MinK-related peptides (MiRPs) are single transmembrane proteins that associate with mammalian voltage-gated K\(^+\) subunits. Here we report the cloning and functional characterization of a MiRP \(\beta\)-subunit, MPS-1, and of a voltage-gated pore-forming potassium subunit, KVS-1, from the nematode *Caenorhabditis elegans*. *mps-1* is expressed in chemosensory and mechanosensory neurons and co-localizes with *kvs-1* in a subset of these. Inactivation of either *mps-1* or *kvs-1* by RNA interference (RNAi) causes partially overlapping neuronal defects and results in broad-spectrum neuronal dysfunction, including defective chemotaxis, disrupted mechanotransduction, and impaired locomotion. Inactivation of one subunit by RNAi dramatically suppresses the expression of the partner subunit only in cells where the two proteins co-localize. Co-expression of *MPs-1* and *KVS-1* in mammalian cells gives rise to a potassium current distinct from the KVS-1 current. Taken together these data indicate that potassium currents constitute a basic determinant for *C. elegans* neuronal function and unravel a unifying principle of evolutionary significance: that potassium channels in various organisms use MiRPs to generate uniqueness of function with rich variation in the details.

MinK-related peptides (MiRPs or KCNEs)\(^1\) are single-transmembrane proteins that associate with pore-forming ion-channel subunits to form stable complexes with channel properties markedly distinct from those of the isolated pore-forming subunits (1, 2). MiRPs were identified recently in an attempt to find a \(\beta\)-subunit for the cardiac potassium channel HERG, which in heterologous expression systems behaves differently than in native cardiomyocytes. Using *in silico* approaches three MiRPs (MiRP1, MiRP2, and MiRP3) were recognized by their homology with MinK, a putative \(\beta\)-subunit of HERG and KCNQ1 (2–5). The last member of the family, MiRP4 was identified later (6). Although MiRPs were initially identified as cardiac proteins, they were soon found to be expressed and function in other tissues and to cause acquired and congenital disease (2, 7–11). For instance, MiRP2 is expressed in skeletal muscle where it associates with Kv4.3 and, when defective, can cause periodic paralysis (8). Mutations in MinK and MiRP1 genes can lead to Long QT syndrome, a specific form of polymorphic ventricular tachycardia characterized by impaired ventricular repolarization (2, 10–12). A well-established characteristic of MiRPs is the capacity to associate with multiple pore-forming subunits in heterologous systems. For instance, MiRP1 can associate with HERG, KCNQ1, HCN, and Kv4.2 subunits (2, 13–15). Indeed, MiRP “promiscuity” has consider-able biomedical implications, because, if a single MiRP co-assembles with multiple pore-forming subunits, genetic mutations would be predicted to lead to disruption of multiple currents simultaneously.

To date MiRPs have been reported only in vertebrates, suggesting that MiRPs might be relatively young in the evolutionary scale. We speculated that they might underlie a more general role, and therefore we sought to identify potential MiRPs homologous in lower organisms such as the nematode *Caenorhabditis elegans*. The comparative simplicity of *C. elegans* invites a comprehensive description of any biological aspect of a gene. This is particularly relevant to studies involving genetic, physiological, and structural aspects of ion channels proteins. Thus, the existence of *C. elegans* MiRPs would be of considerable interest with respect to conservation of K\(^+\) channel subunits and to the possibility of using *C. elegans* as a model system to understand how MiRPs contribute to cellular function and how defects in these proteins might alter cellular signaling.

We report here the cloning and functional characterization of the first MiRP from the nematode *C. elegans* (*mps-1*). *mps-1* is expressed in the *C. elegans* nervous system and represents the first example of MiRP that is essential to neuronal excitability. We also report the cloning and characterization of a *C. elegans* voltage-gated K\(^+\) channel (*kvs-1*) partner for *mps-1*. KVS-1 shares a significant homology with human K\(_{\text{Na}}\)\(_{2}\), a neuronal subunit and well-recognized partner of MiRP1 (15). In this report we show that KVS-1 is abundantly expressed in the *C. elegans* nervous system and that it associates with MPS-1 in a subset of sensory cells where both are required for normal neuronal function.

