The DSC1 Channel, Encoded by the smi60E Locus, Contributes to Odor-Guided Behavior in Drosophila melanogaster

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ABSTRACT

Previously, we generated P-element insert lines in Drosophila melanogaster with impaired olfactory behavior. One of these smell-impaired (smi) mutants, smi60E, contains a P[ArB] transposon in the second intron of the dsc1 gene near a nested gene encoding the L41 ribosomal protein. The dsc1 gene encodes an ion channel of unknown function homologous to the paralytic (para) sodium channel, which mediates neuronal excitability. Complementation tests between the smi60E mutant and several EP insert lines map the smell-impaired phenotype to the P[ArB] insertion site. Wild-type behavior is restored upon P-element excision. Evidence that reduction in DSC1 rather than in L41 expression is responsible for the smell-impaired phenotype comes from a phenotypic revertant in which imprecise P-element excision restores the DSC1 message while further reducing L41 expression. Behavioral assays show that a threefold decrease in DSC1 mRNA is accompanied by a threefold shift in the dose response for avoidance of the repellent odorant, benzaldehyde, toward higher odorant concentrations. In situ hybridization reveals widespread expression of the dsc1 gene in the major olfactory organs, the third antennal segment and the maxillary palps, and in the CNS. These results indicate that the DSC1 channel contributes to processing of olfactory information during the olfactory avoidance response.

Chemosensory Coding

Chemoreception is essential for the survival of virtually all animals and depends on the nervous system’s ability to discriminate odorants, encode their chemical structures and concentrations as patterns of action potentials, and transmit this information to the central nervous system. Insects and vertebrates alike have evolved specialized chemosensory neurons that express receptors for the recognition of numerous odorants. In the mammalian olfactory system, each olfactory neuron expresses only a single receptor from among a large repertoire of olfactory receptor genes (Buck and Axel 1991; Chess et al. 1994). Axons from neurons that express the same odorant receptors converge upon one or a few output neurons (Vassar et al. 1994; Mombaerts et al. 1996) in spherical structures of neuropil (glomeruli) in the olfactory bulbs (Hildebrand and Shepherd 1997). Combinatorial activation of odorant receptors (Malnic et al. 1999) generates spatial and temporal patterns of neural activity (Sicard and Holley 1984), which are represented as specific patterns of glomerular activation that encode odor quality and concentration (Bozza and Kauer 1998; Mori et al. 1999; Rubin and Katz 1999). The insect olfactory system resembles the vertebrate system in its functional organization in that axons of olfactory neurons relay olfactory information to the brain by forming convergent glomerular projections that encode odor representations in the antennal lobes (Joerges et al. 1997; Gao et al. 2000; Vosshall et al. 2000), suggesting that fundamental strategies for odor discrimination are similar (Hildebrand and Shepherd 1997).

Thresholds for odor recognition vary among individuals. Differences in olfactory acuity can arise at the level of odorant recognition, signal propagation, or signal processing. Since olfactory behavior depends on the coordinated expression of multiple genes, quantitative genetic approaches are necessary to identify those genes that contribute to individual variation in olfactory responsiveness and to determine quantitatively the effect of each gene product on the phenotype (Mackay 1996, 2001; Anholt and Mackay 2001).

Drosophila melanogaster provides a powerful system for studies of the genetic architecture of odor-guided behavior, since its genome has been sequenced (Adams et al. 2000; Rubin 2000) and mutations can be generated readily in a controlled genetic background (Mackay 1996, 2001). Furthermore, the olfactory system of Drosophila is comparatively simple; the Drosophila genome encodes 60 putative odorant receptors (Rubin 2000), of which at least 41 are expressed in ~1200 chemosensory neurons housed in sensilla in the third antennal segment and the maxillary palp (Clyne et al. 1999; Gao and Chess 1999; Vosshall et al. 1999, 2000; Gao et al. 2000).
2000). Correspondingly, each antennal lobe contains only ~43 glomeruli (Laisanne et al. 1999) and represents a much more simplified system for odor coding than the ~1800 glomeruli in the mouse olfactory bulb (Pomeoy et al. 1990).

