The *Drosophila* Sodium Channel 1 (DSC1): The founding member of a new family of voltage-gated cation channels

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ABSTRACT

It has been nearly three decades since the identification of the *Drosophila* Sodium Channel 1 (DSC1) gene from *Drosophila melanogaster*. The orthologs of the DSC1 gene have now been identified in other insect species including *BSC1* from *Blattella germanica*. Functional analyses of DSC1/BSC1 channels in *Xenopus* oocytes reveal that *DSC1* and *BSC1* encode voltage-gated cation channels that are more permeable to Ca^{2+} than to Na^+. Genetic and electrophysiological analyses show that knockout of the *DSC1* gene in *D. melanogaster* causes behavioral and neurological modifications. In this review, we summarize major findings from recent studies and highlight a unique role of the DSC1 channel, distinct from that of the sodium channel, in regulating membrane excitability and modulating toxicity of pyrethroid insecticides.

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1. Introduction

Voltage-gated sodium channels are essential for the initiation and propagation of electrical signaling in the insect nervous system. They are the primary targets of pyrethroids, a large class of insecticides structurally derived from naturally occurring pyrethrins from *Chrysanthemum* plant species [1]. Pyrethroids modify the transitions between opening and closing (i.e., gating) of sodium channels, resulting in the disruption of the proper function of the nervous system [2–4]. Pyrethroids are highly insecticidal, but have low toxicity to mammals, and are used as major weapons against a wide range of arthropod disease vectors and agricultural pests. Unfortunately, due to intensive use, numerous mutations in sodium channel genes have been selected in various arthropod species and confer resistance to pyrethroids [5,6]. Development of pyrethroid resistance demands research to discover new insecticides that act on new targets and/or enhance the potency of existing pyrethroids.

In addition to having a classical sodium channel gene, such as *para* in *Drosophila melanogaster* [7], most insects also have a sodium channel-like gene, which was first identified in *D. melanogaster* and was in fact named as *Drosophila* Sodium Channel 1 (*DSC1*). *DSC1* was isolated by probing *Drosophila* genomic DNA libraries with an electric eel sodium channel cDNA [8–10]. For a long time, *DSC1* was

considered to be a putative sodium channel gene because the overall topology and sequence of the DSC1 channel are similar to those of sodium channels, consisting of four homologous repeats, each having six transmembrane segments [11,12]. However, functional analysis of BSC1, a *DSC1* ortholog from *Blattella germanica*, in *Xenopus* oocytes, as will be described below, gave the first indication that BSC1 is not a classical sodium channel, but has high permeability to Ca^{2+} [12,13]. Subsequent functional analysis of DSC1 confirmed this exciting finding [12], suggesting that DSC1 and BSC1 are founding members of a novel type of voltage-gated cation channels in insects. Indeed, *DSC1* orthologs have been found in other insect species including all of those with a complete genome sequence [14–16]. Importantly, recent isolation of two DSC1 knockout lines in *D. melanogaster* by ends-out homologous recombination [17] allowed investigation of the role of the DSC1 channel in insect neurophysiology and neurotoxicology *in vivo*. Below we summarize major findings from these studies.

