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An evolutionarily-unique heterodimeric voltage-gated cation channel found in aphids

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ABSTRACT

We describe the identification in aphids of a unique heterodimeric voltage-gated sodium channel which has an atypical ion selectivity filter and, unusually for insect channels, is highly insensitive to tetrodotoxin. We demonstrate that this channel has most likely arisen by adaptation (gene fission or duplication) of an invertebrate ancestral mono(hetero)meric channel. This is the only identifiable voltage-gated sodium channel homologue in the aphid genome(s), and the channel's novel selectivity filter motif (DENS instead of the usual DEKA found in other eukaryotes) may result in a loss of sodium selectivity, as indicated experimentally in mutagenised Drosophila channels.

1. Introduction

Voltage-gated sodium channels (Na_vs) mediate the rising phase of the action potential in the majority of innervated metazoans. Due to this critical role in neurotransmission the channels are invariably highly conserved across phyla. Eukaryotic Na_vs are multi-domain proteins, consisting of four non-identical domains (DI-DIV), with each domain comprising six transmembrane (TM) segments (S1–S6) containing a voltage sensor (S1–S4) and a membrane-spanning pore region (S5–S6). Typically, their ion (Na^+) selectivity filter consists of one loop from each domain (located between S5 and S6 of the pore region) which collectively form a 'DEKA' (DI-aspartate; DII-glutamate; DIII-lysine; DIV-alanine) amino acid sequence motif at the entry to the pore. In evolution terms, Na_vs are considered to be the most recent members of a large family of ion channels that includes voltage- and ligand-gated K^+ channels, Ca^{2+} channels and several non-selective channels [1]. Prokaryotic channels, which are the likely progenitors, consist of single domain polypeptides that self-assemble to form functional tetrameric channels. These have some striking similarities to vertebrate monomeric four domain (4x6TM) Na_v and Ca_v channels, so it is likely that the multi-domain channels arose by multiple cycles of gene duplication and fusion from an ancestral single-domain protein [2] (Fig. 1). Contemporary Na_v channels are thought to have evolved from a family of channels called Na_v2 [3] which, because of their unique pore sequence (DKEA or DEEA), preferentially conduct Ca^{2+} [4]. Although this family of cation channels has apparently been lost in vertebrates, they can still be found in invertebrates [5].

Higher order metazoans typically contain multiple genes encoding 4x6TM Na_v channels with different characteristics and functions [6]. These isoforms can be differentiated pharmacologically based on their sensitivity to the pore blocking toxin, tetrodotoxin (TTX). In contrast, all insects thus far studied have only one 4x6TM Na_v gene, with alternative splicing of exons imparting functional variability [7] and all channel variants exhibiting high

Abbreviations: BLAST, basic local alignment search tool; bp, base pair; cDNA, DNA complementary to RNA; DDT, dichlorodiphenyltrichloroethane; kb, kilobase(s) or 1000 base pairs; MYA, million years ago; Na_v, voltage-gated sodium channel; NCBI, The National Center for Biotechnology Information; PCR, polymerase chain reaction; RACE, Rapid Amplification of cDNA ends; RNA, ribonucleic acid; TM, trans membrane; TTX, tetrodotoxin

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sensitivity to TTX. The 4x6TM Na_vs also characteristically bind modulatory drugs and other neurotoxins including local anaesthetics, voltage-sensor disabling scorpion toxins and insecticides such as DDT and the synthetic pyrethroids [8].

It was unexpected when genome annotation predictions for the pea aphid, *Acyrthosiphon pisum* (Harris) [9] identified two genes encoding putative $ApNa_v1$ sequences, correlating with LOC100158802 (NCBI accession XP_008183364.1) encoding DI and DII and LOC100164620 (accession XP_001949648.2) encoding DIII and DIV. This suggested that the pea aphid has a two subunit channel. In this study, we further analysed this genomic data and sought corroborating evidence for the existence of a two subunit channel in aphids that reprises the role of a multi-domain Na_v1 as found in other insect species. This led us to the identification of an evolutionarily-unique heterodimeric voltage-gated cation channel in aphids.

2. Results

The preliminary data described above suggested that the *A. pisum* Na_v channel is encoded by, and assembled from, two unique 2x6TM heteromers (Fig. 2a), here designated as H1 and H2. On closer analysis of the genomic data we established that the two putative genes are orientated in opposite directions on scaffold 318, separated by approximately 23 Kb of non-coding sequence (Fig. 3). H1 has two identifiable alternative exons corresponding to exons j and b in the *Drosophila melanogaster* $DmNa_v1$ (para) gene [7], but unusually the mutually exclusive c/d splice variants ($DmNa_v1$ residues 923–976 (Fig. 4)) are absent. Furthermore, within this highly conserved region of the channel, *A. pisum* has an isoleucine (an atc codon) at position 946 (numbering according to the $DmNa_v1$ sequence), whereas in other insects exon c has a valine (g/tn) and exon d a methionine (at/g), suggesting that the *A. pisum* channel may be derived from an ancestral Na_v lacking the exon duplication found in contemporary insect Na_v1s. Within H2, exons 6 and 7 correspond to the mutually-exclusive exons k/l in DIII of insect Na_v1s (Supplementary Fig. 1).