One of the first olfactory mutants identified in *D. melanogaster* was smellblind (shb), an allele of paralytic (para), which is located on the X chromosome and encodes a voltage-gated sodium channel (Rodrigues and Siddiqi 1978; Lilly and Carlson 1989; Lilly et al. 1994). Whereas the function of this sodium channel in neuronal signal propagation is well established, the function of a second channel, homologous to PARA and encoded by the *dse1* gene at cytological location 60E on the second chromosome, has remained enigmatic (Salkoff et al. 1987). Comparisons of partial sequences indicated that the DSC1 and PARA channels evolved after gene duplication early in evolution (Ramawami and Tanouye 1989). Voltage-clamp recordings of sodium currents in embryonic neurons from deletion mutants over the 60E region showed normal action potentials, in line with the notion that the PARA channel is the primary sodium channel responsible for neuronal excitability in embryonic neurons (Germeraad et al. 1992). This study, however, left the function of the DSC1 channel unresolved. A subsequent study showed that the DSC1 channel, in fact, is not expressed at significant levels until pupal and adult stages, when DSC1 expression patterns in the central and peripheral nervous system overlap the expression pattern of PARA (Hong and Ganetzky 1994). Thus, the DSC1 channel may have evolved a more subtle, regulatory function in adult flies that complements the essential role of the PARA channel.

Previously, we used Pelement insertion mutagenesis in a highly inbred strain to identify *smell-impaired* (*smi*) lines that showed reduced responsiveness to the repellent odorant, benzaldehyde (Anholt et al. 1996). Here, we report that Pelement insertion in one of these lines, *smi60E*, disrupts the expression of the DSC1 channel. Behavioral analysis of the *smi60E* mutant shows a threefold displacement in the dose response to benzaldehyde, indicating an increased olfactory threshold. These results demonstrate a role for the DSC1 channel in regulating olfactory acuity.

**Materials and Methods**

**Fly stocks:** The *smi60E* line carries a single *P[AvrB] element* at cytological location 60E, which contains a visible marker that complements the *ry* phenotype of the *Sam;ry* host strain, a *lacZ* reporter gene, and the *pBluescript* cloning vector (Anholt et al. 1996). Flies of the isogenic *Sam;ry* parental line, the *smi60E* line, *EP* lines (obtained from Exelixis, South San Francisco; Rørth 1996), and *P* element excision lines were reared at 25°C in plastic culture vials on agar-yeast-molasses medium at 70% humidity and under a 12-hr light/dark cycle.

**Generation of phenotypic revertants:** Mobilization of the *P[AvrB] element* was achieved by crossing *smi60E* females to *Sam1; SM5, Cy/Sp; TM6, Ubx,S/Aa* 2-3 males and subsequently crossing male offspring of genotype *Sam1; Cy/Sam2* to *ry* females. Single *Sam1; Cy/Sam2* females were then crossed to generate homozygous viable *P* element excision lines in the original *Samarkand* isogenic background. Excision lines with poor homozygous viability or fertility were maintained as heterozygotes against the SM5, *Cy* balancer chromosome.

**Behavioral assay:** All behavioral assays were performed between 1:00 and 4:00 p.m. in an environmental chamber with a controlled temperature at 25°C and constant 70% humidity. Flies, 2–10 days post-eclosion, were removed from their food source 1–2 hr prior to assay and placed in the behavioral chamber. Avoidance responses to the repellent odorant benzaldehyde were quantified, using the “dipstick” assay (Anholt et al. 1996; Fedorowicz et al. 1998). One replicate assay consisted of a single-sex group of five individuals in a test vial. The test vial was divided into compartments by placing markers on the side of the vial 3 and 6 cm from the bottom of the vial. The animals were exposed to an aqueous solution containing the desired concentration of benzaldehyde, which is introduced into the vial on a cotton wool swab and wedged between the cotton wool plug and the side of the vial with the tip of the swab at the 6-cm mark. Following introduction of the odorant, the number of flies migrating to the compartment that was remote from the odor source was measured at 5-sec intervals, from 15 to 60 sec after introduction of the odor source. The “avoidance score” of the replicate is the average of these 10 counts and ranges between 0 (maximal attraction) and 5 (all flies are in the compartment away from the odor source for the entire assay period, i.e., a maximal repellent response). At least 10 replicate assays per sex were done for each line. Since no significant sexual dimorphism was observed for olfactory responses of *smi60E* flies (Anholt et al. 1996), data presented here are based on combined scores for both sexes. The Student’s t test was used to evaluate statistical significance of avoidance scores between lines.