**MATERIALS AND METHODS**

Cloning of *kvs-1* and *mps-1*—Cloning of *kvs-1* and *mps-1* was performed with a Smart Race kit (Clontech) using poly(A)\(^+\) mRNA extracted from total *C. elegans* RNA with a Gilotex kit (Qiagen). The primer for *kvs-1* 5’-RACE was GGGCCACCAAGCTTCTAGTAGGAG-GACTCA and that for 3’-RACE was GACCCTTGATGTATGCCCCA-
A MiRP in *C. elegans*

GAGAATTGCCACCGAACCTC. The primer for mps-1 5’-RACE was GTTACATTAGGAAATATTGCAAAAT to that for 3’-RACE was GAGGATGACATGTCGTGCGGAT. cDNA was amplified by PCR and inserted in pCI-neo vector (Promega) for functional expression in CHO cells. All sequences were confirmed by automated DNA sequencing. Transcripts were quantified with spectrophotometry and compared with control samples separated by agarose gel electrophoresis stained with ethidium bromide. These novel genes have been assigned the GenBank accession numbers AF541979 for kvs-1 and AF541978 for mps-1 by the Genome Data Base Nomenclature Committee.

Construction of Tugged Reporter Fusions to GFP—We constructed both translational and transcriptional GFP reporters. To obtain transcripts encoding the open reading frame of the plate border gene *mps-1* (translation) and *mps-1* (transcription—PCR products) (Fig. 1D), we employed a method developed by Yuan et al. (16). The last ~1 kb of *kvs-1* and *mps-1* were amplified by PCR from genomic DNA and joined in-frame to the GFP reporter gene in the pPD 95.75 vector (1995 Fire Vector Kit, generously provided by Dr. A. Fire). The reporter constructs and the cosmid were linearized and co-injected into the syncytial gonad of adult hermaphrodite N2 nematodes. Because the constructs intentionally lack the promoter and the initial methionine, they are not translated without recombination with the cosmid (which contains the entire gene and its promoter). Thus, this strategy increases the likelihood of detecting all tissue-specific expression, because all upstream regulatory sequences are incorporated, and it ensures high specificity, because it requires the cosmid for recombination. For *kvs-1::gfp* the primers were 5’ (direction) ATGGATCCTATTAAGCTACATGAAGTGATTTTCTATGCTAGTATTTTCTGCGCTAATCACGAGG and ATGCCTGGATACATCATACGCTTCCACCTGTTCTGCAGTCA-TGT in the forward and reverse direction, respectively. For *mps-1::gfp* the primers were ATGGATCCTATTAAGCTACATGAAGTGATTTTCTATGCTAGTATTTTCTGCGCTAATCACGAGG and ATGCCTGGATACATCATACGCTTCCACCTGTTCTGCAGTCA-TGT in the forward and reverse direction, respectively. To create *P_{mps-1}::gfp* a ~3-kb fragment of intronic sequence upstream exon 2 containing 20 bp of the exon was cloned into pPD95.75. Primers were: GCCAATATTCAGCTAGCACTA-AGAGTTAAGCACCTATGAGTAAGTGGCAGCCGCTTTCGCTGTACCTATGGTACAT in the forward and reverse direction, respectively. To construct *P_{mps-1}::gfp* a ~4-kb fragment of intronic sequence upstream exon 2 primers were GCAGTGGGTCCTCACTTCAATCAGGG and TTAGAGTACTGTCAGATGAGTATTAATACGCTCATTACATACGAGG and 5’-TATTATATATATATAGGATGATAGGGAAGACATTACATCGACTAAT- AACACTAATAAGCACCAAG, and cDNA was used as the template. The transcriptional promoter was *linH4-5* (~50 ng/μl). The transgene was transformed into *C. elegans* by RNAi mediated gene silencing using a dsRNA with C29F5.4 (translation) and C29F5.4 (transcription—PCR products) (Fig. 1D), and Clampex software (Axon), filtered at 1 kHz, and sampled at 2 kHz. After 15 min at room temperature. After fixing, cells were washed three times for 5 min with PBS and blocked for 1 h at room temperature with 5% nonfat dry milk in PBS plus 0.1% Tween 20. Cells were incubated with the secondary antibody, Cy3-conjugated goat anti-mouse (Jackson Immunoresearch) (1:2000, in 5% nonfat dry milk in PBS plus 0.1% Tween 20) for 1 h at room temperature, and subsequently washed three times for 5 min with PBS.

