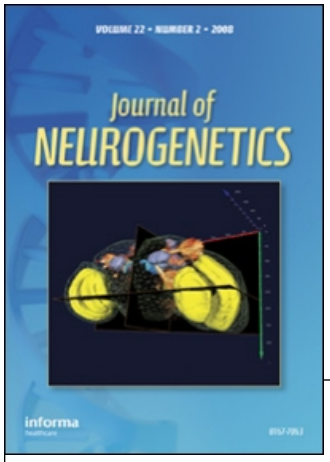

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Nektarios Tavernarakis [a]; Monica Driscoll [a]
[a] Department of Molecular Biology and Biochemistry, Nelson Biological Laboratories, Piscataway, NJ, USA

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Short Communication

CAENORHABDITIS ELEGANS DEGENERINS AND VERTEBRATE ENaC ION CHANNELS CONTAIN AN EXTRACELLULAR DOMAIN RELATED TO VENOM NEUROTOXINS

NEKTARIOS TAVERNARAKIS* and
MONICA DRISCOLL

Department of Molecular Biology and Biochemistry, Nelson Biological Laboratories, 604 Allison Road, Rutgers University, Piscataway, NJ 08855, USA

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The DEG/ENaC (DEGenerin/Epithelial Na^+ Channel) superfamily includes closely related ion channel subunits from divergent species ranging from the simple nematode *Caenorhabditis elegans* to humans. Members of this protein group play roles in several important processes including transduction of mechanical stimuli, sodium re-absorption and blood pressure regulation. Structure/function relationships in members of this superfamily are just beginning to be elaborated. Using a bioinformatics approach, we identified a novel structural element in the extracellular region of DEG/ENaC proteins that exhibits significant similarity to venom neurotoxins. Since venom neurotoxins bind to sodium channels at high affinity, we suggest that the related domain embedded in DEG/ENaC channels may interact with other regions of the channel or channel complex to modulate channel function.

Keywords: Mechanotransduction; neurodegeneration; Kunitz-type protease inhibitors; anti-epilepsy peptide

* Corresponding author. Tel.: (732)-445-7187. Fax: (732)-445-4213.
E-mail: tavernarakis@mbcl.rutgers.edu.

INTRODUCTION

Cell volume regulation, gravitaxis, proprioception, touch sensation, and auditory transduction all depend on the conversion of mechanical energy into cellular responses (reviewed in Sackin, 1995). Still, little is known about the molecular properties of ion channels specialized for mechanotransduction. Genetic studies in the nematode *Caenorhabditis elegans* led to the identification of several genes that encode subunits of candidate mechanically-gated ion channels involved in mediating touch transduction, proprioception and regulation of locomotion (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Tavernarakis *et al.*, 1997). These channel subunits belong to a large family of related proteins in *C. elegans* referred to as degenerins, because unusual gain-of-function mutations in several family members induce swelling or cell degeneration (Driscoll and Chalfie, 1991; Tavernarakis and Driscoll, 1997). *C. elegans* degenerins exhibit approximately 25–30% sequence identity to subunits of the vertebrate amiloride sensitive epithelial Na^+ channels (ENaCs; Chalfie *et al.*, 1993), which are required for ion transport across epithelia (reviewed in Palmer, 1992). Together the *C. elegans* and vertebrate proteins define the DEG/ENaC (degenerin/epithelial sodium channel) superfamily of ion channels (Corey and García-Añoveros, 1996). Members of the DEG/ENaC superfamily are characterized by two transmembrane domains and two cysteine-rich domains (CRDs) (CRDII and CDRIII); an additional CRD, CDRI is present only in nematode degenerins. DEG/ENaC proteins are localized in the plasma membrane with both amino- and carboxy-termini projecting inside the cell and a large region that includes the CRDs situated on the extracellular side (Canessa *et al.*, 1994; Lai *et al.*, 1996).

The high degree of sequence conservation observed within specific extracellular segments of DEG/ENaC superfamily members implies that these domains may contribute to an important, currently undetermined function. We took a bioinformatics approach to investigate the possibility that these domains comprise essential structural elements, not necessarily confined to this category of ion channels. The presence of similar sequence segments in other protein groups suggests a common function and may provide a hint as to what purpose these domains serve in the extracellular region of DEG/ENaC proteins.

MATERIALS AND METHODS

Sequences

Accession numbers for the toxin sequences analyzed are: AEP_MESMA: P15228, SCXC_CENLL: P45667, SCXI_CENSC: P01491, SCX1_CENSC: P01492, SCX2_CENSC: P01493, SCX1_CENNO: P15223, SCX7_CENNO: P45665, NTSR1C: g69540. Accession numbers for members of the DEG/ENaC superfamily are DEL-1: U76403, MDEG: U53211, MEC-4: U53669, MEC-10: P34886, UNC-8: U76402, αrENaC: X70497, βrENaC: X77932, γrENaC: X77933.

