

REVIEW

An Evolutionarily Conserved Family of Accessory Subunits of K⁺ Channels

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Abstract

Accessory subunits are an essential feature of voltage-gated potassium (Kv) channels. They determine trafficking to the plasma membrane, surface expression, gating, permeation, and pharmacology. At least three distinct classes of accessory subunits including the KCNE family can regulate Kv channel function. KCNE genes encode integral membrane proteins with a single transmembrane domain. KCNE genes span the eukaryotic kingdom and, in mutated form, can cause acquired and congenital disease. Here we review genetic, physiological, and biophysical aspects of KCNE proteins with particular emphasis on the *Caenorhabditis elegans* subfamily.

Index Entries: *C. elegans*; KCNE; chemotaxis; kinase.

INTRODUCTION

In 1988, Takumi and colleagues cloned a small protein with a single transmembrane domain (1) that later was termed MinK by Hausdorff and colleagues (2), for “minimal K⁺ channel” because when MinK is expressed in *Xenopus laevis* oocytes it produces a potassium current. MinK belongs to a family of ancillary subunits of K⁺ channels, encoded by the KCNE genes, that span the eukaryotic kingdom from invertebrates to humans (1,3–7). These proteins have the ability to interact intimately with K⁺ channels (8–10), to affect several functional properties simultaneously (11–16), to control the channel’s modulation by signaling molecules (17,18), to partner with multiple K⁺ channels (3,15,16,19–31), and to cause congenital and acquired channelopathies (3,7,28,32–34). Here we review genetic, physiologic, and biophysical aspects of these fascinating proteins with emphasis on the *Caenorhabditis elegans* subfamily.

KCNE Phylogenetic Tree

Members of the KCNE family include four *C. elegans* (6), three *Xenopus laevis* (4), and five human genes (3,7)

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grouped into three major subfamilies (Fig. 1A). KCNE proteins range from approx 100 to 300 amino acids (aa) in length and have a single transmembrane domain, which is the most conserved domain in these proteins (Fig. 1B). Phylogenetic comparison of human, *Xenopus*, and *C. elegans* KCNE primary sequences identifies two major lineages: one is defined by *C. elegans* MPS-2, MPS-4, and human KCNE2; the other by all remaining members (Fig. 1A). Within the second lineage, human and *Xenopus* subfamilies define subgroups with close homology. *C. elegans* MPS-1 and MPS-3 define a subgroup with human KCNE4. There is no apparent homology between *C. elegans* and *Xenopus* KCNE1, KCNE3, and KCNE5, suggesting that they might have evolved independently.

KCNE Proteins Form Multiple Channel Complexes

A striking feature of KCNE accessory subunits is their ability to assemble with multiple K⁺ pore-forming subunits of the same specie and of different species (3,15,16,19–31). Thus far several KCNE-K⁺ channels interactions, some not yet proven to be physiological, have been reported and the number is expected to grow as our knowledge of these proteins progresses (Table 1). The physiological implications for this promiscuity are potentially very significant. Because KCNE proteins act to alter the functional characteristics of the channels



Fig. 1. The family of KCNE proteins. **(A)** Phylogenetic tree of the human, *Xenopus*, and *C. elegans* families. Human and *Xenopus* members are indicated by the suffix "h" and "x," respectively. *C. elegans* members are indicated with the standard three letters code. **(B)** *C. elegans* MPS cDNA sequences and alignments. Red, green, and blue indicate identical, strongly, and weakly similar residues, respectively. The dendrogram in **(A)** and the alignment in **(B)** were computed by ClustalW available at <http://align.genome.jp/>.

they assemble with, they can be seen as a means to generate heterogeneity of K^+ currents with few gene products. Moreover, genetic mutations in KCNE genes can lead to disruption of multiple currents. Abbott and Goldstein have captured some essence of the problem by showing that mutations of two residues conserved among KCNE1, KCNE2, and KCNE3 affect the function of complexes formed with KCNQ1, HERG, and Kv3.4 (although not all KCNE mutations showed channel-regulating defects across the gene family) (35). The existence of mechanisms of channel modulation common to all KCNE proteins seems to go beyond species boundaries. Mammalian KCNE1 proteins can assemble with putative *Xenopus* KCNQ homologues (1) and, reciprocally, *Xenopus* KCNE proteins affect the gating of several heterologously expressed human pore-forming subunits (4,36). The molecular bases for this promiscuity are not well understood, but given the relevance of this issue, it is likely that it will receive increasing attention in the future. Generally speaking, one could argue that the lack of sequence specificity might indicate that the interactions result from the three-dimensional protein conformation rather than strict amino acid sequence motifs.

