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MOLECULAR CHARACTERIZATION AND FUNCTIONAL EXPRESSION OF THE DSC1 CHANNEL

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Abstract

Drosophila Sodium Channel 1 (DSC1) was predicted to encode a sodium channel based on a high sequence similarity with vertebrate and invertebrate sodium channel genes. However, *BSC1*, a *DSC1* ortholog in *Blattella germanica*, was recently shown to encode a cation channel with ion selectivity towards Ca^{2+} . In this study, we isolated a total of 20 full-length cDNA clones that cover the entire coding region of the *DSC1* gene from adults of *D. melanogaster* by reverse transcription-polymerase chain reaction. Sequence analysis of the 20 clones revealed nine optional exons, four of which contain in-frame stop codons; and 13 potential A-to-I RNA editing sites. The 20 clones can be grouped into eight splice types and represent 20 different transcripts because of unique RNA editing. Three variants generated DSC1 currents when expressed in *Xenopus* oocytes. Like the BSC1 channel, all three functional DSC1 channels are permeable to Ca^{2+} and Ba^{2+} , and also to Na^{+} in the absence of external Ca^{2+} . Furthermore, the DSC1 channel is insensitive to tetrodotoxin, a potent and specific sodium channel blocker. Our study shows that *DSC1* encodes a voltage-gated cation channel similar to the BSC1 channel in *B. germanica*. Extensive alternative splicing and RNA editing of the *DSC1* transcripts suggest the molecular and functional diversity of the DSC1 channel.

Keywords

Drosophila Sodium Channel 1; RNA editing; alternative splicing; voltage-gated ion channel

1. Introduction

Voltage-gated ion channels are a diverse group of integral transmembrane proteins that are essential for electrical signaling in neurons, muscle and endocrine cells. In response to changes in membrane potential, these channels mediate rapid ion flux through a highly selective pore. Voltage-gated sodium channels are responsible for inward movement of sodium ions during electrical signaling in cell membranes. Sodium channels consist of a large α -subunit and a variable number of smaller subunits (Yu et al., 2005). The α -subunit

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forms the ion conducting pore while the associated subunits modulate channel expression and gating. The pore-forming α -subunit consists of four repeated homologous domains (I–IV), each having six membrane spanning segments (S1–S6). The S1–S4 segments serve as the voltage sensing module and the S5 and S6 segments and their connecting P loop serve as a pore-forming module (Catterall, 2000).

Analysis of the genome of *Drosophila melanogaster* revealed only two sodium channel-like sequences, *para*, and *DSC1* (*Drosophila Sodium Channel 1*) (Littleton and Ganetzky, 2000). The *para* gene was isolated based on temperature-sensitive paralysis phenotype; and encodes a protein having all of the structural hallmarks of voltage-gated sodium channel α subunits and exhibiting approximately 50% overall amino acid sequence identity to vertebrate sodium channel α subunits (Loughney et al., 1989). It is well-established that *para* and *para*-orthologs in other insect species encode functional sodium channels (Feng et al., 1995; Warmke et al., 1997; Soderlund, 2005; Dong, 2007). Extensive alternative splicing and RNA editing of the transcripts of *para* and *para*-orthologs generate molecular and functional diversity of sodium channels in insects (Loughney et al., 1989; Thackeray and Ganetzky, 1994; O'Dowd et al., 1995; Thackeray and Ganetzky, 1995; Tan et al., 2002; Liu et al., 2004; Song et al., 2004; Dong, 2007; Olson et al., 2008; Lin et al., 2009).