2. BSC1/DSC1 channels belong to a distinct family of voltage-gated cation channels

In our initial electrophysiological characterization experiments, we found that expression of BSC1 in *Xenopus* oocytes increased oocyte endogenous Ca^{2+}-activated Cl^- currents, indicating that BSC1 channels are permeable to Ca^{2+}, and that Ca^{2+} influx through BSC1 channels activated endogenous Ca^{2+}-activated Cl^- channels. In order to separate the currents carried by Ca^{2+}-activated Cl^- channels, Ca^{2+} in the recording solution was replaced with Ba^{2+}, allowing us to isolate inward BSC1 currents with Ba^{2+} acting as a surrogate charge carrier [13]. Interestingly, both Co^{2+} and Cd^{2+} block
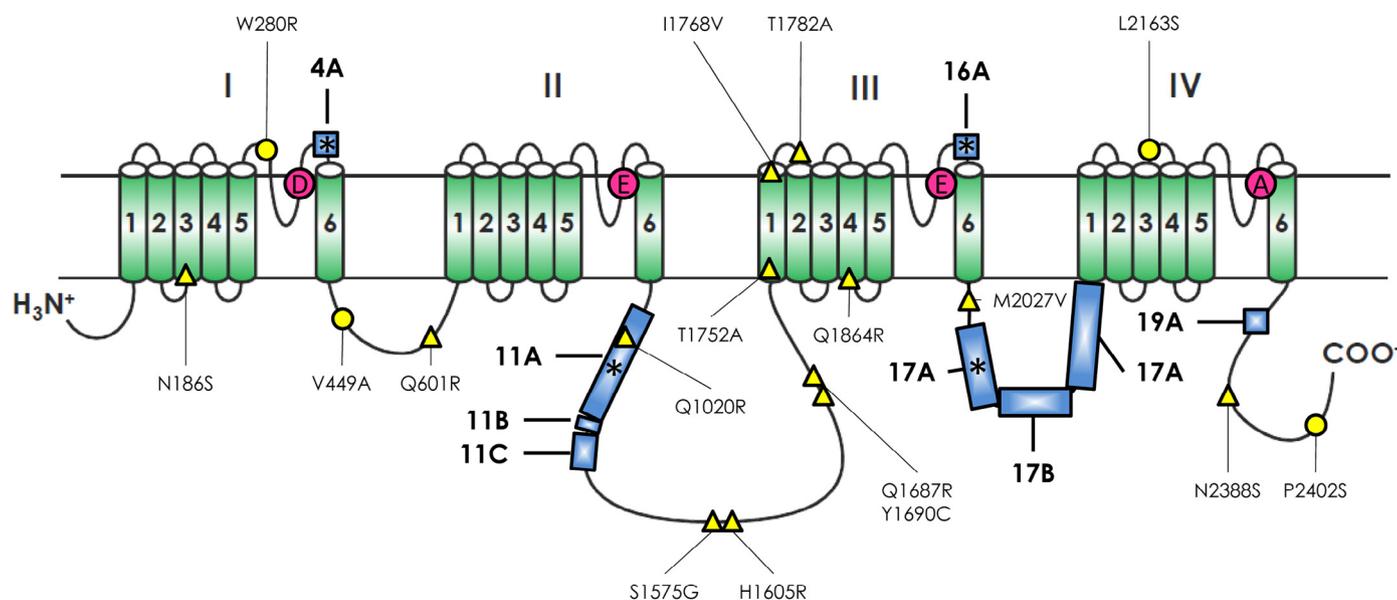
Fig. 1. Topology of the DSC1 channel consisting of four homologous domains (I–IV) composed of 6 transmembrane segments (1–6). The position of the DEEA selectivity filter on the pore-loop region between transmembrane segment 5 and 6 of each domain is indicated by the pink circles. Sodium channels have a DEKA motif instead. Optional exons are shown as blue rectangles. Premature stop codons in optional exons 4A, 11A, 16A, and 17A are indicated by an asterisk (*). The positions of putative A-to-I and C-to-T RNA-editing sites are indicated by yellow triangles and yellow circles, respectively.

the BSC1 channel, similar to their effects on voltage-gated Ca^{2+} channels [18]. The BSC1 channel is permeable to monovalent cations in the absence of external Ca^{2+}. The permeability ratios were $P_{Ba}/P_K \approx 30$ and $P_{Ba}/P_{Na} \approx 22$, indicating that Ca^{2+}/Ba^{2+} are significantly more permeable through the BSC1 channel than either Na^+ or K^+ [13]. Therefore, the BSC1 channel is distinct from classical Ca^{2+} channels, which has a P_{Ba}/P_{Na} of >1000 [18].