Biophysical Attributes of KCNE Proteins

Takumi and colleagues and, independently, Goldstein and Miller were the first to map which residues of

KCNE1 are essential for the protein function (37,38). The first group could construct a minimal peptide of 63 residues that included residues 1–9 and 40–94 maintaining the transmembrane segment (A45-I67). The second group reported on the molecular basis for selectivity. Substantial evidence supports the notion that the molecular determinants for KCNE control of channel function reside in the transmembrane domain and in the adjacent portion of the C-terminus, also considering that the majority of disease-associated mutations found in KCNE1, KCNE2, and KCNE3 genes occur in these domains (3,11,12,28,32,33,35,39–47). Generally, KCNE sequences contain one or two *N*-glycosylation consensus sites, and in heterologous expression systems, mature KCNE proteins appear to be fully glycosylated (3,28,48). The physiologic function of *N*-glycosylation of KCNE proteins is elusive, however. Takumi showed that deletion of the two *N*-glycosylation consensus sequences of KCNE1 had no noticeable effect (37). A single-nucleotide polymorphism that substitutes an alanine for threonine (T8A) in one of the two *N*-glycosylation sites of KCNE2 gives rise to glycosylation-defective proteins that behave normally at baseline (33,44). The lack of sugars, however, makes channels formed with the single-nucleotide polymorphism and HERG subunits more susceptible to the inhibiting action of the antibiotic sulfamethoxazole, placing the individuals that carry the mutation at risk of acquired cardiac disease (43).

Table 1
KCNE Subunits Form Multiple Complexes

		Pore-forming subunit				
Mammalian						
KCNE1	KCNQ1 (15,16,66)	HERG (19,66)	Kv3.1-.2 (20)			
KCNE2	KCNQ1-3 (21,22,74,75)	HERG (3,66,68,76)	Kv3.1-.2 (20)	Kv4.2 (24)	HCN1-2; 4 (23,25,26,77)	
KCNE3	KCNQ1 (27,74)	HERG (27)	Kv2.1 (29)	Kv3.1; -.4 (28,29)		
KCNE4	KCNQ1 (78)		Kv1.1; -.3 (31)			
KCNE5	KCNQ1 (30)					
<i>Xenopus</i>						
KCNE1			Kv2.1 (36)			
KCNE3	KCNQ1 (4)	HERG (4)	Kv2.1 (36)			
KCNE5						
<i>C. elegans</i>						
MPS-1 (5,6,56)						KVS-1
MPS-2						KVS-1 (6)
MPS-3						KVS-1 (6)
MPS-4						KVS-1 (6)

Reported interactions between KCNE subunits and pore-forming subunits. References are indicated in parentheses.

KCNE stoichiometry and topology have been a matter of controversy (8,10,11,38,49–55). Recently, Chen and colleagues (51) using charybdotoxin-sensitive KCNE1 variants determined the stoichiometry of the KCNE1–KCNQ1 complex to be two KCNE1 subunits per KCNQ1 tetramer, confirming previous conclusions from the same laboratory (50). Using a cysteine scanning mutagenesis approach, Tai and Goldstein (8) proposed that the KCNE1 subunits contribute to the ion-conduction pathway of the KCNE1–KCNQ1 complex. Although this revolutionary notion has been challenged by other investigators, Melman and colleagues (9) have recently shown that multiple segments of the KCNQ1 channel pore structure bind to KCNE1.

The *C. elegans* Subfamily

We discovered *C. elegans* KCNE orthologs in the search for animal systems amenable to study the genetic and physiological aspects of KCNE function, which are difficult to understand in the more complex mammals (5,6). The *C. elegans* subfamily is composed of four members, including two isoforms (MPS-2.a and MPS-2.b) that are almost exclusively expressed in the nervous system of the animal. MPS-1 was the first member to be

identified using *in silico* approaches; MPS-2, MPS-3, and MPS-4 were recognized more recently by their homology with MPS-1.