DSC1 was first discovered two decades ago by probing a *Drosophila* genomic DNA library with an eel sodium channel cDNA (Salkoff et al., 1987). It was predicted to encode a sodium channel based on its overall similarity in deduced amino acid sequence and domain organization with eel and mammalian sodium channels (Salkoff et al., 1987; Littleton and Ganetzky, 2000). Sodium currents in embryonic neurons from homozygotes for a chromosome deficiency at 60E5/6, where the *DSC1* gene is located, were similar to those from wild-type neurons, indicating that *DSC1* is not the primary sodium channel gene in embryonic neurons (Germeraad et al., 1992). A *P*-element insertion in the second intron of *DSC1* in a *smell-impaired* (*smi*) mutant, *smi60E*, reduced *DSC1* transcript level by two-fold and results in a three-fold decrease in olfactory response to benzaldehyde (Kulkarni et al., 2002), suggesting that the *DSC1* channel plays an important role in olfaction. Comparative studies revealed different spatial and temporal expression patterns of *DSC1* and *para* transcripts and proteins, particularly in the adult peripheral nervous system, suggesting different functions of *DSC1* and *Para* channels in the nervous system. For example, in adults, expression patterns of *DSC1* and *para* transcripts overlap in the CNS based on the RNA *in situ* hybridization study (Hong and Ganetzky, 1994). However, an immunohistochemical study indicated that *DSC1* channels were only distributed in synaptic regions and axonal tracts while *Para* channels were also localized on cell bodies in the PNS. Only weak *DSC1* signal could be detected from compound eyes where the expression level of *Para* channels is high. The dense localization of the *DSC1* protein was detected in the nerve endings of motor neurons in the thorax, leg muscles and also in the proboscis, tibia and tarsi, where the mechanoreceptors or chemosensory organs are located (Castella et al., 2001).

No functional analysis of the *DSC1* channel has been reported. However, a *DSC1* ortholog, *BSC1* from *Blattella germanica*, was confirmed to encode a novel Ca^{2+} -selective cation channel, not a sodium channel, when expressed in *Xenopus* oocytes (Zhou et al., 2004). The first complete cDNA sequence of *DSC1* was reported based on the genomic sequence of a 20 kb genomic region where the *DSC1* gene resides; and the RT-PCR analysis of partial cDNAs (Kulkarni et al., 2002). Subsequently, with the completion of *Drosophila* genome sequencing, another cDNA sequence of *DSC1* (NM_166696.2) was deduced from an annotated genomic sequence (NT_033778.3). However, there were substantial sequence discrepancies between the two reported cDNA sequences. To elucidate the molecular and functional characterization of *DSC1*, we isolated 20 full-length cDNA clones covering the

entire coding region of the *DSC1* gene from adults of *D. melanogaster*. Sequence analysis revealed that *DSC1* transcripts undergo extensive alternative splicing and potential RNA editing. Functional expression in *Xenopus* oocytes confirmed that, like *BSC1*, *DSC1* encoded a Ca^{2+} -selective cation channel.

2. Materials and methods

2.1. Amplification and cloning of the coding region of the *DSC1* gene by reverse transcription-polymerase chain reaction (RT-PCR)

Five to seven day-old *w¹¹¹⁸* adults were used to isolate total RNA. First-strand cDNA was synthesized using Oligo dT primers (Invitrogen, Carlsbad, CA), and the SuperScript[®] II reverse transcription kit (Invitrogen, Carlsbad, CA). Conditions for the first-strand cDNA synthesis reaction were: 42°C for 2 min followed by a 60 min incubation at 48°C. RNA was removed by a 20 min incubation with RNaseH at 37°C. The PCR primers for amplification of the cDNA were, 5'-CCGCTCGAGGCCACCATGGGTGATGATCAAGCGACG-3', and 5'-TACCCCTAGGAAATATCAGATAGAAAGTTC-3'. To improve the expression efficiency, a Kozak sequence (GCCACCATGGGT) was added to the forward primer and the first nucleotide of the second codon was substituted from A to G to meet the sequence requirement. The *Xho* I and *Avr* II restriction enzyme sites (underlined) were added to the forward and reverse primer, respectively, to facilitate the cloning of the PCR product into a modified pGH19 vector in which an *Xho* I restriction site was removed by site-directed mutagenesis. The original pGH19 (a *Xenopus* expression vector containing the 3' and 5' untranslated regions of the *Xenopus* β -globin gene at the 3' end of T7 promoter and 5' end of SP6 promoter, respectively) was kindly provided by Barry Ganetzky, University of Wisconsin, Madison, WI. The PCR reaction conditions were the same as previously described (Zhou et al., 2004). The reaction mixture (50 μ l) contained 0.5 μ l cDNA, 50 pmol each primer, 200 mM each dNTP, 1 U eLONGase (Invitrogen), 1.5 mM MgCl_2 , and 1 $\times$ PCR reaction buffer. PCR was carried out as follows: one cycle of 94°C for 1 min; 33 cycles of 94°C for 30 s, 58°C for 30 s, 68°C for 8 min; and one cycle of 68°C for 15 min. The PCR products were purified using the QIAEX II Gel Extraction Kit[®] (QIAGEN Sciences, MD) and cloned into the modified pGH19. MAX Efficiency Stbl2-competent cells (Invitrogen) were used as host cells.