**RESULTS**

Using in silico approaches, we identified a predicted MiRP-related *C. elegans* protein, C29F5.4 (mps-1) and confirmed its expression and primary sequence by analysis of reverse transcription-PCR products (Fig. 1A–C). Interestingly, the *mps-1* N terminus and transmembrane domain exhibit significant homology to human MiRP1, but the C terminus is more similar to human MiRP3, suggesting that MiRP1 and MiRP3 might be derived from a common ancestor shared with nematodes (Fig. 1B).

MiRPs do not form channels on their own (2). The *C. elegans* genome encodes many potassium channel homologues that could be potential interaction partners of MiPS-1 (22). We focused on predicted potassium channel subunit C53C9.3 (kvs-1) (Fig. 1D–F) as a candidate partner for MiPS-1, because 1) potassium channel C53C9.3/KVS-1 shares significant homology with human Kv4.2, known to associate with MiRP1 (15) and 2) microarray data suggest *mps-1* and *kvs-1* gene expression is co-regulated in different growth conditions and at different developmental stages (23). We determined expression patterns of *mps-1* and *kvs-1* by...
analyzing transgenic animals harboring translational and transcriptional reporter fusions. We found \textit{mps-1} expression in ASG, ASE, ADF, and ASH neurons (Fig. 2, A–F, and Table I; we confirmed identities of the latter by scoring for DiD filling (24)), AWC (see Fig. 4A and Table I), in ALM and PLM touch-sensing neurons (Fig. 2, G and H), and in the vulva (Fig. 4B). We detected \textit{kvs-1} expression in more than 10 cells in the head, including the amphid neurons ADL, ASK, ASH, ADF, ASE, AWC (Fig. 2, I–Q, and Table I), and ASG (not shown, Table I), in ventral cord neurons (Fig. 2R), in the motoneuron PDA (not shown) in the anal depressor muscle (Fig. 2S) and in sperm (not shown). Thus, MPS-1 has the potential to influence the activities of multiple potassium channels expressed in diverse neurons and cell types. Moreover, the co-expression of \textit{mps-1} and \textit{kvs-1} in AWC, ADF, ASG, ASE, and ASH neurons suggests MPS-1 and KVS-1 may form a functional complex in these amphid neurons, which mediate diverse chemosensory responses (25).

To probe the physiological roles of \textit{mps-1} and \textit{kvs-1} in nervous system, we screened for potential behavioral defects predicted for impaired neuronal function of the cells that appear to express \textit{kvs-1} and \textit{mps-1} using double-stranded RNA-mediated gene inactivation (RNAi). RNAi was fairly effective in knocking down the expression of GFP-tagged MPS-1 and KVS-1 proteins (Figs. 4, C–D and K–L) confirming RNAi targeting of their transcripts. \textit{mps-1} RNAi induced defects in body touch sensation (Fig. 3A), chemotaxis to biotin and lysine, osmotic avoidance, and nose-touch collision (Fig. 3, B–E), phenotypes that correlate well with the detected presence of \textit{mps-1} in ALM and PLM touch-sensing neurons (body touch sensation) and in ASG, ADF, and ASE (chemotaxis to biotin and lysine) and ASH amphid neurons (osmotic avoidance and nose-touch, see Fig. 3 legend and Table I). \textit{mps-1} RNAi did not affect chemotaxis to benzaldehyde (Fig. 3G) and isoamyl alcohol (Fig. 3H) that are mediated by AWC neurons where \textit{mps-1} appears to be expressed, and, as expected, it did not affect other sensory functions such as chemotaxis to diacetyl, octanol avoidance, and thermotaxis that are mediated by neurons that not appear to express \textit{mps-1}. We conclude that \textit{mps-1} is required for the normal function of several neuronal types in which this MiRP is expressed.

