BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **246,** 210–216 (1998)
ARTICLE NO. RC988183

# A New Member of the Amiloride-Sensitive Sodium Channel Family in *Drosophila melanogaster* Peripheral Nervous System

Isabelle Darboux,* Eric Lingueglia,* David Pauron,† Pascal Barbry,* and Michel Lazdunski*,[1]
**Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, UPR 411, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France; and †Laboratoire de Biologie des Invertébrés, INRA, 123, Boulevard Francis Meilland, 06606 Antibes Cedex, France*

Received January 13, 1998

**Amiloride sensitivity is a common characteristic of structurally related cationic channels that are associated with a wide range of physiological functions. In *Caenorhabditis elegans*, neuronal and muscular degenerins are involved in mechanoperception. In animal epithelia, a $Na^+$-selective channel participates in vectorial $Na^+$ transport. In the snail nervous system, an ionotropic receptor for the peptide FMRFamide forms a $Na^+$-selective channel. In mammalian brain and/or in sensory neurons, ASIC channels form $H^+$-activated cation channels involved in nociception linked to acidosis. We have now cloned a new member of this family from *Drosophila melanogaster*. The corresponding protein displays low sequence identity with the previously cloned members of the super-family but it has the same structural organization. Its mRNA was detected from late embryogenesis (14–17 hours) and was present in the dendritic arbor subtype of the *Drosophila* peripheral nervous system multiple dendritic (md) sensory neurons. While the origin and specification of md neurons are well documented, their roles are still poorly understood. They could function as stretch or touch receptors, raising the possibility that this *Drosophila* gene product, called dmdNaC1, could also be involved in mechanotransduction.** © 1998 Academic Press

Amiloride-sensitive sodium channels have been described in a large number of species and tissues. In mammalian brain and/or in sensory neurons, amiloride-sensitive proton activated channels have been recorded and are suspected to be involved in nociception linked to acidosis (1). A highly sodium-selective channel is present in animal epithelia where it participates in the active vectorial sodium transport that is involved in salt homeostasis (2). The *Hirudo medicinalis* leech volume is controlled by a channel related to this epithelial sodium channel (3), and a similar channel is also involved in *Lumbricus terrestris* intestinal sodium transport (4). An amiloride-sensitive ionotropic receptor for the mollusc neuropeptide FMRFamide was characterized in *Helix aspersa* and *Aplysia californica* neurons (5). Most of the corresponding cDNAs have now been cloned. The first one, was the mammalian epithelial sodium channel that is made up of three types of homologous subunits ($\alpha$ENaC, $\beta$ENaC and $\gamma$ENaC) (6-9). The snail FMRFamide activated $Na^+$ channel (FaNaC) was identified as an homo-tetrameric sodium selective channel (10, 11). Acid Sensing Ion Channels (ASIC) are homo- or hetero-multimeric $H^+$-activated cation channels (12-15). All these proteins share significant sequence identity and display the same overall structural organization, characterized by the presence of two hydrophobic domains surrounding a large extracellular loop that includes one cysteine-rich region (16). The family also includes *Caenorhabditis elegans* degenerins. These neuronal (MEC-4, MEC-10, UNC-8) and muscular (UNC-105) proteins may encode amiloride-sensitive channels that are involved in mechanotransduction (17). Mutations in the genes encoding these proteins lead to degeneration of the expressing cells (18).

We report here the identification of a novel member of the degenerin/ENaC/FaNaC/ASIC gene super-family in *Drosophila melanogaster*. Its mRNA was detected after the complete formation of the peripheral nervous system (PNS), at a very late stage of embryogenesis. It is present primarily in a subpopulation of neurons of

[1] Corresponding author. Fax: 33 (0)4 93 95 77 04. E-mail: ipmc@unice.fr.

Abbreviations used: ENac, epithelial $Na^+$ channel; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; md, multiple dendritic; da, dendritic arbor; ASIC, acid sensing ionic channel.

0006-291X/98 $25.00

the embryonic PNS that corresponds to multidendritic neurons (md), and thus was called dmdNaC1 (for *Drosophila m*ulti*d*endritic neuron *Na C*hannel). Since md neurons are thought to function as stretch or touch receptors, a possible role of this protein in mechanoperception, as for the *C. elegans* degenerins, may be postulated.