The inserts of *DSC1* cDNA clones were sequenced by moderate throughput sequencing (ABI 3730xl, Research Technology Support Facility, Michigan State University). Sequence data were analyzed using software Lasergene 6[®] (DNASTAR, Inc., Madison, WI).

2.2. Expression of *DSC1* channels in *Xenopus* Oocytes

Procedures for oocyte preparation and cRNA injection were similar to those described by Tan et al. (Tan et al., 2002). Oocytes were obtained surgically from healthy female *Xenopus laevis* (*Xenopus* I, Ann Arbor, MI) and incubated with 1 mg/ml type IA collagenase (Sigma Inc. St. Louis, MO) in a Ca^{2+} -free ND-96 medium, which contains 96 mM NaCl, 2 mM KCl, 1 mM MgCl_2 , and 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5. Follicle cells remaining on the surface of oocytes were removed manually with forceps. Isolated oocytes were incubated in ND96 culture medium containing 1.8 mM CaCl_2 supplemented with 50 μ g/ml gentamicin, 5 mM pyruvate, and 0.5 mM theophylline (Goldin, 1992). Healthy stage V–VI oocytes were used for cRNA injection. To prepare *DSC1* cRNA, plasmid DNA containing full-length *DSC1* cDNA was linearized with NotI (New England Biolab, Inc., Ipswich, MA), which does not cut the insert, followed by *in vitro* transcription with T7 polymerase using the mMACHINE mMESSAGE Kit (Ambion, Inc., Austin, TX). Each oocyte was injected with 15 ng *DSC1* cRNA. After injection, oocytes were incubated in ND-96 culture solution containing 96 mM NaCl, 2 mM KCl, 1.8

mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, 2.5 mM Na-pyruvate, 0.5 mM Theophylline for 2–3 days before recording.

2.3. Electrophysiological recording

DSC1 currents were recorded using standard two-electrode voltage clamp technique. The borosilicate glass electrodes were filled with filtered 3 M KCl in 0.5% agarose and had resistance at 4–6 MΩ. Currents were measured using the oocyte clamp instrument OC725C (Warner Instrument Corp., Hamden, CT), Digidata 1200A interface (Axon Instrument, Inc., Foster City, CA), and pCLAMP 8.2 software (Axon Instrument, Inc., Foster City, CA). External recording solutions containing only Na⁺, Ca²⁺ or Ba²⁺ were used to record currents in oocytes expressing the DSC1 channel as described in Zhou et al., (2004). The Na⁺ recording solution consisted of 50 mM NaOH, 45 mM TEAOH, 10 mM HEDTA, and 10 mM HEPES. The Ca²⁺ solution consisted of 50 mM Ca(OH)₂, 55 mM TEAOH, and 10 mM HEPES. The Ba²⁺ solution consisted of 50 mM Ba(OH)₂, 55 mM TEAOH, and 5 mM HEPES. All recording solutions were adjusted to pH 7.0 with methanesulfonic acid. For the relative permeability, the recording protocol consisted of a 15-ms depolarization from –100 mV to 80 mV followed by repolarization to –100 mV (Zhou et al., 2004). All experiments were performed at room temperature (22°C–25°C). For the application of tetrodotoxin (TTX), the disposable perfusion system developed by Tatebayashi and Narahashi (1994) was used. The TTX working solutions were diluted from a stock solution (2 mM) using Ba²⁺ external recording solution.