MPS-1 is a Bifunctional Protein

Three KCNE proteins, *C. elegans* MPS-1, MPS-3, and human KCNE4, are characterized by large intracellular domains, ranging from 116 to 192 aa (compared with the average 60 aa of the other KCNE C-terminus domains). This difference suggests that these domains might confer additional or unique regulatory functions to these proteins. This hypothesis has received support by our recent discovery that the cytoplasmic domain of MPS-1 is a serine/threonine kinase (56). Of particular interest are the effects that MPS-1-dependent phosphorylation exerts on the function of voltage-gated K⁺ channel KVS-1, which is a MPS-1 physiologic substrate. Compared with KVS-1 channels alone, heteromeric complexes containing MPS-1 pass less current, inactivate faster, and recover from inactivation more slowly. When the catalytic domain is deleted, however, the resulting channels pass the same current as KVS-1 channels alone, but retain wild-type inactivation and recovery from inactivation kinetics. These data suggest that MPS-1 controls KVS-1 function

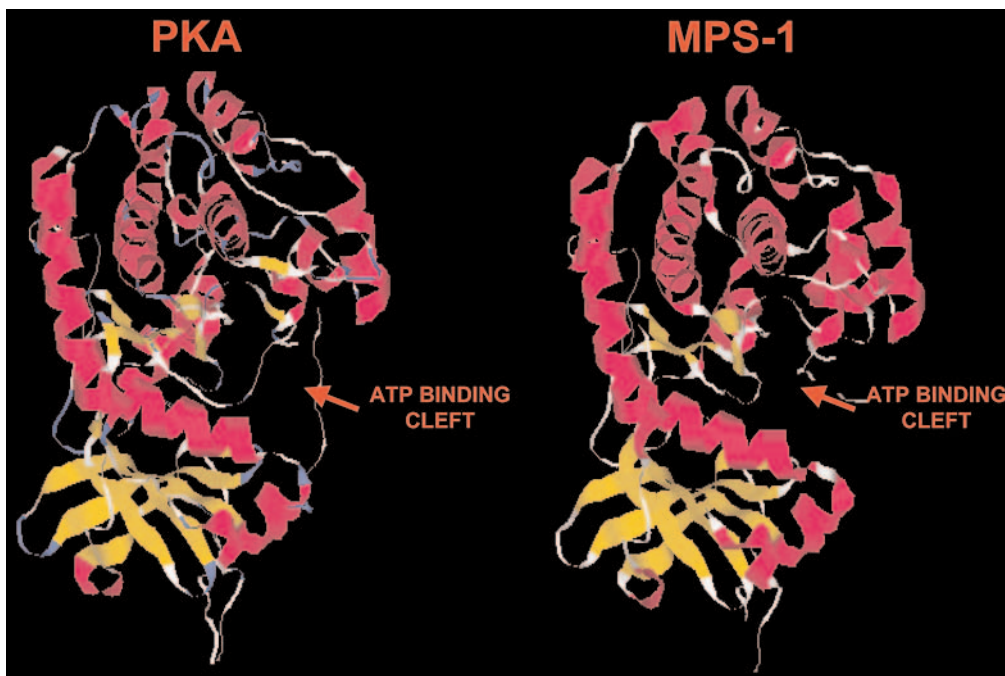


Fig. 2. Putative three-dimensional structure of the catalytic domain of MPS-1. The reconstruction of the putative three-dimensional structure of the catalytic domain of MPS-1 (right) was performed using the crystal structure of PKA as template (left) by Swiss Model software (available at <http://www.expasy.org>). The model was optimized by energy minimization. Even though the low-sequence identity between PKA and MPS-1 (25%) does not guarantee accuracy, the model predicts the correct position of the DFG catalytic site.

through two independent mechanisms: phosphorylation of the channel acts to reduce the current, whereas the rest of the protein controls other physiologically relevant channel functions—inactivation is a key feature of A-type voltage-gated K^+ channels—presumably through mechanisms common to the other KCNE β -subunits. Even though MPS-1 does not display sequence homologies to canonical kinases, the three-dimensional structure of its catalytic domain can be modeled into the crystal structure of PKA remarkably well (Fig. 2). Sequence homology is not a prerequisite for kinase function. α -Kinases, a recently discovered family (57) that includes channel-kinases (58,59), show no detectable sequence homologies to conventional protein kinases yet the majority of structural elements, sequence motifs, and the position of key amino acid residues important for catalysis appear to be remarkably conserved (59). Whether MPS-1-like kinases are present in other genomes is an open question. *In silico* analysis seems to exclude the existence of other MPS-1-like bifunctional proteins in the nematode's genome. The two closest MPS-1 relatives, *C. elegans* MPS-3 and human KCNE4, lack the characteristic kinase signatures in their amino acid sequences (MPS-3 exhibits an aspartate instead of a phenylalanine in the catalytic site), although this does not rule out the possibility that they might have other regulatory functions. A predicted MPS-1 homolog

(predicted protein CBG02619) conserving the catalytic domain is found in the genome of *C. briggsae* (60). *C. elegans* and *C. briggsae* are close relatives, although they diverged evolutionarily approx 50 million yr ago. Nonetheless, the existence of an MPS-1 homolog in another species indicates that bifunctional proteins like MPS-1 may represent a potentially general mode of K^+ channel regulation in invertebrates, whereas (at present) there is no evidence that such proteins might operate in mammals.