Ionic selectivity in voltage-gated sodium and calcium channels is strongly influenced by a ring of amino acids in the pore regions (i.e., the loop connecting S5-S6 in each of the four domains, Fig. 1) [19]. All voltage-gated sodium channels, including insect sodium channels, contain the amino acids D, E, K, and A in the loop connecting S5 and S6 of domains I, II, III, and IV, respectively, whereas voltage-gated calcium channels contain the acidic residues (E, E, E, and E) at the corresponding positions [13]. Interestingly, DSC1 and its orthologs from other species possess an E (as opposed to K) in domain III. In other words, DSC1 and its orthologs all contain a DEEA motif [14]. Substitution of the second E in the DEEA motif with K in BSC1 decreased the permeability to Ba^{2+}, and increased the permeability for Na^+, indicating that the E residue in domain III is important for the selectivity of the BSC1 channel toward divalent ions such as Ba^{2+} or Ca^{2+} [13].

The BSC1 channel expressed in *Xenopus* oocytes also exhibits unique gating kinetics [13]. Unlike sodium channels which activate rapidly within a few milliseconds upon depolarization [5], the BSC1 channel achieves full activation after a 40 ms depolarization. BSC1 is a high-voltage-activated channel with the half-maximal activation voltage of BSC1 channels around 50 mV, whereas cockroach sodium channels have the half maximal activation voltage ranging from −25 mV to −44 mV [20]. The BSC1 channel slowly deactivates, resulting in a large tail current upon repolarization. BSC1 also inactivates slowly. No significant inactivation is observed if BSC1 current traces are recorded with 40-ms depolarization pulses from −50 to 80 mV from the holding potential of −100 mV [13]. However, complete inactivation is evident when the depolarization pulse is extended to 500 ms [13].

Like the BSC1 channel, the DSC1 channel is permeable to Ca^{2+} and Ba^{2+}, and also to Na^+ in the absence of external Ca^{2+} [12]. The gating properties of the DSC1 channel are also similar to those of the BSC1 channel (Fig. 2). These results indicate that DSC1 encodes a voltage-gated cation channel similar to the BSC1 channel. The DSC1 channel is insensitive to tetrodotoxin, a potent and specific sodium channel blocker [12]. Furthermore, TipE-like homologs (TEH1-4) that modulates the gating properties of the Para sodium channel [5,21] do not alter the gating of the DSC1 channel (Fig. 2). The *DSC1* transcript undergoes extensive alternative splicing and RNA editing (Fig. 1). Functional consequences of these two post-transcriptional modifications remain to be determined.

3. DSC1 knockout flies exhibit neurological defects

A *Drosophila* P-element insertion line, smi60E, which has reduced expression of *DSC1* due to a P-element insertion in the second intron of *DSC1*, exhibits a 2-fold impairment in olfactory response to benzaldehyde [22]. We made complete DSC1 knockout lines using targeted gene knockout via homologous recombination [17]. Unlike null mutants of the *para* sodium channel, which are lethal [7], the *DSC1* knockout flies are viable under standard laboratory rearing conditions. Similar to smi60E, *DSC1* knockout flies exhibited reduced repellency by benzaldehyde as well as citronellal [17]. Besides the olfactory defect, two additional defects of the DSC1 knockout mutants are recently reported [17]. First, unlike *para* sodium channel mutants, DSC1 knockout flies do not exhibit temperature-sensitive paralysis. However, upon being returned to room temperature, *DSC1* knockout flies had significantly slower recovery from heat-induced paralysis. Second, *DSC1* knockout flies exhibit a prominent jumpy phenotype when disturbed and this defect is intensified under heat shock and starvation conditions.