3. Results

3.1. Comparison of deduced amino acid sequences among DSC1, BSC1 and Para proteins

A total of 20 *DSC1* cDNA clones that cover the entire coding region of the *DSC1* gene were isolated by RT-PCR using total RNA isolated from adults of *D. melanogaster*. The nucleotide sequence of one of the clones, DSC1-1.1, has been deposited in GenBank (GenBank ID: DQ466888.1) and its deduced amino acid sequence is presented in Figure 1 in comparison with those of BSC1 and Para. The overall topology of the DSC1 channel is similar to that of BSC1 and Para channels: four homologous domains each having six transmembrane segments. The DSC1-1.1 protein shares 52% identity in the overall sequence with BSC1, and 29% with Para. The highest sequence identities are found in the transmembrane domains with 83% – 90% between DSC1-1.1 and BSC1 proteins, and 48% – 54% between DSC1-1.1 and Para proteins. The sequence identities of the first intracellular linker connecting domains I and II and the second linker connecting domains II and III are less than 30% between DSC1-1.1 and BSC1 proteins. The second linker of the DSC1-1.1 channel is much longer (838 amino acids) than those of BSC1 and Para, which have 330 and 257 amino acids, respectively. The linker connecting domains III and IV is highly conserved between DSC1-1.1 and BSC1 (90% identity) or Para (52% identity). It is well established that this linker, particularly the IFM (mammals) or MFM (insects) motif in the middle of the linker, is critical for fast inactivation of sodium channels (Catterall, 2000). Corresponding to the MFM motif in Para, the DSC1-1.1 and BSC1 channels have “VFL” and “MFL,” respectively. The “V” in the VFL motif is the result of an A-to-I editing causing an M to V change (Hoopengardner et al., 2003; and also in our study, see Table 1). Therefore, the conventional motif in the DSC1 channel is also “MFL” like in the BSC1 channel.

The ion selectivity of voltage-gated sodium channels is determined by the amino acids D, E, K, and A in the pore positions of domains I, II, III, and IV, respectively (i.e., the selectivity-filter motif “DEKA”) (Catterall, 2000). However, both the DSC1 and BSC1 channels contain the DEEA motif instead of the DEKA motif. The positively charged residues in the voltage sensor S4 of each domain that are critical for the gating of sodium channels

(Catterall, 2000). The number of the positively charged residues in IS4 and IVS4 is conserved among Para and DSC1/BSC1 (Fig. 1). However, DSC1 and BSC1 channels have an extra positively charged residue in IIS4 compared to the Para channel. Furthermore, DSC1-1.1 has four positively charged residues in IIS4, whereas five in BSC1 and Para channels. These two differences are conserved in all 20 DSC1 variants.

3.2. Sequence comparison of 20 DSC1 cDNA clones reveals extensive alternative splicing of *DSC1* transcripts

Sequence comparison of the 20 cDNA clones with the *DSC1* genomic sequence (NT_033778.3) revealed 20 constitutive exons, exons 1 to 20 (Fig. 2A); and nine optional exons, 4A, 11A, 11B, 11C, 16A, 17A, 17B, 17C, and 19A (named after the constitutive exons upstream) (Fig. 2A and 2B). Most of the optional exons have the consensus GT and AG sequences at the 5' donor and 3' acceptor sites, respectively, except for 11A and 11B which do not have the GT sequence. The size of the optional exons ranges from 15 bp to 861 bp. Exon 11B is 15 bp (5'-ATTTTCAAGAAGAAG-3') long. The nucleotide sequences of other longer optional exons are deposited in GenBank (GenBank ID: HM348600.1-348607.1). Exon 4A encodes part of the loop connecting S5 and S6 in domain I. Exons 11A, 11B, and 11C are three tandem exons that encode part of the linker connecting domains II and III. Exons 17A, 17B, and 17C are another group of tandem exons encoding part of the linker connecting domains III and IV as well as part of IVS1. Exon 16A encodes part of the loop connecting S5 and S6 in domain III. Exon 19A encodes a sequence in the C-terminal region. Intriguingly, exons 4A, 11A, 16A, and 17A contain in-frame stop codons (Fig. 2B). Inclusion of these exons in *DSC1* transcripts creates truncated proteins.

