cDNAs. Top: a diagram of VmNa protein showing the four homologous domains (I-IV), each divided into six transmembrane segments (S1–S6). Clones M1, M2, M3 were isolated by RT–PCR, whereas clones M4 and M5 were isolated by RACE. The size of each fragment is indicated.

3. Results and discussion

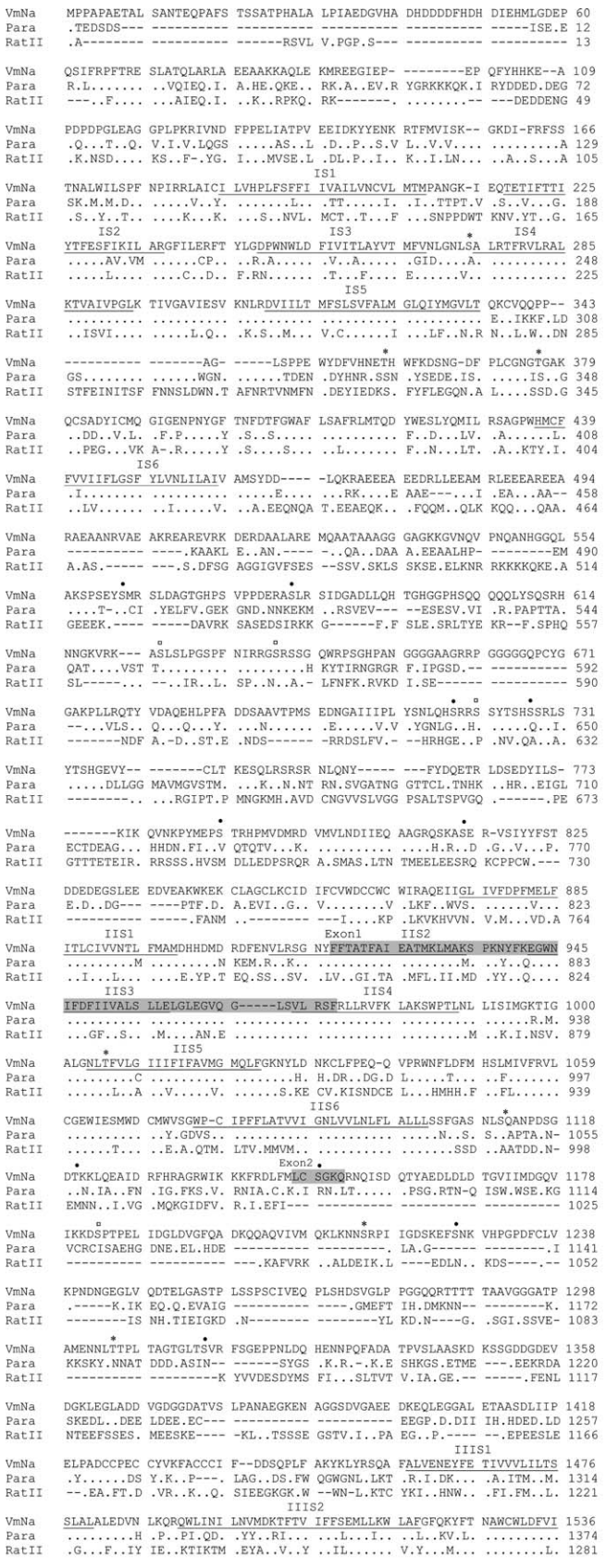
We previously reported the cloning and sequencing of two overlapping clones encoding IIS3 to IVS6 of *VmNa* (Wang et al., 2002) (Fig. 1). Briefly, a 0.9 kb fragment (M1), which covers IIIS6 to IVS5, was amplified using degenerate primers 1 and 2, designed based on the highly conserved amino acid sequences of sodium channel proteins from *D. melanogaster* (Loughney et al., 1989), *Blattella germanica* (Dong, 1997), and *Musca domestica* (Ingles et al., 1996; Williamson et al., 1996). A 2.3 kb fragment (M2) was amplified using degenerate sense primer 4 and gene-specific antisense primer 5 based on the conserved amino acid sequence in IIS3 of known sodium channel proteins and on the M1 fragment sequence, respectively. In this study, we aimed at defining the entire open reading frame (ORF) of *VmNa*. For this purpose, we amplified a 2 kb fragment (M3) using primers 7 and 8 (Fig. 1). Fragments M4 and M5 were obtained by 3′ and 5′ RACE, respectively. The M4 fragment contains 416 bps of the 3′ end of the *VmNa* coding region and 1184 bps of the 3′ untranslated region (UTR). The M5 fragment contains 895 bps of the 5′ end of the *VmNa* coding region and 205 bps of the 5′-UTR.