**Fig. 1.** The \textit{C. elegans} genome encodes a MiRP-related protein and a Kv4.2-related protein that is a candidate interaction partner. A, the genomic organization of \textit{mps-1}. Exons are indicated by boxes, introns by lines. The gene is spliced in six exons in contrast to human MiRPs that are generally contained in a single exon. B, MPS-1 protein sequence and alignments with MiRP1 (20% identity and 50% homology) and MiRP3 (20% identity and 61% homology). Alignment by ClustalW (available at bioweb.pasteur.fr). C, hydropathy plot of MPS-1. Plot was calculated with the Kyte and Doolittle algorithm (available at bioweb.pasteur.fr/seqanal/interfaces/toppred.html). Top, a schematic representing MPS-1 predicted topology. The secondary structure predicts a single-span protein that has the basic topology of mammalian MiRPs and that is an extracellular N terminus, a single transmembrane domain, and an intracellular C terminus. The branch structure indicates a potential N-glycosylation site, a feature common to other members of the family. D, the genomic organization of \textit{kvs-1}. Roughly 10% of the genomic sequence encodes the cDNA. E, the protein sequence of KVS-1 and alignment with the human voltage-gated K\textsubscript{v}4.2 (20% identity and 40% homology). Transmembrane helices (S1–S6) and the potassium-channel signature are indicated. F, hydropathy plot of KVS-1. Top, the predicted topology of KVS-1 forecasts an ion-channel protein with a large intracellular N domain, six transmembrane domains, including an S4 span with multiple positively charged residues arrayed as in other channels activated by voltage and a pore loop with a potassium channel signature sequence.
Importantly, like *mps-1* RNAi-treated animals, *kvs-1* RNAi-treated animals exhibited impaired chemotaxis to lysine and to biotin (Fig. 3, B and C), defective osmotic avoidance (Fig. 3D), and nose-touch responses (Fig. 3E), consistent with compromised ADF, ASG, ASE, and ASH function (Fig. 3 and Table I) (19, 20, 25, 26). Nematodes co-injected with a mixture of dsRNA of both *mps-1* and *kvs-1* did not exhibit significant enhancement in defects in osmotic avoidance and nose-touch (Fig. 3, D and E). This suggests phenotypes we observe reflect maximal disruption of these behaviors and that the two subunits contribute similarly to the behaviors, possibly as parts of the same channel complex.

Consistent with the *kvs-1* cellular expression pattern, RNAi induced defective forward movement (Fig. 3F) and defective chemotaxis to benzaldehyde (Fig. 3G) and to isoaeryl alcohol (Fig. 3H). Benzaldehyde and isoaeryl alcohol chemotaxis are specifically mediated by AWC neurons, which appear to express *kvs-1* (25). Other sensory functions such as thermotaxis and octanol avoidance, controlled by neurons that did not express *kvs-1*, were unaffected.

To test the hypothesis that *mps-1* and *kvs-1* belong to the same channel complex in some sensory neurons, we assessed the effect that epigenetic inactivation of each gene has on expression of its putative partner. For this purpose we exploited *kvs-1::gfp* and *mps-1::gfp* transgenic nematodes that express KVS-1 and MPS-1 subunits fused to GFP proteins. Inactivation of *kvs-1* by RNAi dramatically suppressed *mps-1::gfp* signals only in those cells where the two proteins co-localize, that is ADF and ASE (Fig. 4, A–F, and O), and ASG and ASH (not shown) with the exception of AWC neurons. Reciprocally, *mps-1* RNAi selectively inhibited *kvs-1::gfp* fluorescence in the same neurons (ADF and ASE (Fig. 4, I–N and P) and ASG and ASH (data not shown)). Thus, it appears that in the cells where both proteins co-localize the stability of KVS-1 depends on *mps-1* and vice versa. These data are consistent with the observation that in AWC neurons simultaneous inactivation of both genes produces the same effect as inactivation of each gene separately (Fig. 3, D and E). Furthermore, they may account for the fact that *mps-1* and *kvs-1* RNAi impair neuronal functions to the same extent in the neurons where they co-localize (Fig. 3, B–E). They may also imply that, in the cells where the two subunits do not co-localize, they might associate with other unidentified endogenous subunits rather than exist in homomeric form. Moreover, they suggest that *mps-1* and *kvs-1* might interact to form a channel complex early during protein biosynthesis. Alternatively, each subunit might be required to confer structural stability to the complex at the plasma membrane, although this possibility seems unlikely, because in mammalian expression systems, both *kvs-1* (Fig. 5A) and *mps-1* (Fig. 4, G and H) appear to be stable when expressed alone.