**Plasmid rescue and sequence analysis:** A fragment of genomic DNA adjacent to the *P[AvrB] insertion* site of the *smi60E* mutant was isolated by digestion of *smi60E* genomic DNA with HindIII, ligated, and obtained as an insert in *pBluescript*. This insert, which we refer to as the “rescued fragment,” was sequenced and used to probe a selection of cosmids clones that span the 60E cytological region, generously provided by Dr. Inga Sidén-Kiamos from the European Drosophila Genome Project (Herklioton, Crete; Siden-Kiamos et al. 1990). Cosmid 108A8 hybridized to the rescued fragment and sequence was obtained ~2 kb upstream and 12 kb downstream of the *P* element insertion site. This sequence was compared with a previously reported partial sequence (Salkoff et al. 1987; GenBank accession nos. X14394, X14395, X14396, X14397, and X14398) and with sequence from the Berkeley Drosophila Genome Project (Adams et al. 2000; http://www.fruitfly.org/; FBgn0002920; CT24831). Whereas the sequence we obtained matched the CT24831 sequence, several discrepancies were observed with the sequence published by Salkoff et al. (1987), indicating either sequencing errors in the previously published sequence, including a frameshift in the N-terminal region, or polymorphisms due to strain differences. The DSC1 sequence was aligned with the PARA sequence (accession no. NP_523371) and analyzed for predicted transmembrane regions using the ALIGN, Genestream
and SOSUI programs in the ExPASy suite of proteomics tools (http://www.expasy.ch/tools/). DNA sequencing was performed by the DNA sequencing service facility at the University of Iowa (Iowa City, IA). The exon-intron structure of the dsc1 gene was determined by analyzing sequences from the Berkeley Drosophila Genome Project (FBgn0002920; CT24831) and sequences of expressed sequence tag clones (HL01858, HL01171, and LD03260) together with RT-PCR experiments using different combinations of the following primers: 5'-CGGATCCTGACCAACTAGTCAAGTC (F1), 5'-CGGATCACCTGCTGCTCAAGTTGCT (F2), 5'-CAGCATCAGAGGCAGGTTCAAC (R9), and The Drosophila DSC1 Channel

RESULTS

The smi60E mutant was originally isolated as the result of a P-element insertional mutagenesis screen designed to identify co-isogenic P-element insert lines with quantitative defects in olfactory avoidance behavior (Anholt et al. 1996). The defect observed in smi60E flies is not specific to benzaldehyde, but generalizes to structurally diverse odorants and affects males and females equally, with reporter gene expression evident in the antennae (Anholt et al. 1996).