MPS Proteins Are Expressed in the Nervous System of C. elegans

The cellular expression patterns of the MPS proteins have been determined by analyzing transgenic animals expressing the full MPS proteins fused to green fluorescent protein (GFP). Table 2 lists the cells where these translational gene reporters give detectable signals. As opposed to mammalian KCNE proteins that are expressed and operate in multiple tissues (3,15,28), the *C. elegans* genes are almost exclusively expressed in the nervous system. MPS protein expression is particularly abundant in the sensory apparatus of the worm which is mainly composed of 12 amphid neurons mediating multiple sensory functions (61). GFP requires relatively high levels of protein to give

Table 2
MPS Proteins are Expressed in the Nervous System

Tissue	MPS-1	MPS-2	MPS-3	MPS-4
ADF	XX	XX	XX	
ASH	XX			
AWC	XX			
ASE	XX			
ASG	XX			
ALM	XX			
AIA				X ¹
AUA				X ¹
PLM	XX	X		
Vulva	X			
PVC			X ¹	X ¹
Enteric muscle		XX		XX
PVN			X ¹	
Tail neurons*	X			
Head neurons*	X		X	

Cellular expression patterns of the MPS proteins were determined by translational green fluorescent protein gene reporters (5,6). Transgenic animals were analyzed and photographed with a Zeiss Axioplan 2 microscope equipped with a digital camera. X and XX indicate detectable and strong fluorescence, respectively.

X¹ indicates neurons that were identified tentatively.

*Presently unidentified tail and head neurons.

detectable signals; therefore, it is quite possible that the list in Table 2 is partial and that MPS proteins are expressed more broadly. There are two tissues, the ADF neurons and the enteric muscle, where multiple MPS proteins are present (Table 2). KVS-1, which has been shown to assemble with each individual MPS subunit in heterologous expression systems (6), is also present in ADF neurons. These cells have therefore provided a good system to study the interactions of multiple KCNE subunits in a physiological context.

MPS Proteins Associate With Voltage-Gated K⁺ Channel KVS-1

There is evidence—mainly based on phenotypic analysis—indicating that, as with their mammalian relatives, MPS proteins assemble with multiple pore-forming subunits in *C. elegans* (5). Only one MPS partner, KVS-1, has been identified; the number and the identity of other putative channels remains elusive. In heterologous expression systems (CHO cells), coexpression of each MPS subunit with the voltage-gated K⁺ channel KVS-1 is associated to changes in the macroscopic attributes of the current. A similar situation exists with human KCNE proteins, where each subunit can individually assemble with KCNQ1 in heterologous expres-

sion systems and, in the case of KCNE1 and KCNE2, also in native tissues (3,15,27,30,31,62). That two proteins interact in vitro does not necessarily imply that the complex they form exists in native tissues however. Coimmunoprecipitation is probably the most direct criteria to assess whether two proteins interact in vivo. Biochemistry is not always feasible when dealing with native tissues and, unfortunately, this is often the case with *C. elegans*. Alternatively, three criteria can be used. First, the proteins must colocalize in the same cell at the same time. Second, heterologously expressed complexes must recapitulate the attributes of native channels. Third, knockdown of each protein must produce the same phenotype. These criteria *per se* do not guarantee that two proteins interact in vivo, for instance the fact that a complex recapitulates native attributes ignores the putative contribution of other unknown proteins and gene knockdown does not account for the possibility of compensatory or up- or downregulation of other genes, but nevertheless these criteria are good guidelines, especially when they are satisfied simultaneously. This is the case for the MPS-1-KVS-1 complex. Native currents recorded from ASE right (ASER) neurons exhibit an inactivating component that can be suppressed by KVS-1 or MPS-1 RNA interference (RNAi). Moreover, RNAi had no effect on reporters that express green fluorescent protein driven by KVS-1 or MPS-1 promoter sequences indicating that RNAi does not interfere with gene transcription (6). These observations have validated the notion that a MPS-1-KVS-1 complex is not only functional in ASER neurons, but also in the other amphid neurons where it is expressed. In addition, the finding that MPS-1 RNAi suppresses the MPS-1-KVS-1 current has corroborated the empirical notion that in *C. elegans*, knock down of any subunit belonging to an integral membrane complex is sufficient to destabilize the entire channel, an effect observed with several channel complexes (63,64). This offers a significant experimental advantage because RNAi techniques and gene reporter analysis can be coupled to determine subunit-subunit interactions. Using this approach, we proposed that MPS-1, MPS-2, and MPS-3, which are all detected in ADF neurons, combine with KVS-1 to form two distinct channel complexes: binary complexes containing only MPS-1 subunits (MPS-1-KVS-1) and ternary complexes containing MPS-2, MPS-3, and KVS-1 (MPS-2-MPS-3-KVS-1) (6). It is interesting to note that the subunit composition of ternary complexes appears to be regulated at least in ADF cells: combinations in principle perfectly plausible such as MPS-1-MPS-2-KVS-1 and MPS-1-MPS-3-KVS-1 are apparently forbidden (that is, RNAi of MPS-1 does not diminish the fluorescence of MPS-2 for instance). The ability to form mixed complexes might be a general peculiarity of