Based on optional exon usage, the 20 cDNA clones can be grouped into eight unique splice types, DSC1-1 to DSC1-8 (Fig. 2C). The six cDNA clones in splice type 1 are designated as DSC1-1.1 to DSC1-1.6 because each clone possesses several unique amino acid changes (see below). For the same reason, the five clones in splice type 2 are named DSC1-2.1 to DSC1-2.5. Two optional exons, 17B and 19A, are present in both splice type 1 and 2 variants (Fig. 2C). Splice type 2 variants also have a third optional exon, 11B. A total of seven cDNA clones in splice types 3, 4, 5, 6 and 9 are predicted to encode truncated proteins because of inclusion of stop codon-containing alternative exons (Fig. 2B and 2C). DSC1-3 and DSC1-4 variants would lack domain IV and the C-terminus. DSC1-5, DSC1-6 and DSC1-9 variants contain only the N-terminus plus domain I.

3.3. Identification of potential RNA editing

Comparing the deduced amino acid sequences of the 20 variants with the cDNA sequence (NM_166696.2) deduced from an annotated genomic sequence (NT_033778.3), we found 9 to 27 scattered amino acid changes in individual variants due to nucleotide changes. Because we isolated these full-length clones by RT-PCR, some of nucleotide changes could be the results of PCR mistakes. However, if a nucleotide change occurs in two or more variants that belong to different splice types, such a change is likely due to RNA editing, not a PCR mistake. 13 A to G changes, three T to C changes and one C to T change were detected in more than one variant, resulting in a total of 17 amino acid changes. The rest of the nucleotide changes were found only in individual variants. Therefore, the 13 amino acid changes are likely caused by A-to-I RNA editing and the other four are likely due to U-to-C or C-to U editing (Table 1). Most of the editing sites are in the linkers connecting domains or the short loops connecting segments. One A-to-I editing resulting in M2027V change in the linker connecting domains III and IV was previously reported in *DSC1* transcripts (Hoopengardner et al., 2003). We found this editing event in nine variants including DSC1-1.1.

Nucleotide substitutions introduced premature stop codons in four clones, two of which also contain stop codon-containing exons. The DSC1-7.1 variant contains an insertion of a 19 bp sequence (5'-CTCAACGTAAAAAACTAA-3') at the N-terminus. Surprisingly, the 19-bp sequence was part of exon 6 which encodes part of the linker connecting domains I and II. Somehow the 19 bp sequence was duplicated into the N-terminus. This insertion causes a frame shift in the downstream sequence, resulting in a premature stop codon. Therefore, a total of 10 variants contain premature stop codons. In addition, a six nucleotide sequence, CCGTCT, was found in all 20 cDNA clones, but not in the genomic DNA sequence (NT_033778.3). This difference might be caused by sequence polymorphisms between fly lines used in the genome sequence project (*y; cn bw sp; +; +*) and our study (*w¹¹¹⁸*).