Because our data suggested that *mps-1* associates with KVS-1 in some amphid neurons, we conducted electrophysiological studies to compare the functional properties of KVS-1 channels alone and with *mps-1*. We expressed KVS-1 and *mps-1* in Chinese hamster ovary (CHO) cells and used the whole-cell configuration of the patch clamp to characterize currents. When we transfected KVS-1 cDNA alone, we found a novel voltage-gated, potassium-selective ion channel opened by depolarization. The opposing effect of rapid activation and inactivation generated a typical “A-type” profile, characteristic of the human K_v1.1 and K_v4 potassium channel sub-families (Fig. 5A) (27). By progressive substitution of bath potassium with sodium, we found the KVS-1 channel to be selective for potassium, which shifted reversal potential by \( kT/e \approx 20.5 \pm 1.7 \text{ mV} \) (Fig. 5D). We assessed monovalent cation selectivity by determining bi-ionic permeability ratios and found the KVS-1 profile corresponded to a type IV series, potassium > rubidium > cesium > sodium = lithium, with sodium -100-fold less permeant than potassium (\( P_{K}/P_{Na} = 0.01 \) \( n = 7 \); data not shown). Thus, *C. elegans* KVS-1 encodes a K-v4-like channel with properties similar to mammalian channels of the Kv4 family, indicating functional conservation suggested by sequence homology.
The expression of the two genes in each of the listed amphid neurons was determined by translational constructs (mps-1::gfp and kvs-1::gfp).

| Gene    | ADP+ | ADL | ASE+ | ASG+ | ASH+ | ASK | AWC |
|---------|------|-----|------|------|------|-----|-----|
| mps-1::gfp | +/+P  |     |      |      |      |     |     |
| mps-1 RNAi | Yes  |     |      |      |      |     |     |
| kvs-1::gfp | +/+P |     |      |      |      |     |     |
| kvs-1 RNAi | Yes  |     |      |      |      |     |     |

* Neurons in which mps-1 and kvs-1 were co-expressed and produced similar phenotypes when inactivated.

+ + indicates detectable GFP fluorescence, + + indicates strong intensity. The suffix "P" indicates that expression was also detected with a transciptional construct confirming the complete translation of mps-1::gfp and kvs-1::gfp (see "Materials and Methods"). Transcriptional reporters revealed only partial expression suggesting the existence of regulatory sequences not contained in the promoter region. The cases in which one subunit was expressed in a certain neuron type and its epigenetic inactivation by RNAi was associated with altered function of the same neuron are indicated by "yes."

Fig. 3. kvs-1 and mps-1 RNA interference results in a broad spectrum of neuronal dysfunction. A, inactivation of mps-1 but not of kvs-1 causes insensitivity to body touch. Animals were tested six times for response to light touch to the head behind the pharyngeal bulb and to the tail with an eyelash. Responses to head and tail touches were recorded as backward and forward movement, respectively. The overall response to touch causes insensitivity to body touch. Animals were tested six times for response to light touch to the head behind the pharyngeal bulb and to the tail glutamatergic neurotransmission in C. elegans, was employed as "sensory-defective" positive control. Chemotaxis index (C.I.) = (number of animals at odorant − number of animals at diluent)/(total number of animals). A C.I. of 0 indicates complete attraction; a C.I. of 0 indicates a random distribution of worms on the assay plate. Attraction to biotin is mainly mediated by ASE and to a lesser extent by ASG, ASI, and ADF neurons (20). C, under the same conditions as in B mps-1 or kvs-1 RNAi worms do not respond to lysine. Chemotaxis to lysine depends on the partly redundant functions of ASE, ASG, ASI, and ASK (20). D, mps-1 and kvs-1 RNAi worms do not avoid high osmotic strength. Fraction that avoid = (number of animals retained by the glycerol ring)/(total number of animals assayed). A fraction of 1.0 represents complete osmotic avoidance; a fraction of 0 indicates that all animals escaped the ring. Osmotic strength sensing is characteristic of ASH neurons (36). E, mps-1 and kvs-1 RNAi worms do not respond to touch to the nose. Animals were scored 10 times each for response to nose touch (collision test). Avoidance was quantitated as the percentage of trials in which animals responded to touch with an eyelash by stopping forward movement or reversing. This function is mainly carried out by ASH neurons (26). F, worms treated with dsRNA encoding for kvs-1 but not for mps-1 exhibit marked locomotion defects as assayed by the "thrashing" test. Worms at the L4 larval stage were picked to a drop of M9 buffer on an agar plate. After 2 min of recovery, thrashes were counted for 2 min. A thrash was defined as a change in the body bend at the mid-body point. G and H, benzaldehyde (G) and isoamyl alcohol (H) attraction was defective in dsRNA kvs-1- but not in mps-1-treated nematodes. AWC neurons mediate this function (21). For all panels, error bars represent S.E. Significant differences from N2 (p < 0.05 and 0.01) are indicated with * and **, respectively. Each data point represents the average of at least three independent assays using a minimum of 20 animals per assay.