## MATERIALS AND METHODS

*Cloning of dmdNaC1.* Two subclones of the P1 clone DS 06238 (D26 6 c2 and D26 1 c7, accession numbers L39712 and L39713, respectively) were used to design a sense primer (5′-CAAATAACTAACATTACTGTTTG-3′) and an anti-sense primer (5′-TTCTCAGATTTTTCCTCTGGTAA-3′). These primers allowed amplification by PCR of a 320 base pair fragment from whole *Drosophila* cDNA. This fragment was used to screen a *Drosophila* head cDNA library. A clone of 2740 base pairs was sequenced. It corresponds in fact, to a genomic EcoRI/ EcoRI fragment whose sequence is completely included in the P1 subclones. A search for putative protein coding exons was performed using the Baylor College of Medicine Gene Finder programs. A combination of DSPL program (search for potential splice sites in *Drosophila* sequences) and HSPL program (search for potential human splice sites), together with general protein sequence searches, allowed the identification of a putative cDNA sequence. Two primers surrounding the predicted coding sequence were designed, a sense primer carrying a 5′ EcoRI restriction site (5′-TCGAATTCCACGAGTCCTGCTCTT-3′) and an anti-sense primer carrying a 5′ XhoI site (5′-CCCTCGAGGGCCTTTAAGACCATCGACTAG-3′). These primers were used to amplify a 1867 base pair fragment by PCR with the Expand High fidelity PCR system (Boehringer Mannheim) from approximately 500,000 clones of an adult *Drosophila* cDNA library. After digestion by EcoRI and XhoI, the fragment was subcloned in the EcoRI and XhoI sites of pBSK-SP6-Globin vector (19). Three independent clones were sequenced on both strands. They display an open reading frame of 1818 nucleotides.

*Fly stocks.* All flies stocks were maintained under standard culture conditions. "Wild-type" controls were either Oregon R or w1118.

*RT-PCR analysis of total RNA.* Total RNA was prepared from individual stages, and subsequently treated with DNase I (GIBCO BRL). First strand cDNA for PCR analysis was generated from RNA samples using Superscript II reverse transcriptase (Gibco-BRL). The 20 $\mu$l reaction contains 1 $\mu$g of total RNA, 100 pmoles of oligodT primers, 300 units of Superscript II and dNTPs at a final concentration of 0.5 mM. After incubation at 37 °C for 1 hr, 1 $\mu$l of each reaction was amplified in a 20 $\mu$l reaction containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit of Taq DNA polymerase (Promega) and 10 pmoles of both the sense (5′-CATATGTCGTGTCCACTGAC-3′) and antisense primers (5′-ACTGAGTTCTGCTGTTGTAT-3′). PCR was carried out for 35 cycles, each consisting of denaturation at 96 °C for 1 min; annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. The predicted size of the amplification product was 568 base pairs. Half of the samples were electrophoresed on 1.5% agarose gels and transferred to nylon membranes (Hybond N, Amersham). Blots were hybridized with a dmdNaC1 probe generated by random priming and exposed to Kodak X-OMAT AR film for 14 days at −70 °C with intensifying screens. To estimate the relative amounts of cDNA in each reverse transcription sample, amplification with specific primers of the *Drosophila* rpl17 gene (20) was performed.

*In situ hybridization of whole-mount embryos.* dmdNaC1 transcripts were localized by whole-mount *in situ* hybridization with a non-radioactive probe as described by Tautz and Pfeifle (1989) (21). A digoxigenin-labeled RNA probe corresponding to the entire cDNA sequence was synthesized by random priming according to the manufacturer's instruction (Promega, Riboprobe, Gemini II-Core system). The probe was hybridized overnight at 48°C. Following several washes in PBS, 0.1% Tween, embryos were incubated with antidigoxigenin antibodies conjugated to alkaline phosphatase for 1 hr at room temperature, washed and incubated with alkaline phosphatase color substrates. For double-labelling, embryos were treated for the *in situ* hybridization to visualise the dmdNaC1 transcripts, and subsequently immunocytochemically stained with the monoclonal antibody 22C10 (1:100 dilution). Embryos were mounted in 80% glycerol and viewed with a microscope under Nomarsky optics.

*In situ hybridization to polytene chromosomes.* *Drosophila* salivary gland polytene chromosome squashes were hybridized with biotin-labelled DNA probes, synthesized according to the manufacturer's instruction (Boeringher Mannheim). Hybridization was detected after coloration with diaminobenzidine (Sigma).