3.4. Three DSC1 channel variants are functionally expressed in *Xenopus* oocytes

To determine whether the DSC1 channel is also a cation channel like the BSC1 channel (Zhou et al., 2004), we examined the 20 DSC1 variants in *Xenopus* oocytes using two-electrode voltage clamp. To record DSC1 currents, we adopted the protocol previously used for the BSC1 channel (Zhou et al., 2004). The membrane potential was depolarized to 80 mV for 15 ms from a holding potential of -100 mV and then repolarized back to -100 mV (Fig. 3A). A small outward current during depolarization followed by a large tail current associated with repolarization was recorded from oocytes expressing DSC1-1.1 in Ba²⁺ recording solution (Fig. 3A). Smaller outward and tail currents were detected in Ca²⁺ recording solution; and a larger outward current and a much smaller tail current were detected in Na⁺ recording solution (Fig. 3A). The larger outward current during the depolarization in the Na⁺ solution is most likely caused by the increased driving force resulting from the relatively high internal K⁺. This phenomenon was also observed in the study of the BSC1 channel (see Fig. 3A in Zhou et al., 2004). To facilitate the comparison between currents from different recording solutions, the tail currents recorded from each oocyte in Ca²⁺ recording solution or Na⁺ recording solution were normalized to the tail current obtained in Ba²⁺ recording solution from the same oocyte (Fig. 3B). These results indicate that like the BSC1 channel, the DSC1-1.1 channel is more permeable to Ca²⁺ and Ba²⁺, and also to Na⁺ in the absence of external Ca²⁺. As expected, none of the 10 variants that encode truncated proteins produced any currents using this recording protocol. However, only two other variants, DSC1-1.2 and DSC1-2.2, are also functional generating currents similar to those from the DSC1-1.1 channel (Fig. 3B). We did not observe any detectable currents from other seven *DSC1* clones that do not contain any premature stop codon.

The sodium channel blocker TTX inhibits insect sodium channels at nanomolar concentrations (Tan et al., 2002; Du et al., 2009). To determine whether TTX also inhibits DSC1 channels, we recorded DSC1 currents in Ba²⁺ recording solution in the presence of TTX (0.1 μM, 1 μM and 10 μM) using the recording protocol described above. Even at 10 μM, TTX did not reduce the amplitude of either the outward current or the tail current, indicating that DSC1-1.1 channel is TTX-insensitive (Fig. 3C).

4. Discussion

In this study, sequencing analysis of 20 cDNA clones reveals extensive alternative splicing and potential RNA editing of *DSC1* transcripts. A total of nine optional exons were identified, four of which contain in-frame stop codons. It is striking that seven of 20 clones are predicted to encode truncated proteins due to the inclusion of stop codon-containing optional exons. Interestingly, like the stop codon-containing exon 11A located in the linker connecting domains II and III in DSC1, two BSC1 optional exons in the linker connecting domains II and III also contain a premature stop codon (Liu et al., 2001). Transcripts containing these *BSC1* optional exons were detected only in leg muscle, suggesting a

possibly tissue-specific role of these truncated proteins (Liu et al., 2001). The conservation of this splicing site in two phylogenetically distant species raises the possibility that two-domain truncated proteins might play a role in insect neurophysiology. Premature stop codon-containing alternative exons are also detected in voltage-gated sodium and calcium channel genes, although the role of these truncated proteins is unclear. For example, transcripts containing two mutually exclusive exons, 18A and 18N, encoding IIIS3-S4, are found in mouse $\text{Na}_v1.6$ (Plummer et al., 1997). Exon 18N contains a stop codon and exon 18N-containing transcripts are detected in fetal brain and non-neuronal cells (Plummer et al., 1997). Moreover, this stop codon-containing exon is conserved in sodium channel genes of human, pufferfish (Plummer et al., 1997), and the German cockroach (Tan et al., 2002). Other sodium channel genes also produce premature stop codon-containing alternative exons, such as exon 17A in $\text{Na}_v1.2$ and $\text{Na}_v1.3$, exon 17B in $\text{Na}_v1.3$, and exon 16A in $\text{Na}_v1.7$ in mouse and human (Kerr et al., 2008). Like exon 18N, the inclusion of exon 17A is tissue- or developmental stage-specific (Kerr et al., 2008).

Only three of the 20 clones produced DSC1 currents. While it is not surprising that the 10 truncated DSC1 proteins did not produce any current, it is not clear why seven clones that contain no premature stop codon did not produce currents. We previously encountered a similar problem with functional expression of *BSC1* variants in *Xenopus* oocytes. While one *BSC1* variant gave robust currents (Zhou et al., 2004), no currents were detected from other two *BSC1* clones that do not contain premature stop codons (Chung and Dong, unpublished data). It is likely that unidentified accessory subunits or other chaperone proteins are required for the expression of these variants in *Xenopus* oocytes.