As expected from work with mammalian MiRPs, when we co-transfected MPS-1 with KVS-1, we identified several novel features of the introduced current, suggesting co-assembly (Fig. 5C). As expected, MPS-1-KVS-1 channels were selective for potassium (kTle = 19.0 ± 1.7 mV, Fig. 5D). Heterologously expressed macroscopic KVS-1 currents showed half-
maximal activation ($V_{1/2}$) at 44.6 ± 1.8 mV with a slope factor ($V_0$) of 21.7 ± 1.7 mV (Fig. 5E). Co-expression with MPS-1 induced a left shift in the midpoint for activation ($V_{1/2}$ = 32.6 ± 1.2 mV) without significantly affecting the voltage-dependence ($V_0$ = 20.1 ± 1.1). Although KVS-1 subunits inactivated with a rate of $\tau$ = 39.9 ± 4.4 ms at +120 mV, MPS-1-KVS-1 channels were faster ($\tau$ = 21.3 ± 3.0 ms, Fig. 5F). We quantified recovery from inactivation with whole-cell currents using 1.2-s and 0.2-s depolarizing pulses at +60 mV spaced out by progressively longer periods at −80 mV (Fig. 5G). The time course was best fit to a single-exponential function with $\tau$ = 9.1 ± 0.2 ms for KVS-1 alone and $\tau$ = 20.0 ± 0.6 ms with MPS-1-KVS-1. MPS-1 effects extended to pharmacology. We tested tetraethylammonium and 4-aminopyridine (4-AP), two classic potassium channel blockers that have been assessed on ASE native potassium currents and on Kv4 family members (29–31). We found that both cloned channels were resistant to tetraethylammonium (20 mM). In contrast, 4-AP inhibited MPS-1-KVS-1 complexes more efficiently than channels formed with KVS-1 subunits alone. Dose-response curves revealed equilibrium inhibition constants of $K_i$ = 6.2 ± 1.0 mM for MPS-1-KVS-1 channels and $K_i$ = 25.6 ± 4.1 mM for channels formed with KVS-1 subunits alone. In both cases, the relationships of fractional current and 4-AP fit well to the Hill function with $n$ ~ 1 suggesting that a single 4-AP molecule inhibits one channel complex (Fig. 5I).

Taken together, electrophysiological and pharmacological analyses establish that a C. elegans MiRP can alter the properties of a specific C. elegans K+ channel, demonstrating functional conservation of MiRP activities and suggesting that a MPS-1-KVS-1 channel may assemble in vivo to influence neuronal function.