To gain insight into neural/physiological processes that directly or indirectly affect jumpy response to heat shock and starvation, we examined the activities of a well-defined neural circuit, the giant fiber system (GFS), which controls the jump-and-flight escape reflex [17]. In these experiments, electric stimuli at different intensities were delivered to head or thorax; and then the responses from jump or flight thoracic muscles, respectively, were recorded. We detected specific defects in the circuitry of the GFS, which are consistent with a critical
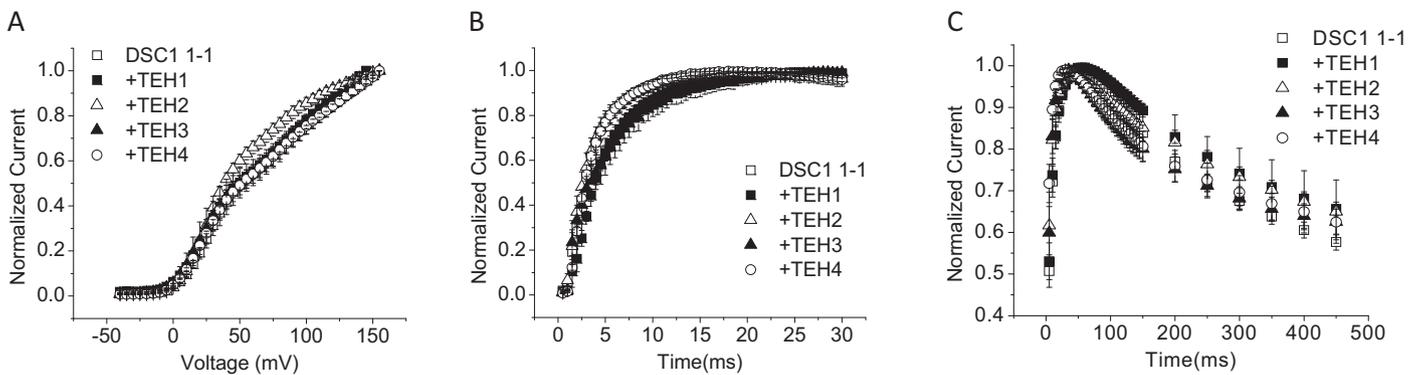
Fig. 2. Coexpression of DSC1 channels with TEH1-4 proteins in *Xenopus* oocytes. (A) Voltage dependence of activation. Voltage dependence of activation was tested by step depolarization from a holding potential of –100 mV to a range of potentials from –40 mV to 150 mV in 5 mV increments, followed by a repolarization to –100 mV. Peak tail currents during the repolarization were normalized to the maximal peak tail current and plotted against depolarizing voltage. (B) Kinetics of activation. Activation kinetics were determined by depolarization from –100 mV to 80 mV for 0.5–30 ms with 0.5 ms increments, followed by repolarization to –100 mV. Peak tail currents during the repolarization were normalized to the maximal peak tail current and plotted versus the time of depolarization. (C) Kinetics of inactivation. Inactivation kinetics were determined by depolarization from –100 mV to 80 mV for up to 500 ms in 5 ms increments for the first 150 ms and 50 ms increments for the remaining 350 ms, followed by repolarization to –100 mV. Peak tail current during the repolarization were normalized to the maximal peak tail current and plotted versus the time of depolarization. Each data point is shown as mean ± SEM with the number of oocytes between 6 and 13. The procedures for oocyte preparation, cRNA injection and current recording are identical to those described previously by Zhang et al. [12]. DSC1 1-1 cRNA was co-injected into oocytes with individual TEH1-4 cRNAs in 1:1 molar ratio. DSC1 currents were recorded using standard two-electrode voltage clamp technique at room temperature. External recording 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.

function of the DSC1 channel in maintaining the stability of the GFS and modulating the activities of the giant fiber (GF) and the central neurons presynaptic to the GF, particularly under the conditions of heat shock and starvation. This finding corroborates very well with a high level of *DSC1* expression observed in the GF as well as in the sensory system, such as the antennal nerves and optic lobes, which send sensory input directly or indirectly to the GF [23]. All results considered, we speculate that the DSC1 channel functions as a "breaker" to keep the insect nerve firing property in check, especially under stressful conditions, such as heat shock and starvation, which tend to hyper-stimulate the nervous system. How the DSC1/BSC1 cation channel exerts stability control over stress-enhanced neuronal hyperexcitability remains to be worked out.