Association of the smi60E insertion site with the smell-impaired phenotype: To identify flanking regions of the P[ArB] insertion site, a 1413-bp fragment of host genomic DNA was rescued in pBluescript. In situ hybridization of the rescued fragment to larval polytene salivary gland chromosomes of the sam;ry506 host strain verified localization to the correct cytological location. Sequence analysis localized the P[ArB] insertion site to the second intron of the dsc1 gene, which follows the first 81 N-terminal amino acids of the coding region (Figure 1). The dsc1 gene comprises ~20.9 kb and contains 19 introns (Figure 1). Nested in the second intron is a second gene in the opposite orientation encoding a heat-denatured antisense and sense riboprobes were generated using SP6 and T7 RNA polymerases, respectively. For in situ hybridization 50 heads of Drosophila were dissected, fixed in 10% buffered formalin for 16 hr at 4°C, followed by dehydration in graded alcohols and embedding in paraffin, and 10-μm sections of randomly oriented heads were prepared. The slides were heated to 60°C for 15 min in an air deparaffinizer, deparaffinized in xylene, rehydrated through graded alcohols, and fixed in 4% paraformaldehyde for 20 min. After extensive washing in 4.3 mM NaH2PO4, 1.4 mM KH2PO4, 137 mM NaCl, 2.7 mM KCl, 0.1% Tween 20, pH 7.4 (PBT), they were treated with 200 mM HCl for 10 min. Following additional washes with PBT, the slides were treated for 10 min with acetic anhydride and triethanolamine-HCl to reduce background. Following this treatment, the slides were washed with PBT and incubated with 5 μg/ml proteinase K for 1 hr at 37°C. After an additional incubation with PBT for 5 min at 4°C, they were dehydrated through graded alcohols and rinsed in chloroform. The sections were hybridized for 16 hr at 55°C in hybridization buffer (50% formamide, 5× SSC, 100 μg/ml tRNA, 50 μg/ml heparin, and 0.1% Tween 20) with either a heat-denatured antisense or a sense probe. Following hybridization, the slides were washed at 55°C with hybridization buffer for 1 hr followed by 20 min sequential washes at 55°C with 75% hybridization buffer and 25% PBT, 50% hybridization buffer and 50% PBT, and 25% hybridization buffer and 75% PBT. This was followed by a 15-min wash at room temperature with PBT. Hybridization products were visualized using the digoxigenin-dUTP nonradioactive in situ detection system from Roche Molecular Biochemicals (Indianapolis) after a 16-hr incubation at 4°C with a rabbit antidigoxigenin antibody conjugated to alkaline phosphatase in PBT (Roche Molecular Biochemicals). Reaction products were detected via the alkaline phosphatase reaction in 100 mM Tris, 100 mM NaCl, 50 mM MgCl2, and 0.1% Tween 20, pH 9.5, using nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate as substrates. After dehydration through graded alcohols and xylene the slides were cover-slipped in Permount and examined under a Nikon Eclipse E800 microscope. Images were digitally captured and processed with Adobe Photoshop.

To further demonstrate that aberrant olfactory behav-
ior results from transposon insertion in the 

ior results from transposon insertion in the \( dsc1 \) gene and not from a random mutation, the \( P[\text{ArB}] \) element was mobilized and \( P \) element excision lines were generated. We obtained 20 excision lines, of which 10 showed normal olfactory avoidance responses (Figure 3). P-element excision was in all cases verified by the \( \text{rosy} \) pheno-
type and absence of the pBluescript moiety of the \( P[\text{ArB}] \) transposon as assessed by Southern blots (data not shown).

Evidence that disruption of the \( dsc1 \) gene accounts for the smell-impaired phenotype: We performed Northern blots to evaluate reduction of the expression of the DSC1 and L41 messages in \( \text{smi}60E \) flies compared to the \( \text{Sam;ry}^{506} \) controls. Northern blots hybridized to a mixture of fragments corresponding to the \( dsc1 \) gene coding sequence reveal a band at the expected size of 7.4 kb. In contrast to the PARA channel (\( \text{Loughney et al.} \) 1989; \( \text{Thackeray and Ganetzky} \) 1995), \( dsc1 \) splice variants are not immediately evident, although their existence cannot be entirely excluded, since the hy-

bridizing band is broad and may harbor heterogeneous messages (Figure 4A). In addition, a minor hybridizing species at 4.4 kb was also observed. Hybridization of the same blots with an L41 probe visualized an \( \sim250\)-bp band (Figure 4B). Actin was visualized as an internal standard for densitometric quantification (Figure 4C).

In the \( \text{smi}60E \) mutant, expression of the DSC1 channel is decreased by 66 \( \pm \) 2\%, whereas the L41 message shows a 41 \( \pm \) 2\% reduction (Figure 4, A, B, and D). To evaluate to what extent the smell-impaired phenotype could be attributed to either the \( dsc1 \) gene or the nested \( L41 \) gene, we asked whether, among one or more of our phenotypic revertants, an imprecise excision event could have occurred that would differentially affect the expression of these two genes. Indeed, in revertant 16 (rev16) a 53-bp DNA fragment including the terminal inverted repeat of the \( P[\text{ArB}] \) transposon remained at the original \( P \)-element insertion site following excision (Figure 4E). This revertant shows full restoration of the DSC1 message (Figure 4, A and D).