The composite *VmNa* cDNA contains 6645 nucleotides with an ORF encoding 2215 amino acids, which gives a calculated molecular mass of 254 kDa. The first ATG is assigned as the start codon because it is the first in-frame methionine codon in the ORF that yields a sodium channel-like amino acid sequence. The designated stop codon (TGA) is located at nucleotide 6645. A poly(A) tail was found at the 3′ end of the composite cDNA. However, we did not find an 'AATAAA' polyadenylation site in any of the *VmNa* cDNA sequences.

Alignment of the deduced amino acid sequence of VmNa with those of *Drosophila* Para and $Na_v1.2$ (Rat brain II) (Fig. 2) reveals an overall organization of four large hydrophobic domains, each composed of six membrane-spanning segments (Fig. 2). The deduced amino acid sequence of VmNa shares 51.2% and 41.1% overall identities with *Drosophila* Para and $Na_v1.2$, respectively. The transmembrane segments are well conserved (57.5–72.2%); so is the third intracellular linker connecting domains III/IV (65.5–87%). However, the first two intracellular linkers connecting domains I/II and II/III are more divergent (14.6–37.7%). VmNa shows the strongest similarity with a southern cattle tick sodium channel protein (He et al., 1999) in a region covering domains 3 and 4 and the third intracellular linker, with 80.7–98% identities at the amino acid level. The sequence of the remaining region of the cattle tick sodium channel gene is not available for comparison. There are four potential cAMP-dependent protein kinase (PKA) phosphorylation sites in the deduced VmNa protein. Three of them (Ser-623, Ser-637, and Ser-722) are in the first cytoplasmic linker, the forth (Ser-1183) is in the second cytoplasmic linker. A total of ten potential protein kinase C (PKC) sites are present in the first and second cytoplasmic linkers. In addition, there are twelve potential N-linked glycosylation sites.

The VmNa protein possesses all the hallmarks of a voltage-dependent sodium channel protein. First, the S4 transmembrane segments in all known sodium channel proteins contain 4–8 positively charged amino acids arranged at the every third position, serving as a voltage sensor that initiates voltage-dependent activation by presumably moving outward under the influence of the electric field (Catterall, 2000). This S4 feature is well conserved in the VmNa protein. Second, the highly conserved short intracellular linker (L3) connecting domains III and IV plays an important role in the fast inactivation of sodium channels (Catterall, 2000). An IFM motif in the linker L3 of $Na_v1.2$ is essential for fast inactivation (Catterall, 2000). An MFM tripeptide motif is present at the equivalent position in VmNa, *Drosophila* Para, and Para-orthologs. Third, all functional sodium channel proteins possess a characteristic DEKA signature (D, E, K, and A, respectively, in the putative pore positions in the short segment 2 connecting S5 and S6 of domains I– IV), which are critical for Na^+ ion selectivity (Catterall, 2000). The DEKA motif is completely conserved in the VmNa protein. The conservation of all these features strongly suggests that *VmNa* encodes a functional sodium channel protein.

In the course of analyzing the cDNA clones of the M1-5 fragments, we found length polymorphism in domains II and III, the intracellular linker connecting domains II and III, and the C-terminus. A stretch of 174 bps encoding part of IIS2-4 was missing in three of twenty M3 clones. An insertion of an 18 bp fragment in the linker connecting domains II and III was found in 13 of 48 M2 clones. Eighteen of 48 M2 clones lacked a stretch of 123 bps encoding IIIS3-4. In the C-terminus, we found an inclusion of 210 bps in one clone. No length polymorphism was detected in 58 M1 and 20 M5 clones. To confirm that the observed deletions or insertions of these short fragments were the result of alternative splicing, we determined the genomic organization of these regions by amplifying and sequencing the corresponding genomic DNA fragments using primers listed in Table 1 (Fig. 3). Primer pairs, 14/8 and 15/16 were used in PCR to amplify fragments containing two introns (1.2 and 1.7 kb) flanking exon 1. Fragments containing two introns (3.9 and 4 kb) flanking exon 3 were amplified using primer pairs 17/19 or 18/20. A 0.5 kb fragment containing a 210 bp retained intron was amplified using the primer pair 21/22. Comparison between cDNA and genomic DNA sequences revealed the exon/intron organization and confirmed that these short fragments are optional exons. The consensus splice donor and acceptor sequence, GT and AG, respectively, were