4. DSC1 knockout flies are more sensitive to pyrethroid insecticides

Generation of *DSC1* knockout flies also prompted us to assess the susceptibility of these flies to insecticides that act on components in the nervous system [17]. *DSC1* knockout flies are not more resistant to any of the insecticides we tested, which include four pyrethroids, indoxacarb/DCJW and fipronil (Fig. 3). These results indicate that the DSC1 channel is not a primary insecticidal target of these compounds. However, *DSC1* knockout flies were more susceptible than control (*w1118*) flies to pyrethroids, but not to DCJW and fipronil (Fig. 3). This suggests a specific effect of the DSC1 channel on modulating pyrethroid sensitivity because indoxacab/DCJW is a sodium channel blocker insecticide and has a mode of action opposite to pyrethroids (i.e., inhibiting neuronal excitability) [3] and fipronil causes neuronal hyperexcitability like pyrethoids, but by blocking the GABA-gated Cl⁻ channel [24]. The specific effect of *DSC1* knockout on pyrethroid sensitivity suggests that *DSC1* knockout flies are more susceptible to neuronal stimulation by sodium channel activators. Enhanced hypersensitivity of the GFS to pyrethroids in DSC1 knockout flies further supports the notion that the DSC1 channel normally suppresses the response of the nervous system to pyerthroid exposure [17].

In summary, we have identified DSC1 and its orthologs as a novel family of voltage-gated cation channels in insects. The DSC1-family cation channel could be targets for a new generation of safer insecticides because they are found only in arthropods, but not in vertebrates.

In addition, because the DSC1 mutants are hyper-susceptible to pyrethroids, development of DSC1 channel blockers may be a promising approach to enhance the efficacy of pyrethroids, which are major weapons against numerous agriculturally and medically important arthropod pests, including malaria-transmitting mosquitoes.
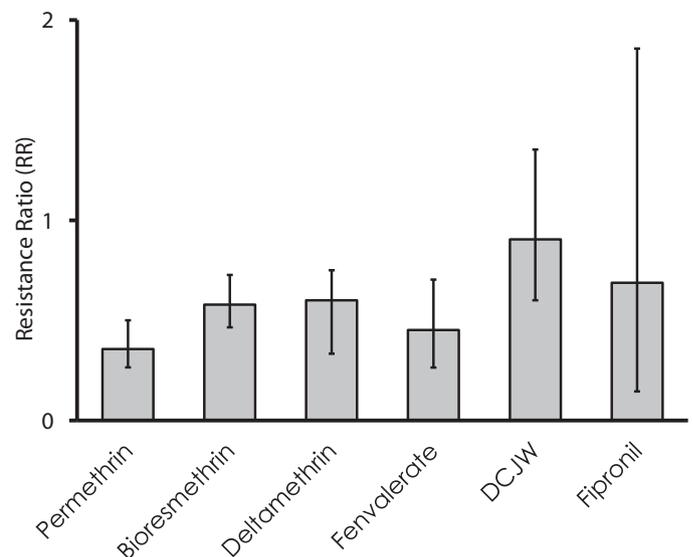
Fig. 3. *DSC1* knockout flies are specifically more sensitive to pyrethroid insecticides. The Resistance Ratios (RR) are calculated as LC$_{50}$ values of *DSC1* knockout flies relative to those of w1118 flies. The RRs that are significantly below 1 for permethrin, bioresmethrin, deltamethrin, and fenvalerate show increased sensitivity of *DSC1* knockout flies to pyrethroids. There is no change in the RRs of DCJW or fipronil in *DSC1* knockout flies. Figure generated from data in table 1 from Zhang et al. [17].

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References

[1] M. Elliott, Synthetic Pyrethroids, American Chemical Society, Washington, DC, 1977.

[2] T. Narahashi, K.S. Ginsburg, K. Nagata, J.H. Song, H. Tatebayashi, Ion channels as targets for insecticides, Neurotoxicology 19 (1998) 581–590.

[3] K.S. Silver, Y. Du, Y. Nomura, E.E. Oliveira, V.L. Salgado, B.S. Zhorov, et al., Voltage-gated sodium channels as insecticide targets, 2014.

[4] D.M. Soderlund, Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances, Arch. Toxicol. 86 (2012) 165–181.