Figure 1.—Genomic organization of the coding region of the \( dsc1 \) gene. Solid boxes represent exons. The insertion site and orientation of the \( P[\text{ArB}] \) element are indicated by open arrows. The bottom solid bar represents the largest possible transcript. The open box represents the nested \( L41 \) gene in the opposite orientation. The insertion site and orientations of six \( EP \) lines, 58 bp away from the \( P[\text{ArB}] \) insertion site, are indicated by arrows.

Figure 2.—Olfactory avoidance responses of \( \text{Sam;ry}^{506} \) wild-
type flies, homozygous \( \text{smi}60E \) mutant flies, and heterozygous offspring from \( \text{smi}60E \) flies crossed with \( EP \) insertion lines (\( \text{Rørth} \) 1996). The number of replicate measurements for each cross is in-
dicated above the bar and is composed of the same number of mea-
surements for males and females. Error bars indicate standard er-
ors of the mean. Avoidance scores were obtained at 0.3\% (\( \text{v/v} \) benzaldehyde and were com-
pared to the avoidance score for \( \text{Sam;ry}^{506} \) (3.62 \( \pm \) 0.20) for statisti-
cally significant differences using the Student’s \( t \)-test. (*) \( P < 0.05 \); (**) \( P < 0.01 \); (*** \( P < 0.001 \).
Figure 3.—Olfactory avoidance responses of Sam;ry506 wild-type flies, smi60E mutant flies, and 10 phenotypic revertants (indicated by numerals) obtained by P[lArB] excision from the smi60E mutant. The number of replicate measurements for each cross is indicated above the bar and is composed of the same number of measurements for males and females. Error bars indicate standard errors of the mean. Avoidance scores were obtained at 0.3% (v/v) benzaldehyde and were compared to the avoidance score for Sam;ry506 (3.48 ± 0.12) for statistically significant differences using the Student’s t-test. None of the revertant avoidance scores were significantly different from the Sam;ry506 score. (***) P < 0.001.

and wild-type olfactory behavior (Figure 3). However, the P[lArB] remnant in this line resulted in a dramatic further reduction of the L41 message (85 ± 4% of wild type; Figure 4, B and D), providing conclusive evidence that the smell-impaired phenotype results exclusively from disruption of the dsc1 gene.

Behavioral characterization of the smi60E insertion: Reduction in dsc1 gene expression results in altered responsiveness of the smi60E flies to benzaldehyde (Figures 2 and 3). Aberrant olfactory avoidance behavior of the smi60E mutant becomes especially apparent at lower odorant concentrations. A comparison of the dose-response curves of smi60E flies and their co-isogenic Sam;ry506 parental line shows statistically significant reductions in avoidance scores at concentrations of benzaldehyde between 0.03 and 1.0% (v/v; Figure 5A) and a threefold shift of the half-maximal effective concentration toward higher odorant concentrations (Figure 5B). Revertants derived from smi60E also show restoration of wild-type levels of dsc1 mRNA concomitant with the return of normal olfactory avoidance responses (Figures 3 and 4).