The human MiRP1 and Kv4.2 subunits co-assemble in heterologous expression systems (15). Channels formed by hKv4.2 subunits, alone and with hMiRP1, have been characterized in Xenopus oocytes (15, 31). Homodimeric hKv4.2 channels inactivate and recover from inactivation at rates ~13 and ~300 ms,
respectively (at +60 mV), and are inhibited by 4-AP in the millimolar range (~1 mM). Co-assembly with hMiRP1 slows inactivation kinetics (~44 ms), does not affect recovery from inactivation, and increases 4-AP residency time probably by hindering conformational changes of the channel pore (15). The analogy of this channel complex with *C. elegans* MPS-1/KVS-1...
Fig. 6. MPS-1 and hMiRP1 share common structural determinants. A, whole-cell hMiRP1-KVS-1 currents elicited with voltage jumps from −80 mV to +120 mV in 20-mV increments with a 1-s interpulse interval. Scale bars, 100 pA and 20 ms. B, inactivation rates for hMiRP1-KVS-1 complexes. Time constants were obtained as described in Fig. 5. E, co-expression with hMiRP1 makes KVS-1 channels inactivating faster (τ = 17.5 ± 3.1 ms at +120 mV) than homomeric KVS-1 channels (τ = 39.9 ± 4.0 ms, dotted line). Data from eight cells. C, hMiRP1 slows recovery from inactivation. Like MPS-1, hMiRP1 slows down the recovery rate from τ = 9.1 ± 0.2 ms (KVS-1 alone, dotted line) to 25 ± 3.0 ms. Data from four cells. D, hMiRP1 increases sensitivity to 4-AP. Fit to the Hill equation gave Kᵢ = 12.5 ± 3.1 mM and n = 1.0 ± 0.1, whereas for KVS-1 channels (dotted line) Kᵢ = 25.6 ± 4.1 mM. Data from four cells. E, whole-cell MPS-1-hKv4.2 currents elicited with voltage jumps from −80 mV to +120 mV in 20-mV increments with a 1-s interpulse interval. Scale bars, 90 pA and 20 ms. F, MPS-1 slows down inactivation kinetics of hKv4.2. Homomeric channels (filled triangles) inactivated with τ = 12.3 ± 3.2 ms at +120 mV. Co-expression with MPS-1 (hollow triangles) yielded τ = 24.8 ± 3.4 ms. Data from groups of eight cells. G, MPS-1-hKv4.2 complexes recovery from inactivation like hKv4.2 channels alone. Recovery rates were τ = 294 ± 34 ms and τ = 357 ± 44 ms for hKv4.2 and MPS-1-hKv4.2 channels, respectively. Data from groups of four cells. H, MPS-1 decreases hKv4.2 susceptibility to 4-AP. Inhibition constants were obtained from fits to the Hill equation of dose-response curves. Kᵢ = 1.7 ± 0.3 mM for hKv4.2 and 6.8 ± 0.8 mM with MPS-1. The Hill coefficient was at unity in both cases. Data are from groups of four cells.

**DISCUSSION**

**KVS-1 Is a Novel K⁺ Subunit That Contributes to the C. elegans Neuronal Current Iₖ**—We report here the identification of a novel C. elegans K⁺ channel gene, kvs-1, that is required for the normal function of several C. elegans neurons. KVS-1 is broadly expressed in nervous system, in anal depressor muscle, and in sperm. Disruption of KVS-1 function by RNAi is associated with a variety of neuronal defects ranging from impaired locomotion to defective chemotaxis. These defects correlate well with the cellular expression pattern of the KVS-1 protein. More specifically, kvs-1 RNAi leads to defective chemotaxis to lysine, biotin benzaldehyde isoamyl alcohol, osmotic avoidance and nose-touch, which are specific functions of neurons such as ASK, ASH, ASG, ASE, AWC, and ADF (25).

Unlike vertebrates, C. elegans neurons can signal effectively without classic sodium action potentials (the C. elegans genome does not encode voltage-gated sodium channel genes) (22, 30, 32). Elegant studies on ASE neurons suggest that voltage-dependent potassium currents play a fundamental role for the
functions of *C. elegans* neurons by contributing to both the maintenance and modulation of cell sensitivity (30). Our data support this notion by the identification and functional characterization of this novel voltage-gated potassium channel.

**MPS-1 Associates with KVS-1 in a Subset of Amphid Neurons—** *C. elegans* *mps-1* is the first reported invertebrate MiRP homologue. We propose that in ADH, ASG, ASE, and ASH neurons, MPS-1 works in conjunction with specific K⁺ channel KVS-1 to modulate the electrical activity of these neurons. First, *kvs-1* and *mps-1* expression overlaps in these cells. Second, *mps-1* RNAi is consistent with altered function of these sensory neurons. In particular, the simultaneous inactivation of *mps-1* and *kvs-1* leads to effects on ASH function that are identical to those provoked by inactivation of each gene separately arguing that the two subunits could contribute to the same channel complex. Third, each subunit controls expression and/or stability of the other in neurons where both are expressed. Although the *mps-1* knockdown effects on KVS-1 expression are consistent with previous observations showing that mammalian MiRPs alter protein expression levels of some pore-forming subunits (4, 14, 33), *kvs-1* RNAi effect on MPS-1 protein levels is a novel observation suggesting that neither subunit is dispensable in physiological cell types in which a complex is programme to be assembled. Fourth, in CHO cells KVS-1 forms functional channels whose characteristics are markedly altered upon co-expression with MPS-1. Thus MPS-1 alters voltage dependence of activation, inactivation, residual current, recovery from inactivation, and susceptibility to the K⁺ channel blocker 4-aminopyridine. Interestingly, both KVS-1 and MPS-1 in CHO cells are required to produce a current that has similar characteristics to native currents found in neurons, such as ASEs (29, 30, 32), that endogenously expressed. Overall, several lines of evidence corroborate the notion that in some amphid neurons KVS-1 and MPS-1 associate to form a potassium channel complex that plays an important physiological role. Further efforts will be required to ascertain, however, whether additional endogenous subunits might contribute in these cells to form native MPS-1-KVS-1 complexes.