[5] K. Dong, Y. Du, F. Rinkevich, Y. Nomura, P. Xu, L. Wang, et al., Molecular biology of insect sodium channels and pyrethroid resistance, Insect Biochem. Mol. Biol. 50 (2014) 1–17.

[6] F.D. Rinkevich, Y. Du, K. Dong, Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids, Pestic. Biochem. Physiol. 106 (2013) 93–100.

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

[8] H. Okamoto, K. Sakai, S. Goto, E. Takasu-Ishikawa, Y. Hotta, Isolation of *Drosophila* genomic clones homologous to the eel sodium channel gene, Proc. Jpn. Acad. 63 (B) (1987) 284–288.

[9] M. Ramaswami, M.A. Tanouye, Two sodium-channel genes in *Drosophila*: implications for channel diversity, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 2079–2082.

[10] L. Salkoff, A. Butler, A. Wei, N. Scavarda, K. Giffen, C. Ifune, et al., Genomic organization and deduced amino acid sequence of a putative sodium channel gene in *Drosophila*, Science 237 (1987) 744–749.

[11] N.H. Kulkarni, A.H. Yamamoto, K.O. Robinson, T.F.C. Mackay, R.R.H. Anholt, The DSC1 channel, encoded by the smi60E locus, contributes to odor-guided behavior in *Drosophila melanogaster*, Genetics 161 (2002) 1507–1516.

[12] T. Zhang, Z. Liu, W. Song, Y. Du, K. Dong, Molecular characterization and functional expression of the DSC1 channel, Insect Biochem. Mol. Biol. 41 (2011) 451–458.

[13] W. Zhou, I. Chung, Z. Liu, A.L. Goldin, K. Dong, A voltage-gated calcium-selective channel encoded by a sodium channel-like gene, Neuron 42 (2004) 101–112.

[14] Y.J. Cui, L.L. Yu, H.J. Xu, K. Dong, C.X. Zhang, Molecular characterization of DSC1 orthologs in invertebrate species, Insect Biochem. Mol. Biol. 42 (2012) 353–359.

[15] Z. Liu, I. Chung, K. Dong, Alternative splicing of the *BSC1* gene generates tissue-specific isoforms in the German cockroach, Insect Biochem. Mol. Biol. 31 (2001) 703–713.

[16] Y. Park, M.F. Taylor, R. Feyereisen, Voltage-gated sodium channel genes hscp and hDSC1 of *Heliothis virescens F.* genomic organization, Insect Mol. Biol. 8 (1999) 161–170.

[17] T. Zhang, Z. Wang, L. Wang, N. Luo, L. Jiang, Z. Liu, et al., Role of the DSC1 channel in regulating neuronal excitability in *Drosophila melanogaster*: extending nervous system stability under stress, PLoS Genet. 9 (2013) e1003327.

[18] B. Hille, Ion Channels of Excitable Membranes, Sinauer, Sunderland, MA, 2001.

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

[20] W. Song, Z. Liu, J. Tan, Y. Nomura, K. Dong, RNA editing generates tissue-specific sodium channels with distinct gating properties, J. Biol. Chem. 279 (2004) 32554–32561.

[21] L. Wang, Y. Nomura, Y. Du, K. Dong, Differential effects of TipE and a TipE-homologous protein on modulation of gating properties of sodium channels from *Drosophila melanogaster*, PLoS ONE 8 (2013) e67551.

[22] N.H. Kulkarni, A.H. Yamamoto, K.O. Robinson, T.F. Mackay, R.R. Anholt, The DSC1 channel, encoded by the smi60E locus, contributes to odor-guided behavior in *Drosophila melanogaster*, Genetics 161 (2002) 1507–1516.

[23] C. Castella, M. Amichot, J.B. Bergé, D. Pauron, DSC1 channels are expressed in both the central and the peripheral nervous system of adult *Drosophila melanogaster*, Invert. Neurosci. 4 (2001) 85–94.

[24] V. Raymond-Delpech, K. Matsuda, B.M. Sattelle, J.J. Rauh, D.B. Sattelle, Ion channels: molecular targets of neuroactive insecticides, Invert. Neurosci. 5 (2005) 119–133.