The functional DSC1 variants are permeable to Na^+ , Ca^{2+} , and Ba^{2+} , which is similar to those recorded from *BSC1* channels (Zhou et al., 2004). Like *BSC1*, *DSC1* encodes a voltage-gated cation channel that is more permeable to bivalent cations. It is well established that the ion selectivity of voltage-gated sodium channels is determined by the amino acids D, E, K, and A in the pore positions of domains I, II, III, and IV, respectively (i.e., the selectivity-filter motif “DEKA”) (Catterall, 2000). In contrast, four glutamic acids (EEEE) at the corresponding positions determine the ion selectivity for voltage-gated calcium channels (Catterall, 2000). It has been shown that a K to E substitution in the DEKA motif in a mammalian sodium channel altered the sodium selectivity to more toward calcium (Heinemann et al., 1992). Both the DSC1 and *BSC1* channels contain the DEEA motif instead of DEKA or EEEE motifs. Substitution of the second E with K in the DEEA motif reduced the ion selectivity of the *BSC1* channel for Ba^{2+} , demonstrating its critical role in modulating ion selectivity of the *BSC1* channel (Zhou et al., 2004). It is likely that such a substitution will have a similar effect on the ion selectivity of the DSC1 channel.

It is well recognized that the four S4 voltage sensors in sodium channels, rich in positively charged amino acids, detect membrane depolarization and move outward initiating channel activation (Catterall, 2000). Most of the positively charged residues are also conserved in DSC1 and *BSC1* channels with two minor variations (Fig. 1). First, DSC1 and *BSC1* channels have an extra positively charged residue in IIIS4 compared to the Para channel. Whether the difference contributes to the differences in gating properties between Para and *BSC1* channels (Zhou et al., 2004) remains to be determined. Second, DSC1-1 has four positively charged residues in IIS4, whereas five in *BSC1* and Para channels, suggesting potential differences in voltage sensing between *BSC1* and DSC1 channels. We are currently investigating gating properties of the DSC1 variants; and possible contributions of these sequence variations in voltage sensors in *BSC1* and DSC1 channel gating.

In sodium channels, the selectivity-filter motif “DEKA” and the outer-ring motif “EEMD” (mammals) or “EEID” (insects) in the pore region of domain I–IV are critical for TTX

binding (Catterall, 2000). In DSC1/BSC1 channels the amino acid sequences “DEEA” and “EEIN,” respectively, are found in the corresponding positions. Because the DSC1 channel is insensitive to TTX (Fig. 3C), we speculate that the K to E substitution in the DEEA motif of the DSC1/BSC1 channels might be involved in TTX resistance since a charge-reversal substitution of the K residue in the DEKA motif renders rNa_v1.2 channels extremely resistant to TTX (Catterall, 2000). On the other hand, the N residue in the EEIN motif may not be involved in TTX resistance because an S to N substitution made varroa mite sodium channels more sensitive to TTX (Du et al., 2009). Besides these two motifs, a non-aromatic residue, cysteine or serine, immediately after the first E of the EEMD motif has been shown to be responsible for TTX resistance of rNa_v1.5, rNa_v1.8 and rNa_v1.9 channels (Satin et al., 1992; Sivilotti et al., 1997). Interestingly, both DSC1 and BSC1 channels have a non-aromatic residue (N359 in DSC1, N366 in BSC1) in this position. Therefore, N359/N366 may also contribute to TTX-insensitivity of DSC1/BSC1 channels.

In conclusion, *DSC1* gene encodes a voltage-gated cation channel that is permeable to Ba²⁺, Ca²⁺, and Na⁺. *DSC1* transcripts undergo extensive alternative splicing and RNA editing. These posttranscriptional mechanisms likely play a critical role in regulating DSC1 expression level or in producing channels with distinct gating properties.

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Figure 1.