**mps-1 Is a Gene Influencing Several Neuronal Functions—** MPS-1, like KVS-1, is involved in the control of a variety of neuronal functions. Some defects are common with *kvs-1* phenotypes. Others, such as body touch sensation, are characteristic only of *mps-1* and argue that MPS-1 might promiscuously partner with multiple pore-forming subunits. The identity of other putative partners for MPS-1 has still to be ascertained, however, the physiological relevance of MiRP promiscuity, as well as the potential for leading to multiple dysfunction when *mps-1* is disrupted, is now validated. We did notice one cell type in which MPS-1 and KVS-1 are co-expressed but do not appear to co-assemble, the well-characterized AWC neurons. Specific chemosensory functions mediated by AWC neurons such as chemotaxis to benzaldehyde and isomyl alcohol (25) are unaffected by *mps-1* RNAi but are disrupted by *kvs-1* RNAi. Consistent with the observation, *kvs-1* knockdown does not alter *mps-1* expression in this cell type. AT present the role of *mps-1* in AWC cells as well as the identity of interaction partners remains elusive, but we note that subtle defects in adaptation or developmental specificity may have been missed in our general chemosensory assays.

**MiRPs Share Common Structural Determinants across Phy- llla—**Our data suggest that MiRPs might play conserved roles in modulation of K⁺ channel function. MPS-1, which displays a high level of homology with human MiRPI, especially in the N terminus, can modulate hKv4.2 function and so too hMiRP1 modifies KVS-1 functional attributes. 4-AP binding and hKv4.2 channel inactivation are mutually exclusive, arguing that the structures that are important for inactivation might also be involved in 4-AP binding (31). Probably 4-AP binds to a site at, or adjacent to, the domains involved in channel inactivation and induces conformational changes in the channel preventing ion permeation through the pore (31). MiRPs simultaneously influence channel inactivation and 4-AP blockade, thus disclosing an intimate correspondence between the role played by MiRPs in the two channel complexes. Although it is not clear yet whether MiRPs alter susceptibility to 4-AP by acting on the binding site or by modifying the geometry of the pore, these data suggest the existence of general structural and functional principles that seem to be conserved among MiRPs of diverse species. This correspondence also validates the possibility to use in the future the *C. elegans* MPS-1-KVS-1 complex as a useful tool to investigate biochemical and biophysical aspects of its human homologue MiRP1-Kv4.2.

**MiRPs May Provide Functional Diversity in the *C. elegans* Nervous System—**Our data provide molecular evidence to the general idea that a significant degree of heterogeneity in neuronal potassium flux underlies functional differences among neurons (30). Such complexity is also observed in the human nervous system (34) highlighting a general principle conserved across species. The molecular bases for neuronal diversity may arise through differential expression of potassium channel α-subunits with further diversity accomplished by combination of MiRPs with K⁺ channel α-subunits. This combinatorial arrangement is advantageous from a genetic point of view, because it provides multiplicity and uniqueness through combinations of only a few gene products. In this study we found that both *mps-1* and *kvs-1* are expressed in, and required for the function of, many neuronal types. In humans MiRPs are essential to many biological functions and can lead to inherited and/or acquired disease (2, 8, 10, 11, 35). Here we show that MiRPs are not restricted to higher organisms; rather, MiRP modulation of channel function may represent an ancient mechanism to achieve functional diversity in the nervous system.

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