## RESULTS AND DISCUSSION

A *Drosophila melanogaster* genomic sequence that displays similarities to the degenerin/ENaC/FaNaC/ASIC gene super-family was found in the GenBank data base in two overlapping subclones of a P1 phage (accession numbers L39712 and L39713), localized to the alcohol dehydrogenase region on the second chromosome. Analysis of the sequence using the Baylor College of Medicine Gene Finder programs revealed a putative complete cDNA sequence included in an EcoRI/ EcoRI fragment of the gene. Two primers surrounding the predicted coding sequence were designed and used to amplify by PCR, from a whole *Drosophila* cDNA library, a 1867 base pairs cDNA fragment (Fig. 1A). This fragment contains an open reading frame of 1818 nucleotides preceded by a stop codon (located just before the 5′ EcoRI site at position +1 in Fig. 1A), and codes for a protein of 606 amino acids (Fig. 1A). Comparison between the cDNA and the genomic structure revealed in the genomic DNA region that contains the coding sequence, the presence of 6 introns, ranging from 55 base pairs to 184 base pairs (Fig. 1A). *In situ* hybridization to wild-type salivary gland chromosomes showed that dmdNaC1 maps to the 35B1-4 region (Fig. 2A), in the *Adh* region on the left arm of the second chromosome (22), confirming the data presented for the P1 phage, i.e. a localization in 35B1-3.

The corresponding protein contains all the conserved motifs characteristic of the family, including two hydrophobic domains flanking a large region that includes a cysteine-rich domain (Fig. 1A, B). It has significant but low sequence identity with the other members of the family (below 20%, Fig. 1B) and cannot be linked to any of them by phylogenetic analyses (Fig. 1B), except for another protein identified in *Drosophila* gonads and early embryo, dGNaC1, which displays 38% identity with dmdNaC1 (Darboux et al., submitted). The strongest similarity with the other proteins occurs around the first and second transmembrane domains, while it is low in the large extracellular loop. The overall structure of dmdNaC1 is closely related to that of the other *Drosophila* protein dGNaC1 (Fig. 1B).