KCNE proteins. Lundquist and colleagues (65) speculated that in human heart, a balance of KCNE accessory subunits may be important for cardiac K⁺ channel function. Binary complexes formed by HERG and MinK (66) or HERG and MiRP1 (67,68) have been detected in mammalian hearts. The behavior of *C. elegans* MPS proteins raises the possibility that ternary complexes might also exist and operate in mammalian tissues, underscoring once more the potential of *C. elegans* for studying fundamental aspects of channel function.

Physiological Role of Channel Complexes Formed With KVS-1 and the MPS Proteins

MPS proteins are primarily expressed in the sensory apparatus of the animal; therefore, phenotypic analysis has focused on exploring how MPS proteins affect sensory activities. Knocking down MPS-1 expression by RNAi induces defective body touch sensation, chemotaxis to water-soluble attractants, osmotic avoidance, and mechanosensation (nose-touch collision), all phenotypes that correlate well with the broad presence of MPS-1 in the nervous system of animal. Functions such as body touch sensation are mediated by neurons in which KVS-1 is absent, arguing that MPS-1 partners with other, presently unknown, pore-forming subunits and underscoring the physiologic relevance of the promiscuity of KCNE proteins, as well their potential for leading to multiple dysfunction. Knocking down of MPS-2 and MPS-3 is also associated with chemosensory defects; RNAi produces increased taste for sodium, whereas the taste for other attractants such as chloride, cAMP biotin, and lysine is unaffected. Taken together, these experiments have produced a model that predicts that: channel complexes formed by KVS-1 and the MPS proteins play a central role in the neurosensory mechanisms of the animal; the MPS-2-MPS-3-KVS-1 ternary complex is required to fine tune the responsiveness of the animal for sodium ions; and a behavioral response is triggered only when the concentration of the attractant is sufficient to cross a specific sensory threshold (6). Chemotaxis is an essential function for *C. elegans* because the animal feeds on bacteria and exploits the byproducts of bacterial metabolism such sodium, chloride vitamins, and amino acids to track its sources of food. Therefore, the use of sensory thresholds seems to be an efficient strategy to filter and to balance inputs coming from the diverse sources present in the natural environment of the animal. This mechanism would increase fidelity and it may be seen as a means to lower background noise because it is likely that traces of sodium and other attractants are always present in the animal's environment.

The molecular mechanisms by which channel complexes formed by KVS-1 and the MPS proteins control neuronal excitability are at present poorly understood. Native K⁺ currents in ASE and other amphid neurons

exhibit a significant inactivating component (6,69–71). Generally speaking, inactivating K⁺ currents modulate the time interval between consecutive action potentials in invertebrate neurons (72,73). The role of MPS-1-dependent phosphorylation could be framed in this context. Because K⁺ fluxes stabilize the cell membrane potential, phosphorylation/dephosphorylation of KVS-1 might play a key role in mechanisms that require reversible modulation of cell excitability, such as sensory adaptation. However, amphid neurons do not fire classic action potentials (70); therefore, issues concerning the overall electrical properties of *C. elegans* sensory neurons, as well the role of MPS-1-KVS-1 channels in these cells, lie ahead and will need careful investigation in the near future.

CONCLUSIONS

Accessory subunits are a fundamental feature of potassium channels that determine trafficking to the plasma membrane, location, abundance, sensitivity to stimulation, and pharmacology. The KCNE family is emerging as a general class of ancillary subunits of voltage-gated K⁺ channels. Issues that will receive attention in the near future are likely to revolve around KCNE role in trafficking and other related cellular functions. Other important issues include the identification of KCNE peptides functioning in eukaryotes and in the two other kingdoms. The identification/characterization of new channel complexes is an ongoing effort. In this respect, the use of simple animal systems seems a reasonable strategy to overcome the limitations of higher organisms without losing the ability to work in a true physiological context.

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