Database Mining

The Blockmaker algorithm (Henikoff *et al.*, 1995; Henikoff and Henikoff, 1991) was run on the Blocks Server web site: (http://blocks.fhcrc.org/). Blocks identified were subjected to Multiple EM for Motif Elicitation algorithms (Timothy and Elkan, 1994; Timothy and Gribskov, 1998) run on the Multiple Expectation Maximization for Motif Elicitation (MEME) System, web based, server (http://www.sdsc.edu/MEME/meme/website/). BLAST searches (Altschul *et al.*, 1997) were performed with the National Center for Biotechnology Information web based servers (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/).

Alignments

Multiple sequence alignments were generated with the ClustalW algorithm (Thompson *et al.*, 1994) and displayed with SeqVu (The Garvan Institute of Medical Research, Sydney, Australia).

RESULTS AND DISCUSSION

To scan for conserved motifs within DEG/ENaC superfamily members, we applied the Blockmaker algorithm (Henikoff *et al.*, 1995; Henikoff and Henikoff, 1991) on a group of eight representative protein sequences (DEL-1, MDEG, MEC-4, MEC-10, UNC-8, αrENaC,

βrENaC, γrENaC). Six conserved amino acid sequence blocks were identified in the eight sample proteins (not shown). Based on these blocks, a position-specific scoring matrix was generated and used to search protein databases for sequences that contain one or more of the six identified motifs. The MEME system was employed for these database searches (Timothy and Elkan, 1994; Timothy and Gribskov, 1998). As expected this scan detected all the sequences of DEG/ENaC superfamily members documented in the databases. Interestingly, several short venom neurotoxin sequences were additionally detected that shared significant similarity with a region which partially overlaps with CRDII of both mammalian and *C. elegans* DEG/ENaC superfamily members (we refer to this as the neurotoxin-related domain NTD; see Fig. 1). To further explore this similarity we generated a multiple sequence alignment between the eight channel protein sequences that were used as probe for the described searches and a representative sample of eight of the neurotoxin sequences detected, with the ClustalW algorithm (Thompson *et al.*, 1994). As shown in Fig. 1A the short neurotoxin sequences align to the channel sequences in and around CRDIII with six of the eight conserved cysteine residues of neurotoxins preserved in DEG/ENaC superfamily members. However, the overall similarity between the two groups of protein sequences appeared to be low. This low level of similarity did not allow detection of one of the two protein groups in BLAST searches (Altschul *et al.*, 1997), with the other group used as query. Therefore, we assessed the statistical significance of the similarity by generating blocks from both groups of sequences and aligning them with ClustalW. The blocks aligned with a *p*-value of 1.92e-07 (not shown). Next, the quality of the alignment was calculated to a *p*-value of 1.76e-05 using the FASTA package PRSS3 algorithm (Pearson, 1990) to uniformly shuffle the sequences. We concluded that there is a low but significant level of sequence similarity between an extracellular region of DEG/ENaC proteins and certain neurotoxins.

A few points regarding this sequence similarity are noteworthy. First, it is interesting that all of the detected neurotoxins belong to the beta subfamily of scorpion venom toxins, with only the anti-epilepsy peptide (AEP_MESMA), representing an alpha-type neurotoxin. This bias for specific detection strengthens the significance of the similarity between these toxins and DEG/ENaC channels, as other types of toxins with preponderance of closely spaced cysteine residues are not detected.