It was impossible to record any channel activity after

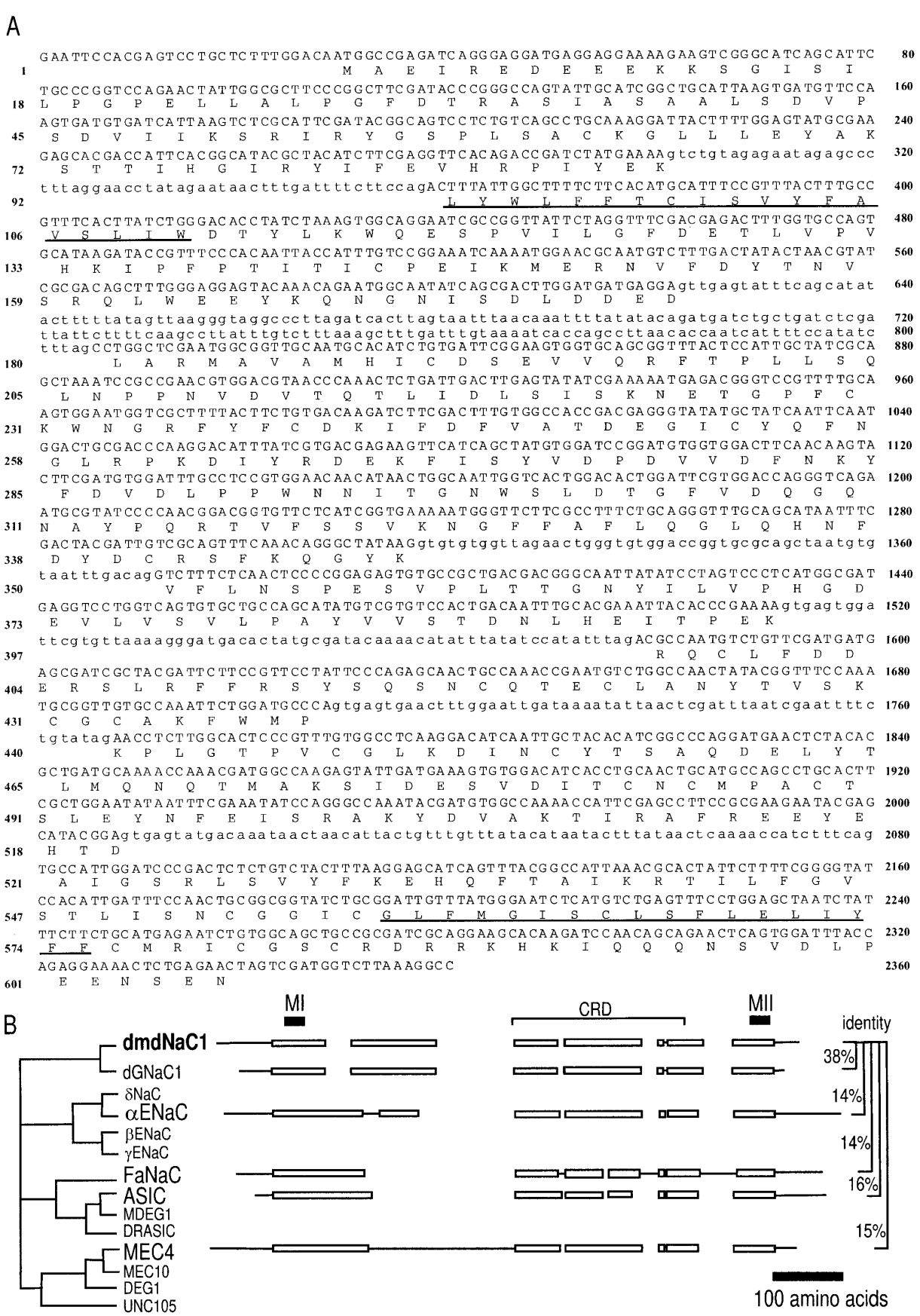


**FIG. 1.** Sequence and structure of the dmdNaC1 gene and of the corresponding protein. (A) Introns, cDNA and predicted protein sequences of the dmdNaC1 gene. The sequence of the dmdNaC1 cDNA is shown, along with the positions and sequences of 6 introns as

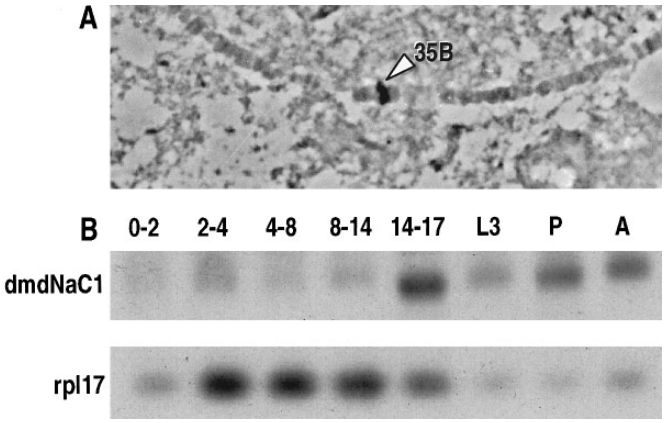


**FIG. 2.** Chromosomic localization and developmental expression of dmdNaC1. (A) *In situ* hybridization of *Drosophila* polytene chromosomes. A cDNA probe labelled with biotin was hybridized to salivary gland chromosomes. The region of hybridization (arrow) is located at position 35B1-4 on the left arm of the second chromosome. (B) RT-PCR analysis of dmdNaC1 gene expression during development. (Top) Southern blot analysis of the 568 base pairs dmdNaC1 PCR product amplified from total RNA extracted from different developmental stages. Lanes are marked according to the specific developmental stage: numbers refer to hours of development after fertilization. L3, third larval stage; P, pupal stage; A, adult stage. Whole dmdNaC1 cDNA was used as a probe. Each sample was subjected to intense amplification (35 cycles). The primers used allowed differentiation between the genomic and cDNA amplification products (sizes of 1015 base pairs and 568 base pairs, respectively). (Bottom) Southern blot analysis of the amplification product for the ribosomal protein L17A gene (rpl17) (20), to estimate the relative amount of cDNA in each RT experiment.