Structure of the DSC1 channel protein and its relation to the PARA channel: The DSC1 protein consists of 2516 amino acids with a calculated molecular weight of 286,476 D. Positions of intron/exon splice sites in the dsc1 coding region are indicated by arrows in Figure 6 and were confirmed by sequence analysis of RT-PCR products. Sequence comparisons show similar organization between the PARA and DSC1 channels with the characteristic four tandem repeats, each containing six predicted transmembrane helical domains. The fourth transmembrane helix of each domain contains a voltage sensor with arginine residues spaced at regular intervals and a hairpin loop that contributes to the ion conducting pore presumably located between the fifth and sixth transmembrane segments of each repeat (Catterall 2000). The DSC1 protein shares 32% sequence identity with the PARA channel. Sequence conservation is considerably greater in the transmembrane regions, where 57% amino acid identity is observed. The principal difference between the two channel proteins is the presence of an expanded 676-amino-acid intracellular loop in the DSC1 protein in the center of the molecule between residues 1054 and 1730, which carries a net negative charge (Figure 6). This major difference in structure between the PARA and DSC1 channels is schematically represented in Figure 7.
Expression of the DSC1 channel in brain and chemosensory organs: Previous studies indicated that the expression of the DSC1 channel occurs with late onset and overlaps the expression pattern of the PARA channel (Hong and Ganetzky 1994). Expression of the lacZ reporter gene in the smi60E mutant showed extensive expression in the antenna (Anholt et al. 1996). We used in situ hybridization to visualize expression of DSC1 mRNA in heads of Sam;ry56 flies. Cross sections through the head show extensive staining in all areas where neuronal cell bodies are located (Figure 8A). In the ocelli, stained cell bodies continuous with their axons can be observed, indicating that a DSC1 message is indeed expressed in neurons, at least in the ocellar pathway (arrows in Figure 8A). Staining is also evident in cell bodies throughout the third antennal segment (Figure 8C) and maxillary palps (Figure 8E), the major olfactory organs of D. melanogaster. Staining is observed only with antisense probes (Figure 8, A, C, and E), but not with sense probes (Figure 8, B, D, and F), indicating that the observed staining is specific.

The widespread expression of the DSC1 channel in the central nervous system (CNS) and in chemosensory organs (Figure 8), and the threefold reduction in message (Figure 4) together with the threefold shift in dose response in the behavioral assay (Figure 5), lead us to conclude that the DSC1 channel plays a role in processing olfactory information during the avoidance response to repellent odorants.

DISCUSSION

P-element insertional mutagenesis in a controlled genetic background, combined with a sensitive statistical
behavioral assay, previously enabled us to identify 14 smi lines with statistically significant reductions in olfactory avoidance behavior (Anholt et al. 1996). In one of these lines, smi60E, insertion of the transposon in the second intron of the dsc1 gene has resulted in a threefold reduction in expression of the dsc1 gene (Figure 4, A and B). This is accompanied by a threefold shift in the dose response for avoidance behavior to benzaldehyde (Figure 5).

We have strong evidence that indicates that the gene affected by the P[ArB] insertion is indeed the dsc1 gene. First, the P[ArB] transposon is located in an intron of the dsc1 gene (Figure 1). Second, quantitative complementation tests between the smi60E mutant and a series of EP insert lines map the smell-impaired phenotype to the smi60E insertion site (Figure 2). Third, wild-type behavior is restored upon excision of the P element, indicating that the P[ArB] insertion indeed causes the mutant phenotype (Figure 3). Finally, evidence that reduction in expression of the DSC1 channel rather than of the L41 ribosomal protein is responsible for the smell-impaired phenotype comes from a phenotypic revertant in which imprecise excision of the P element restores the DSC1 message while further reducing L41 expression (Figure 4, A and B). Removal of the bulk of the 18.5-kb P[ArB] transposon restores full expression of the dsc1 gene, but the small 53-bp remnant of the inverted repeat of the P element (Figure 4C) is likely to form a hairpin structure at the promoter of the L41 gene, reducing expression of this gene even below that observed in the smi60E mutant. Finally, behavioral assays show that a threefold decrease in DSC1 message (Figure 4) is accompanied by a threefold shift in the dose response for avoidance of the repellent odorant, benzaldehyde (Figure 5).

A previous study reported that as little as a 10% difference in the activity of a cyclic GMP-dependent protein kinase in D. melanogaster profoundly affects larval foraging behavior (Osborne et al. 1997). Similarly, only modest changes in the activity of calcium-calmodulin-dependent protein kinase II produce major disruptions in associative learning in flies (Griffith et al. 1993). Our observations stand in contrast with these studies, in which small changes in gene expression have profound phenotypic effects, in that a large reduction in expression of the DSC1 channel impacts olfactory avoidance behavior in a subtle way. Similarly, this relatively subtle phenotypic effect resulting from a large reduction in the expression of the DSC1 channel contrasts with the complete anosmia observed for the smellblind mutant, an allele of paralytic (Rodrigues and Siddiqi 1978; Lilly and Carlson 1989; Lilly et al. 1994).