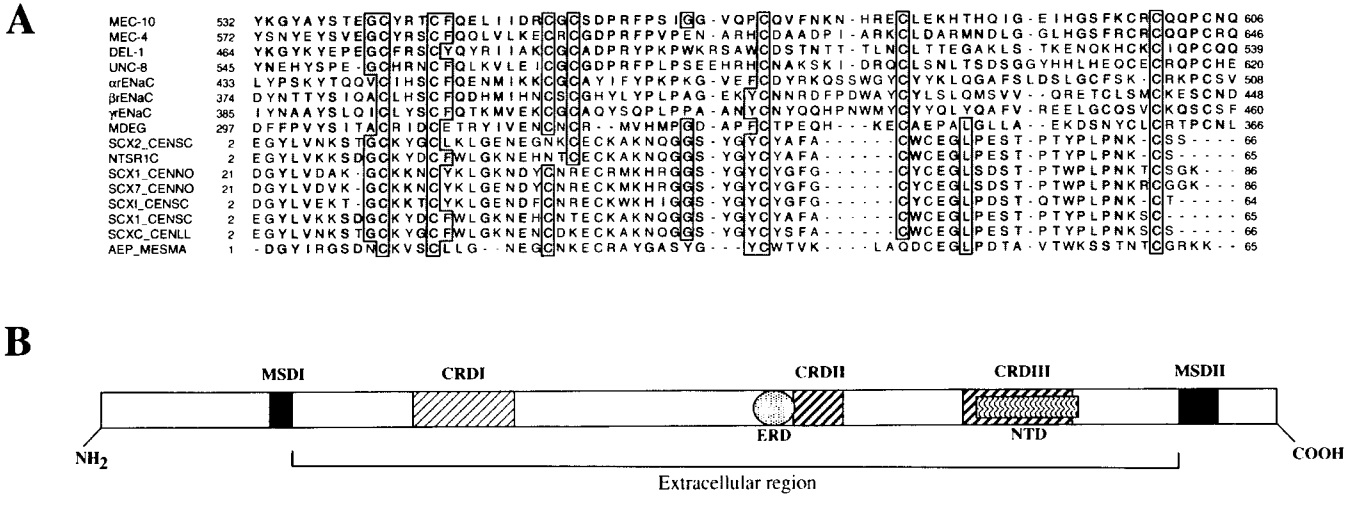

FIGURE 1 Similarity of neurotoxins to a DEG/ENaC ion channel domain. (A) Amino acid alignment of eight DEG/ENaC superfamily members and eight venom neurotoxins. Identical residues that occur in more than 60% of the sequences are boxed. Gray-shaded residues represent sequence similarity (>85%; for homology analysis the first sequence is used as primary, i.e. homology is displayed with respect to MEC-10 sequence). Residue positions are noted on both sides of the alignment for each of the designated sequences. Representative members of the DEG/ENaC superfamily characterized to-date were included in the alignment (without orthologs in different species). Anti-epilepsy peptide (AEP_MESMA) belongs to the alpha subfamily of neurotoxins while the remaining seven toxin sequences are typical of the beta class. (B) Schematic representation of DEG/ENaC ion channel subunit structure. The position of the toxin-related region is shown with respect to other characteristic features of the channel (NTD; depicted by the wavy-line shaded rectangle). Defined channel modules are indicated by shaded boxes. These include the two membrane spanning domains (MSDs; dark-dotted shading), and the three CRDs (CRDs; thick-line shading; the first CRD is absent in mammalian channels and is depicted by thin-line shading). The small light-dotted oval depicts the putative extracellular regulatory domain (ERD) identified by García-Añoveros and co-workers in *C. elegans* degenerins (García-Añoveros *et al.*, 1995).

In addition, DEG/ENaC superfamily members are the only channels that exhibit similarity to venom neurotoxins. Second, NTD is also distantly related to domains in several other proteins including the *Drosophila crumbs* protein, required for epithelial organization (Tepass *et al.*, 1990), *agrin*, a basal lamina protein that mediates aggregation of acetylcholine channels (Rupp *et al.*, 1992), and the selectins which participate in cell adhesion (such as ELAM-1; Bevilacqua *et al.*, 1989). Both alpha and beta type neurotoxins identified contained Kunitz-type basic protease inhibitor motifs. However, the Kunitz domain signature is not intact in the NTD of DEG/ENaC ion channels. Nevertheless, amino acid sequence similarity spans the entire length of the toxins (an average of 65 amino acid residues). Third, the preserved arrangement of cysteines in such sequences could dictate similar higher structures in the corresponding protein domains. However, although the structure of several neurotoxin peptides is known (Zlotkin *et al.*, 1991), such information is not available for any of the DEG/ENaC proteins.

The noted similarities also hold intriguing implications for the function of these domains and channel biology. First, since it is known that venom neurotoxins are potent and specific blockers of Na^+ channels (Zlotkin *et al.*, 1991; Becerril *et al.*, 1993), it is possible that a related domain embedded within a channel protein could regulate channel activity. Interestingly, a region close to NTD has been implicated in closing of *C. elegans* DEG-1 and MEC-4 degenerin channels (ERD for extracellular regulatory domain; see Fig. 1; García-Añoveros *et al.*, 1995). The boundaries of this domain have not been experimentally delineated and might therefore extend into NTD. Further evidence supporting an intra-channel regulatory activity mediated by an extracellular channel domain comes from a channel-activating mutation in the *C. elegans* UNC-8 proprioception channel, which also maps to the NRD region (Tavernarakis *et al.*, 1997).

Second, and more generally, venom neurotoxins bind with high affinity to Na^+ channels which implicates the venom-like domain within DEG/ENaC channels in high affinity interactions. Domains that facilitate such high affinity interactions are thought to be essential for tethering mechanically-gated channels to "molecular springs" that deliver required gating tension. It is intriguing that some mutations that disrupt MEC-4 function, map to the NTD (K. Hong and M. Driscoll, unpublished observations), further suggesting that this region is

functionally significant. The presence of similar domains in other proteins such as *crumbs* and *agrin* implies that such domains might act as interaction modules that mediate analogous interactions needed for tissue organization or protein clustering. We hypothesize that the appearance of neurotoxin related domains in a specific class of ion channels might be the result of convergent evolution, driven by the requirement for high affinity interaction modules in these proteins.

Acknowledgments

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