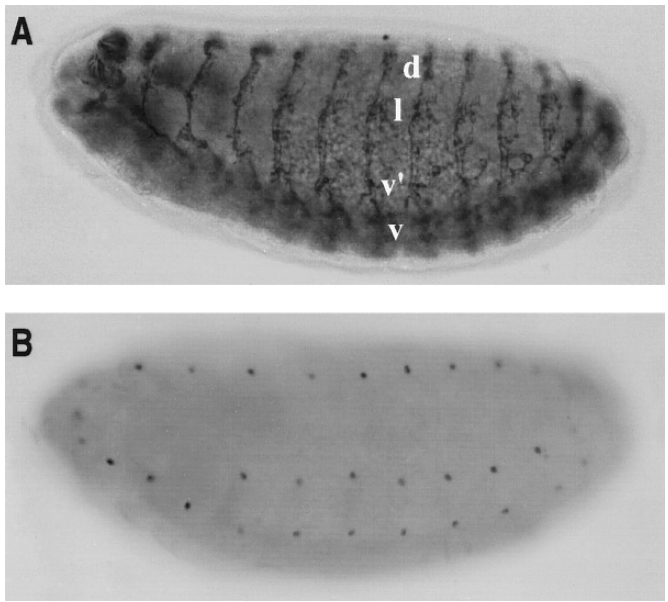


**FIG. 3.** dmdNaC1 expression in stage 15/16 embryo. (A) Double-labelling experiment with a digoxygenin-labelled antisense dmdNaC1 RNA probe and the monoclonal antibody 22C10 (Mab 22C10). In wild-type stage 15/16 embryo, the PNS cells are arranged in a segmental and highly stereotyped fashion along the dorso-ventral axis, forming three groups of dorsal (d), lateral (l) and ventral (v′ and v) clusters per hemisegment, as clearly visualized with Mab 22C10. (B) *In situ* hybridization using a digoxigenin-labelled dmdNaC1 RNA probe. Each abdominal hemisegment contains three clusters of labelled cells; each thoracic hemisegment contains two clusters of labelled cells.

expression of dmdNaC1 *in vitro* transcribed cRNA in *Xenopus* oocytes. A drop of the external pH, activation of protein kinases A and C or addition of FMRFamide and related peptides were not able to activate the channel, nor was the introduction of the S551F or S551L mutations just before the second hydrophobic region (not shown). Corresponding mutations, i.e. substitution of a particular glycine (a small amino acid) for a larger amino acid such as a valine or a phenylalanine results in constitutive activation of the $H^+$-activated channels MDEG1 and ASIC (13, 23). Equivalent mutations in the *C. elegans* degenerins cause cell swelling and neuronal death (18). The lack of dmdNaC1 activity in *Xenopus* oocytes raises the possibility that one or several subunit(s) needed for the channel maturation and/or for the channel activity are missing. They can correspond to homologous subunits, as for the epithelial $Na^+$ channel where the $\beta$ENaC and $\gamma$ENaC subunits are not active by themselves and the full channel activity needs the presence of the $\alpha$ENaC subunit in the complex (7, 9), or as for the MDEG2 subunit of Acid Sensing Ion Channel which is a silent subunit that can modulate the activity of $H^+$-activated cationic channels such as DRASIC and MDEG1 (12). Important modulation by auxiliary non homologous subunit(s) may also exist, as for the *Drosophila* voltage-dependant $Na^+$ channel $\alpha$ subunit encoded by the paralytic

determined from genomic DNA. Intron sequences are in lowercase letters. cDNA sequence is in uppercase letters ; the numbers at left refer to amino acids of the predicted dmdNaC1 protein (which are shown below each codon); those at right refer to nucleotides within the gene. Some differences between the genomic and cDNA sequences exist and are presumably due to strain polymorphisms. They result in silent changes in the protein sequence, except for the K603N substitution. The first in-frame AUG is flanked by a sequence of nucleotides that closely matches to the *Drosophila* consensus for efficient translation initiation (31). Initiation at this AUG would result in a basic protein of 606 amino acids (69.4 kDa). The two putative transmembrane regions MI and MII are indicated by bold lines at the bottom of the sequence. (B) Phylogenetic analysis and structural comparison with other members of the ENaC/ASIC/FaNaC/degenerins family. The phylogenetic tree was established using the GCG Distances program with Kimura substitution followed by the GCG Growtree program with the UPGMA option, from an alignment obtained with the GCG Pileup program. The percentages indicated at the left correspond to protein identity. MI and MII, putative transmembrane domains; CRD, Cysteine-Rich Domain. $\gamma$NaC is an $\alpha$ENaC-like subunit (32). Accession numbers for $\alpha$ENaC, FaNaC, ASIC and MEC4 are X76180, X92113, U94403 and U53669, respectively. Accession numbers for dmdNaC1 and dGNaC1 are Y16225 and Y16240, respectively.

(*para*) locus, which required the presence of the TipE protein for its correct expression in *Xenopus* oocytes (24). On the other hand, some members of the family, such as FaNaC and ASIC, correspond to ligand-gated channels, and the possibility of a direct activation of dmdNaC1 by a specific ligand that would remain to be identified cannot be ruled out.