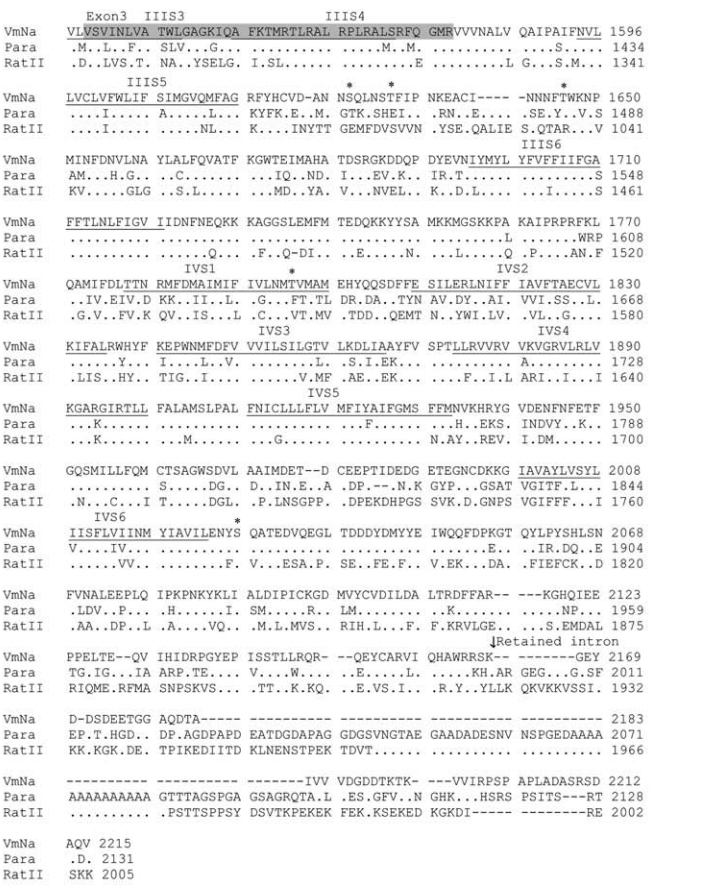


Fig. 2. Alignment of the deduced amino acid sequence of VmNa with *Drosophila* Para and Rat $Na_v1.2$ sodium channel proteins. Dots represent identical residues, and dashes indicate gaps introduced to obtain optimal alignment. Numbers on the right indicate the amino acid positions in each protein. The six transmenbrane segments (S1–S6) in each of four homologous domains (I–IV) are underlined. Predicted N-glycosylation sites (∗), PKA (o) and PKC (●) sites in the VmNa protein are indicated. Three optional exons (exon 1, 2 and 3) are highlighted and the position of a retained intron is indicated.

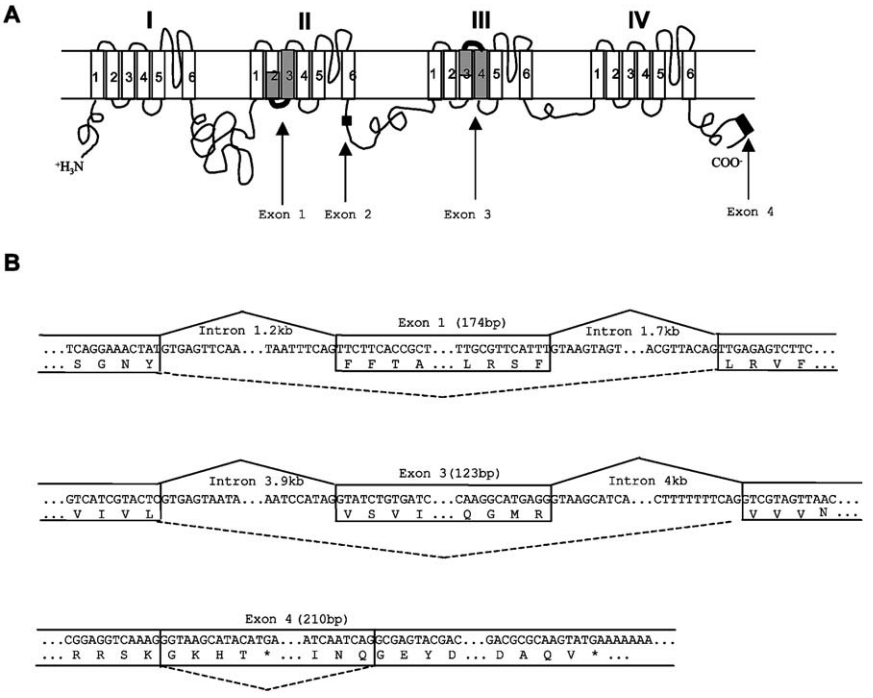


Fig. 3. Alternative exons in *VmNa*. A. Location of alternative exons in *VmNa*. B. Genomic organization of the regions where alternative exons are located. Exons are boxed and the sizes indicated. The consensus splice donor and acceptor sequences, gt/ag, at each exon/intron border are conserved. The exon 4 is a retained intron containing a premature stop codon.