Alignment of amino acid sequences of DSC1-1.1, BSC1 and Para proteins. Dots represent amino acid residues in BSC1 and Para that are identical to those in DSC1-1.1. Dashes indicate gaps introduced to maximize sequence alignment. The four homologous domains (I to IV) and six transmembrane segments (S1 to S6) in each domain are marked above the sequences. Optional exons 17B and 19A which are included in DSC1-1.1 are highlighted. Locations of other optional exons (which are not included in DSC1-1.1) are marked with solid arrowheads. Empty arrow heads indicate the position of introns. Amino acid residues in DSC1-1.1 and BSC1 corresponding to the MFM motif in Para (i.e., IFM motif in mammalian sodium channels) that is critical for fast inactivation are boxed. The amino acid residues which are important for ion selectivity and TTX binding are indicated with asterisks. The GenBank accession numbers DQ466888.1, AF312365.1 and M32078.1 for DSC1-1.1, BSC1 and Para, respectively.

Figure 2. Molecular characterization of 20 *DSC1* cDNA clones. **A.** The genomic organization of the coding region of the *DSC1* gene deduced from the sequence of DSC1-1.1 (GenBank ID: DQ466888.1). Solid boxes represent exons. Positions of the alternative exons are labeled with arrows. **B.** Schematic drawing of the topology of the *DSC1* protein indicating locations of alternative exons. All alternative exons are optional. The asterisks indicate stop codons. **C.** Exon usage of 20 *DSC1* cDNA clones. Splice variants are named according to the splice types. Variants of each splice type are different due to scattered amino acid differences. For example, the two variants in splice type 3 are designated as DSC1-3.1 and DSC1-3.2.

Figure 3.

Functional expression of three DSC1 variants in *Xenopus* oocytes. **A.** DSC1 currents recorded from *Xenopus* oocytes expressing DSC1-1.1 channels. Recording protocol consisted of a 15-ms depolarization from -100 mV to 80 mV followed by repolarization to -100 mV. Superimposed Na^{+} , Ca^{2+} and Ba^{2+} currents recorded from an oocyte two days after injection with 15 ng *DSC1-1.1* cRNA. **B.** DSC1 variants are more permeable to Ca^{2+} and Ba^{2+} than to Na^{+} . Tail currents were normalized to the maximal current from recording in Ba^{2+} recording solution. The data represent the mean $\pm$ SD for at least eight oocytes. The asterisks indicate significant differences from the Ba^{2+} current ($p < 0.05$). **C.** The DSC1-1.1 channel is insensitive to TTX. Superimposed DSC1-1.1 currents recorded using Ba^{2+} recording solution from an oocyte two days after injection with 15 ng *DSC1-1.1* cRNA before and after exposure to 10 μM TTX using the protocol illustrated in A.

Table 1

Potential RNA Editing Events

Amino acid change	Nucleotide change	Position	<i>DSCI</i> Clones
N186S	a to g	DIS3	2-2, 8-1
W280R	t to c	IS5~IS6	3-1, 3-2
V449A	t to c	Linker (DI~DII)	1-4, 7-2
Q601R	a to g	Linker (DI~DII)	1-1, 1-3, 2-5
Q1020R	a to g	Linker (DII~DIII)	5-1, 6-1
S1575G	a to g	Linker (DII~DIII)	5-1, 2-3
H1605R	a to g	Linker (DII~DIII)	4-2, 2-3, 5-1, 2-1
Q1687R	a to g	Linker (DII~DIII)	1-5, 5-1, 2-2, 2-3, 8-1
Y1690C	a to g	Linker (DII~DIII)	2-1, 1-5, 5-1, 2-3, 8-1, 4-2
T1752A	a to g	D3S1	2-1, 1-5, 5-1, 2-2, 2-3, 8-1, 4-2
I1768V	a to g	D3S1	2-1, 1-5, 5-1, 2-2, 2-3, 4-2
T1782A	a to g	D3S1~D3S2	1-1, 2-1
Q1864R	a to g	D3S4~D3S5	2-1, 1-5
M2027V	a to g	Linker (DIII~DIV)	1-1, 1-2, 2-1, 3-1, 5-1, 4-1, 6-1, 1-6, 4-2
L2163S	t to c	D4S3~D4S4	7-1, 7-2
N2388S	a to g	C terminus	1-2, 2-2, 1-6
P2402S	c to t	C terminus	1-2, 1-4, 1-5, 2-3, 1-6, 3-2