Expression of dmdNaC1 transcript during *Drosophila* development was assessed by Northern blot analysis and *in situ* hybridization. Unfortunately, no signal could be detected on Northern blot, even with polyA$^+$ RNA, prepared at a variety of developmental stages. This could be due to low abundance of the dmdNaC1 transcript in embryos. We then performed a more sensitive analysis using RT-PCR. As shown in Fig. 2B, the level of dmdNaC1 transcripts is developmentally regulated. In early embryos, dmdNaC1 was slightly detected, and then its level was largely increased at the end of embryogenesis (14-17 hours). The expression remained significant level during larval and pupal stages and in adults. To determine the spatial expression pattern of dmdNaC1 mRNA during embryogenesis, we performed *in situ* hybridization experiments with digoxygenin-labeled antisense RNA probes on whole-mount non-staged embryos. Expression of dmdNaC1 is not detected until the latest stage of em-

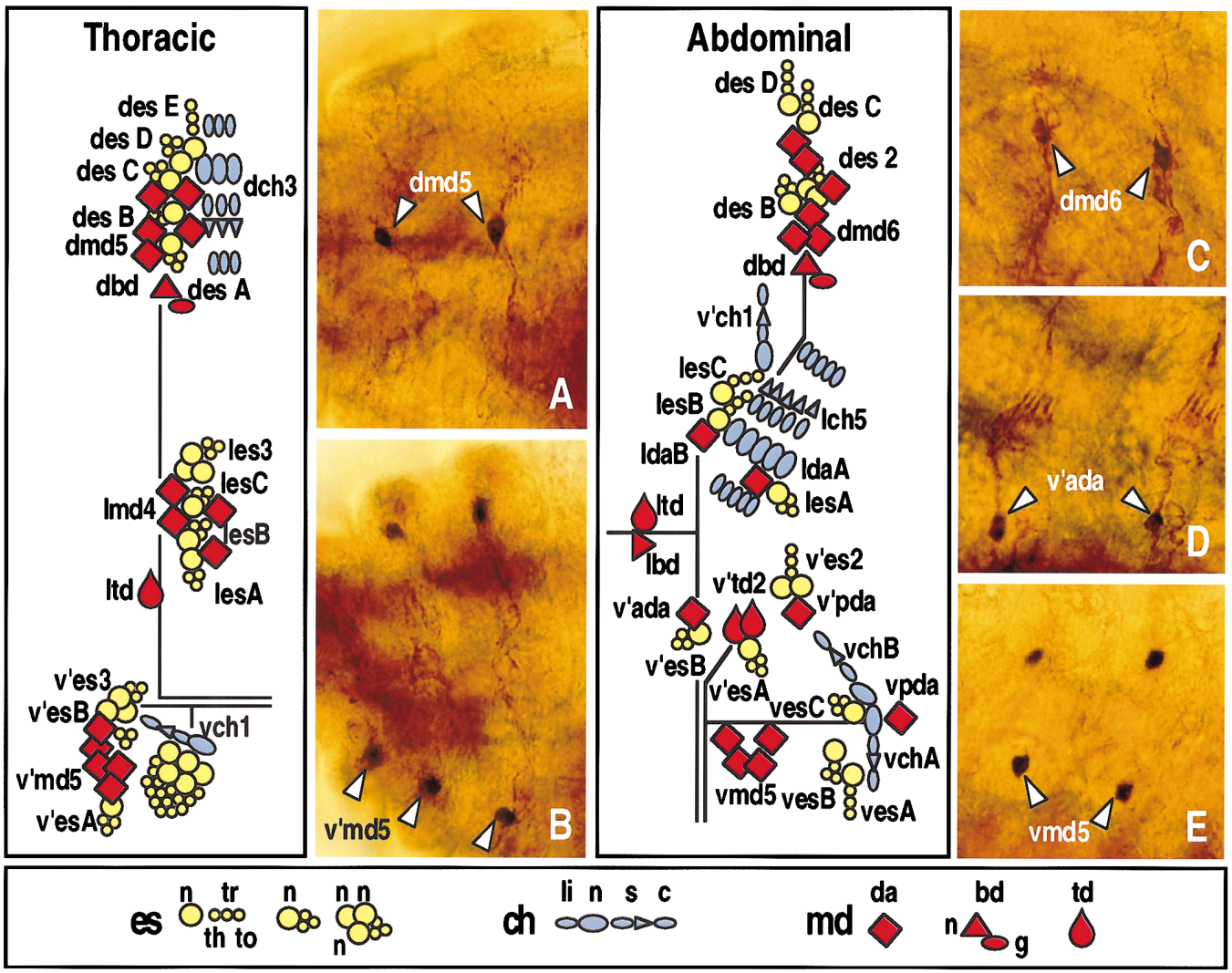


**FIG. 4.** dmdNaC1 is expressed in a subset of multidendritic neurons. The identity of dmdNaC1 expressing cells is determined by double-labelling to visualize cells expressing dmdNaC1 (black, RNA transcripts revealed by peroxidase activity), and PNS neurons (brown, monoclonal antibody 22C10 revealed by alkaline phosphatase activity). Details of the dmdNaC1 transcripts staining are shown in thoracic (A and B) and abdominal (C, D and E) hemisegments with the corresponding diagram of the PNS (redrawn from Bodmer and Jan, (33)). In yellow: external sensory organs (es) ; In blue: chordotonal organs (ch); In red : multiple dendritic neuron (md); (n) neuron; (th) thecogen cell; (tr) trichogen cell; (to) tormogen cell; (li) ligament cell; (s) scolopal cell; (c) cap cell; (da) dendritic arbor neuron; (bd) bipolar dendrite neuron; (g) glial cell; (td) trachea innervating cell. Dorsal is up and anterior is on the left.