present in the boundaries of all confirmed alternative exons.

Extensive alternative splicing is reported in *Drosophila para* as well as *para*-homologous sodium channel genes *vssc1* and *para*^{CSMA} (Thackeray and Ganetzky, 1994; Lee et al., 2002; Tan et al., 2002). A total of nine alternatively spliced exons are identified in *D. melanogaster para*, with seven exons (a, i, b, e, f, j and h) in the first or second intracellular linker, and two mutually exclusive exons, c or d, in domain II (Loughney et al., 1989; Thackeray and Ganetzky, 1994). Significantly, these alternative splice sites are conserved in the *D. virilis para* (Thackeray and Ganetzky, 1995) and the housefly sodium channel gene *Vssc1* (Lee et al., 2002). Most of the splice sites are also found in the cockroach *para*^{CSMA} gene (Liu, Nomura and Dong, unpublished data). However, three of the four alternative splice sites identified in VmNa in this study were novel and have not been identified in sodium channel genes. The unique exon 1 encoding IIS2-3 is two amino acids away from mutually exclusive alternative exons c/d in *para* that encode IIS4-5. The unique exon 2 is located in the second intracellular linker near IIS6 that contains a potential protein kinase C site. This site could modulate channel activity via phosphorylation, as demonstrated for several mammalian sodium channels (Catterall, 2000). The third unique exon is exon 4, a 210 bp retained intron. Interestingly, this alternative exon contains a stop codon at nucleotide 6705. Consequently, inclusion of this intron would produce a VmNa protein with a C-terminus 45 residues shorter than that of the full-size VmNa protein. A recent study showed that the C-terminal domain plays critical roles in sodium channel functions (Mantegazza et al., 2001). Therefore, alternative splicing at this site may significantly modulate VmNa function in vivo.

In contrast to the above three alternative exons, the fourth alternative exon, exon 3, is not unique to VmNa. The precise position and size of exon 3 are conserved in three insect sodium channel genes, and mammalian $Na_v1.6$ (Scn8a) and its human and fish counterparts (Tan et al., 2002; Plummer et al., 1997). Therefore, splicing in this region appears to be of a very ancient origin and was preserved in organisms ranging from arthropods to humans. Exon 3 in VmNa is optional, whereas the corresponding alternative exons in other sodium channel genes are mutually exclusive, such as mutually exclusive exons G1/G2/G3 in *para*^{CSMA}. One of the mutually exclusive exons, exon G3 in *para*^{CSMA} and exon 18N in $Na_v1.6$ genes, contains a pre-mature stop codon and would produce a truncated two-domain protein. The truncated Para^{CSMA} channel does not produce any sodium current when expressed in *Xenopus* oocytes (Tan et al., 2002). Interestingly, exclusion of exon 3 in *VmNa* could also generate a non-functional channel due to the absence of IIIS3-4. Apparently this splice event in *VmNa* occurs quite frequently; eighteen of the forty-eight M2 clones lack exon 3. In the German cockroach, the corresponding exon (G3) is detected only in the peripheral nervous system. The other two alternative exons

(G1/G2) were detected in both the peripheral and central nervous systems (Tan et al., 2002). Exon 18N in $Na_v1.6$ genes is expressed in fetal brain and non-neuronal tissues (Plummer et al., 1997). These truncated proteins may be biologically significant as evidenced by the wide conservation of this unique splice form in arachnid, insect, fish, rat and human.

In conclusion, we have succeeded in defining the entire ORF of a varroa mite sodium channel. The availability of the full-length VmNa provides a critical step toward a comprehensive functional characterization of arachinid sodium channels and an understanding of acaricide resistance to sodium channel insecticides.

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References

Catterall, W.A., 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26, 13–25.

Dong, K., 1997. A single amino acid change in the Para sodium channel protein is associated with knockdown-resistance (*kdr*) to pyrethroid insecticides in German cockroach. Insect Biochem. Mol. Biol 27, 93–100.