To determine if the predicted heterodimeric organisation of the $ApNa_v1$ is unique to *A. pisum*, we used degenerate PCR and RACE to amplify corresponding full-length Na_v1 cDNAs from the closely related and agriculturally important pest aphid, *Myzus persicae* (Sulzer). The *M. persicae* channel was also found to be encoded by two genes (submitted NCBI Accessions FN601405 and FN601406), organized identically to those of the *A. pisum* gene prediction; the only exception being that the cDNA for H1 does not incorporate alternative exon j. The *M. persicae* and *A. pisum* H1 and H2 sequences are highly conserved at the amino acid level and have high (64% (DI–DII); 68% (DIII–DIV)) amino acid identity with equivalent domains in *D. melanogaster* $DmNa_v1$ channels (Supplementary Fig. 1). For both aphids the heteromers encode the full set of positively charged residues in the S4 helices of the voltage-sensing domains required to sense changes in membrane potential and initiate channel activation (Supplementary Fig. 2). The conserved tripeptide 'MFM' motif, unique to invertebrate Na_v1s, which forms the fast inactivation particle in the intracellular loop between DIII and DIV [10], is present in H2 only, as are the adjacent charged residues that modulate fast inactivation [11]

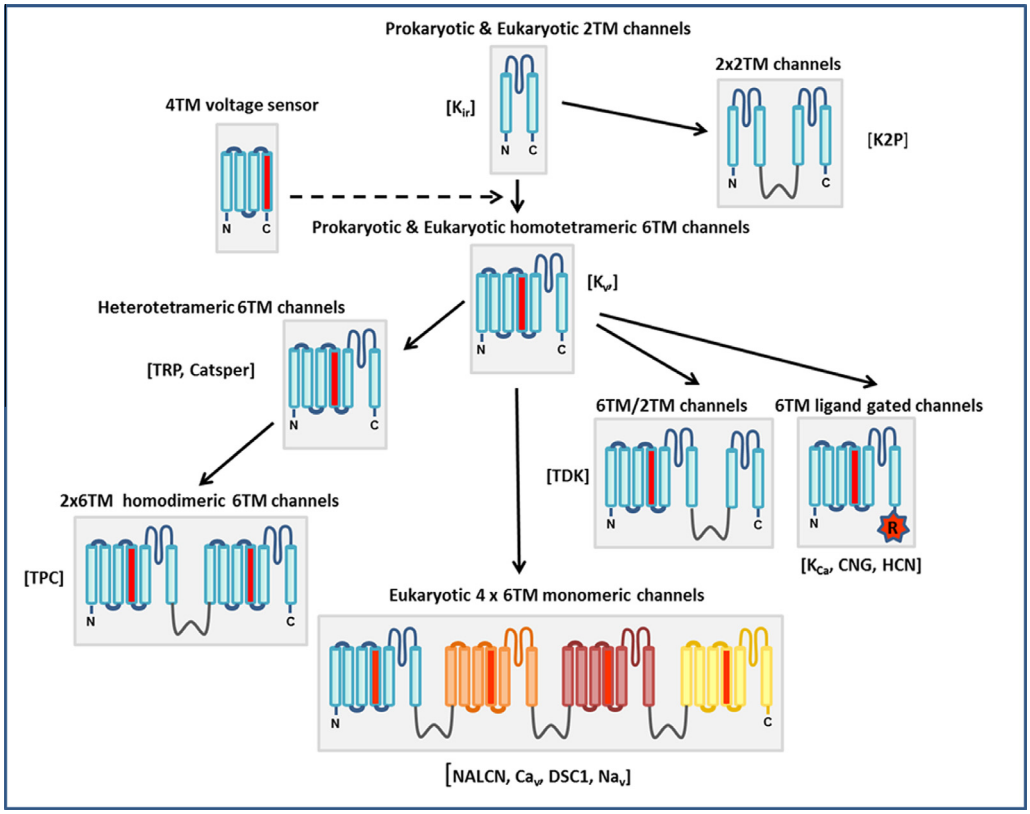

Fig. 1. Scheme of the proposed evolutionary relationships between members of the voltage-gated ion channel family. The inwardly rectifying K^+ channels (K_{ir}) represent the simplest structural motif in the ion channel superfamily in eukaryotes; their 2TM structure is highly representative of the pore domain found in many ancestral prokaryotic and eukaryotic channels. In the majority of eukaryotic channels, the 2TM motif has been augmented by 4 additional TM segments comprising a voltage-sensing domain (similar to that in the proton channel H_v1). Thus K_v channels (and prokaryotic Na_vs) are homo-tetrameric assemblies of 6TM subunits. It is currently thought that the single-domain 6TM K_v channels underwent two rounds of internal gene duplication leading to multi-domain Na_vs and Ca_vs, which resulted in superior kinetics and modulation of channel activation, inactivation, and recovery from inactivation. Two-repeat 6TM homodimeric channels, which may be an evolutionary intermediate, exist in the two-pore channel (TPC) family of Ca^{2+}-permeable channels [51].