bryogenesis. At stage 17 (according to Campos-Ortega and Hartenstein, (25)) a segmentally repeated pattern of expression appeared (Fig. 3B) in a restricted subset of cells corresponding to those of the PNS as confirmed by double-labelling experiments with the monoclonal antibody 22C10 (Mab 22C10) (Fig. 3A). Mab 22C10 is directed against an antigen present in all cell bodies, dendrites and axons within the PNS and a subset of neurons within the CNS (26). The pattern of expression of dmdNaC1 in thoracic hemisegments is different from those of abdominal hemisegments. Two groups of cells are stained in the thoracic segments and three groups are stained in abdominal segments (Fig. 3B).

The PNS of *Drosophila* is composed of three major types of neurons as shown in the diagram in Fig. 4: the external sensory neurons (es) which innervate the mechano- or chemoreceptors in the cuticle, the chordotonal neurons (ch) which innervate internal stretch receptors, and the multiple dendritic neurons (md), which could function as stretch or touch receptors (27, 28). They are all arranged in a segmental and highly stereotyped fashion along the dorso-ventral axis, forming three groups of dorsal (d), lateral (l) and ventral (v′ and v) clusters clearly observed with Mab 22C10 (Fig. 3A). By comparing the diagram of the embryonic PNS with the double staining in whole-mount embryo, we have found that the position of the stained cells in the PNS coincides with those of a subtype of multiple dendritic neurons (md). In the thoracic hemisegments, dmd5 in the dorsal (d) cluster and v′md5 in the ventral (v′) cluster expressed dmdNaC1 (Fig. 4A and B, respectively). In the abdominal hemisegments, dmd6 in dorsal (d) cluster, v′ada and vmd5 neurons located respectively in ventral (v′) and (v) clusters, expressed dmdNaC1 (Fig. 3C, D and E, respectively). Thus, dmdNaC1 transcripts seem to be specific to the third class of sensory neurons in the *Drosophila* PNS corresponding to the md neurons also known as type II sensory neurons. These cells are grouped in three subclasses based on their morphology (28, 29, 30); md-da neurons which extended dendritic arbors below the epidermis, md-bd neurons which extended bipolar dendrites and md-td neurons which extended their dendrites along tracheal branches. All md neurons expressing dmdNaC1 belong to the most abundant subclass of md neurons, the md-da neurons. Their origin and specification are now well studied (27, 29), but their functions are poorly understood.

The late dmdNaC1 expression in md-da neurons coincides with the end of elaboration of the nervous system, after the complete determination and differentiation of neuroprecursors cells in the nervous system. Thus, dmdNaC1 may not be involved in the differentiation of the PNS, but rather in the function of md-neurons. One of the md neurons proposed functions is linked to stretch or touch reception, and it is thus tempting, as other members of the family are involved in similar functions, to propose a role in mechanotransduction for the dmdNaC1 channel subunit.

## ACKNOWLEDGMENTS

This work was supported by the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (INSERM), and the Association Française de Lutte contre la Mucoviscidose (AFLM). I.D. is recipient of a grant from the Association Claude Bernard. We thank Dr. Guy Champigny for the electrophysiological experiments on *Xenopus* oocytes. We are grateful to Drs. John Roote, Christine Dambly-Chaudière, Hideki Sakai, Pierre Léopold, Sylvie Coscoy, and Michel Piovant for fruitful discussions. We thank Dr. Jean-Luc Imler for the generous gift of the adult *Drosophila* cDNA library and Christian Klämbt for the gift of Mab22C10. We thank Dr. Amanda Patel for careful reading of the manuscript. Thanks are due to Franck Aguila for help with the artwork and to Valérie Friend, Martine Jodar, Nathalie Leroudier, and Dahvya Doume for technical assistance.