Feng, G., Deak, P., Chopra, M., Hall, L.M., 1995. Cloning and functional analysis of TipE, a novel membrane protein that enhances *Drosophila* Para sodium channel function. Cell 82, 1001–1011.

He, H., Chen, A.C., Davey, R.B., Ivie, G.W., George, J.E., 1999. Identification of a point mutation in the para-type sodium channel gene from a pyrethroid-resistant cattle tick. Biochem. Biophys. Res. Commun 261, 558–561.

Ingles, P.J., Adams, P.M., Knipple, D.C., Soderlund, D.M., 1996. Characterization of voltage-sensitive sodium channel gene coding sequences from insecticides-susceptible and knockdown-resistant house fly strains. Insect Biochem. Mol. Biol 26, 319–326.

Lee, S.H., Ingles, P.J., Knipple, D.C., Soderlund, D.M., 2002. Developmental regulation of alternative exon usage in the house fly *Vssc1* sodium channel gene. Invert. Neurosci 4, 125–133.

Loughney, K., Kreber, R., Ganetzky, B., 1989. Molecular analysis of the *para* locus, a sodium channel gene in *Drosophila*. Cell 58, 1143–1154.

Mantegazza, M., Yu, F.H., Catterall, W.A., Scheuer, T., 2001. Role of the C-terminal domain in in activation of brain and cardiac sodium channels. Proc. Natl Acad. Sci. USA 98, 15348–15353.

Mota-Sanchez, D., Bills, S.P., Whalon, M.E., 2002. Arthropod resistance to pesticides: status and overview. In: Wheeler, B. (Ed.), Pesticides in Agriculture and the Environment. Marcel Decker, Gainesville, FL, pp. 241–272.

Narahashi, T., 2000. Neuroreceptors and ion channels as the basis for drug action: past, present and future. J. Pharmacol. Exp. Ther. 294, 1–26.

Plummer, N.W., McBurney, M.W., Meisler, M.H., 1997. Alternative splicing of the sodium channel *CN8A* predicts a truncated two-domain protein in fetal brain and non-neuronal cells. J Biol. Chem. 272, 24008–24015.

Smith, T.J., Lee, S.H., Ingles, P.J., Knipple, D.C., Soderlund, D.M., 1997. The L1014F point mutation in the house fly *Vssc1* sodium channel confers knockdown resistance to pyrethroids. Insect Biochem. Mol. Biol. 27, 807–812.

Soderlund D.M. and Knipple D.C., in press. The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem. Molec. Biol.

Tan, J., Liu, Z., Nomura, Y., Goldin, A.L., Dong, K., 2002. Alternative splicing of an insect sodium channel gene generates pharmacologically distinct sodium channels. J. Neurosci. 22, 5300–5309.

Thackeray, J.R., Ganetzky, B., 1994. Developmentally regulated alternative splicing generates a complex array of *Drosophila para* sodium channel isoforms. J. Neurosci. 14, 2569–2578.

Thackeray, J.R., Ganetzky, B., 1995. Conserved alternative splicing patterns and splicing signals in the *Drosophila* sodium channel gene *para*. Genetics 141, 203–214.

Vais, H., Williamson, M.S., Goodson, S.J., Devonshire, A.L., Warmke, J.W., Usherwood, P.N.R., Cohen, C., 2000. Activation of *Drosophila* sodium channels promotes modification by deltamethrin: reductions in affinity caused by knock-down resistance mutations. J. Gen. Physiol. 115, 305–318.

Wang, R., Liu, Z., Dong, K., Elzen, P., Pettis, J., Huang, Z.Y., 2002. Association of novel mutations in a sodium channel gene with fluvalinate resistance in the mite, *Varroa destructor*. J. Apicultural Res. 40 (17-2), 5.

Warmke, J.W., Reenan, R.A.G., Wang, P., Qian, S., Arena, J.P., Wang, J., Wunderler, D., Liu, K., Kaczorowski, G.J., Van der Ploeg, L.H.T., Ganetzky, B., Cohen, C.J., 1997. Functional expression of *Drosophila para* sodium channels: modulation by the membrane protein tipE and toxin pharmacology. J. Gen. Physiol. 110, 119–133.

Williamson, M.S., Martinez-Torres, D., Hick, C.A., Devonshire, A.L., 1996. Identification of mutations in the housefly *para*-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. Mol. Gen. Genet. 252, 51–60.