Previously, the para channel was identified as the principal sodium channel that mediates neuronal excitation in D. melanogaster (Kelly 1974; Germeraad et al. 1992; Hong and Ganetzky 1994; Littleton and Ganetzky 2000). The function of the DSC1 channel, however, remained unresolved. Previous in situ hybridization studies showed that in adult flies para and dsc1 transcripts are coexpressed in the same neurons, suggesting that neuronal activity may be determined by functional interactions between these two channels (Hong and Ganetzky 1994). Indeed, our own observations show that expression of the DSC1 channel is widespread in adult flies, with mRNA being readily detectable in cell bodies in the third antennal segment, the maxillary palps, and the cortical regions of the CNS (Figure 8). Although the data presented in Figure 8 cannot resolve whether expression is exclusively localized to neurons or whether the DSC1 channel may also be expressed in glia, expression in at least one sensory pathway is clearly neuronal as stained cell bodies of ocellar neurons are seen to be continuous with their axons. Our demonstration that disruption of the dsc1 gene in the smi60E mutant results in a reduction in olfactory sensitivity is the first association of this gene with a phenotype.

![Figure 5](image-url)
Figure 6.—Amino acid sequence alignments of the DSC1 and PARA channels. Arrowheads indicate intron-exon boundaries with respect to the \textit{dsc1} coding region. Predicted transmembrane regions are overlined and arginines in the fourth transmembrane helix of each homology repeat are underlined. Identical amino acids are indicated in boldface type. Numbering is according to the DSC1 sequence. Note the large insertion between the second and third homology repeat in the DSC1 protein, which is not present in the PARA channel.

The DSC1 and PARA channels show a similar organizational plan characteristic of the family of voltage-gated sodium channel \textit{H} \textit{n} \textit{H} \textit{K} \textit{H} \textit{n} \textit{K} \textit{n} \textit{K} subunits (Figures 6 and 7; Catterall 2000). Duplication of an ancestral gene early in evolution and the insertion of the large negatively charged intracellular linker between the second and third homology repeats may have allowed the DSC1 channel to evolve an auxiliary regulatory role to that of the PARA channel in mediating neuronal action potentials (Ramaswami and Tanouye 1989). Because of the absence of detailed electrophysiological information, the ion selectivity of the DSC1 channel remains to be determined.
has been demonstrated previously in lobster olfactory neurons (Zhainazarov and Ache 1997; Zhainazarov et al. 1998). A detailed characterization of the electrophysiological properties of the DSC1 channel in a side-by-side comparison with the PARA channel is, ultimately, needed to provide a better understanding of interactions between these channels at the cellular level.

Whether the DSC1 channel is a voltage-gated sodium channel or a voltage-gated calcium channel, its widespread expression pattern suggests that it also contributes to neural activity outside the olfactory pathway. Thus, it is remarkable that a major reduction in message levels for this ubiquitous channel has only subtle phenotypic consequences. In fact, a sensitive statistical detection assay was necessary to reveal smell impairment of olfactory avoidance responses in the smi60E mutant. The most likely explanation for these observations is that the DSC1 channel has an auxiliary function in fine tuning signal propagation in the chemosensory pathway.

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**Figure 7.**—Schematic representation of the transmembrane organization of the PARA and DSC1 channels. Roman numerals indicate the four tandem repeats (for simplicity, hairpin loops and voltage sensors in each repeat have not been indicated in the diagram). Note the expanded 676-amino-acid intracellular loop in the DSC1 protein in the center of the molecule between repeats II and III.

**Figure 8.**—*In situ* hybridization visualizing DSC1 mRNA in the brain (A and B) and olfactory organs (C–F). Sections in A, C, and E were hybridized with an antisense *dsc1* probe, whereas adjacent sections in B, D, and F were stained with a sense probe to control for nonspecific staining. Staining in the brain (A) is prominent in regions where neuronal cell bodies are located. “l,” lamina; “m,” medulla; “L,” lobula; “al,” antennal lobes; “oc,” the ocelli. The arrows indicate axon tracts arising from ocellar neurons of which the cell bodies are stained. C and E show stained cells in the third antennal segment and the maxillary palp, respectively.
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**LITERATURE CITED**


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