## REFERENCES

1. Akaike, N. , and Ueno, S., (1994) *Prog. Neurobiol.* **43,** 73–83.
2. Barbry, P. , and Hofman, P., (1997) *Am. J. Physiol.* **273,** G571–G585.
3. Weber, W. M., Dannenmaier, B., and Clauss, W., (1993) *J. Comp. Physiol. B* **163,** 153–159.
4. Cornell, J. C., (1984) *Comp. Biochem. Physiol. A* **78,** 463–468.
5. Green, K. A., Falconer, S. W., and Cottrell, G. A., (1994) *Pflügers Archiv.* **428,** 232–240.
6. Canessa, C. M., Horisberger, J. D., and Rossier, B. C., (1993) *Nature* **361,** 467–470.
7. Canessa, C., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J. D., and Rossier, B. C., (1994) *Nature* **367,** 463–467.
8. Lingueglia, E., Voilley, N., Waldmann, R., Lazdunski, M., and Barbry, P., (1993) *FEBS Lett.* **318,** 95–99.
9. Lingueglia, E., Renard, S., Waldmann, R., Voilley, N., Champigny, G., Plass, H., Lazdunski, M., and Barbry, P., (1994) *J. Biol. Chem.* **269,** 13736–13739.
10. Coscoy, S., Lingueglia, E., Lazdunski, M., and Barbry, P., (1997) Submitted for publication.
11. Lingueglia, E., Champigny, G., Lazdunski, M., and Barbry, P., (1995) *Nature* **378,** 730–733.
12. Lingueglia, E., de Weille, J. R., Bassilana, F., Heurteaux, C., Sakai, H., Waldmann, R., and Lazdunski, M., (1997) *J. Biol. Chem.* **272,** 29778–29783.
13. Bassilana, F., Champigny, G., Waldmann, R., de Weille, J. R., Heurteaux, C., and Lazdunski, M., (1997) *J. Biol. Chem.* **272,** 28819–28822.
14. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C., and Lazdunski, M., (1997) *Nature* **386,** 173–7.
15. Waldmann, R., Bassilana, F., De Weille, J. R., Champigny, G., Heurteaux, C., and Lazdunski, M., (1997) *J. Biol. Chem.* **272,** 20975–20978.
16. Renard, S., Lingueglia, E., Voilley, N., Lazdunski, M., and Barbry, P., (1994) *J. Biol. Chem.* **269,** 12981–12986.
17. García-Añoveros, J., and Corey, D. P., (1997) *Annu. Rev. Neurosci.* **20,** 567–594.
18. Driscoll, M., and Chalfie, M., (1991) *Nature* **349,** 588–593.
19. Waldmann, R., Champigny, G., and Lazdunski, M., (1995) *J. Biol. Chem.* **270,** 11735–7.
20. Noselli, S., and Vincent, A., (1992) *Gene* **118,** 273–278.
21. Tautz, D., and Pfeifle, C., (1989) *Chromosoma* **98,** 81–85.

22. Ashburner, M., Thompson, P., Roote, J., Lasko, P. F., Grau, Y., el Messal, M., Roth, S., and Simpson, P., (1990) *Genetics* **126,** 679–694.
23. Waldmann, R., Champigny, G., Voilley, N., Lauritzen, I., and Lazdunski, M., (1996) *J. Biol. Chem.* **271,** 10433–6.
24. Feng, G., Deak, P., Chopra, M., and Hall, L. M., (1995) *Cell* **82,** 1001–11.
25. Campos-Ortega, J. A., and Hartenstein, V. (1985) The Embryonic Development of *Drosophila melanogaster,* Springer-Verlag, Berlin.
26. Fujita, S. C., Zipursky, S. L., Benzer, S., Ferrus, A., and Shotwell, S. L., (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7929–7933.
27. Brewster, R., and Bodmer, R., (1996) *Dev. Genet.* **18,** 50–63.
28. Ghysen, A., Dambly-Chaudiere, C., Aceves, E., Jan, L. Y., and Jan, Y. N., (1986) *Roux's Arch. Dev. Biol.* **195,** 281–289.
29. Brewster, R., and Bodmer, R., (1995) *Development* **121,** 2923–2936.
30. Bodmer, R., and Jan, Y. N., (1987) *Roux's Arch. Dev. Biol.* **196,** 69–77.
31. Cavener, D. R., (1987) *Nucleic Acids Res* **15,** 1353–61.
32. Waldmann, R., Champigny, G., Bassilana, F., Voilley, N., and Lazdunski, M., (1995) *J. Biol. Chem.* **270,** 27411–27414.
33. Bodmer, R., Carretto, R., and Jan, Y. N., (1989) *Neuron* **3,** 21–32.