DEG/ENaC channels: a touchy superfamily that watches its salt

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Summary

To the surprise of many, studies of molecular mechanisms of touch transduction and analyses of epithelial Na^+ transport have converged to define a new class of ion channel subunits. Based on the names of the first two identified subfamilies, the *Caenorhabditis elegans* degenerins and the vertebrate epithelial amiloride-sensitive Na^+ channel, this ion channel class is called the DEG/ENaC superfamily. Members of the DEG/ENaC superfamily have been found in nematodes, flies, snails, and vertebrates. Family members share common topology, such that they span the membrane twice and have intracellular N- and C-termini; a large extracellular loop includes a conserved cysteine-rich region. DEG/ENaC channels have been implicated a broad spectrum of cellular functions, including mechanosensation, proprioception, pain sensation, gametogenesis, and epithelial Na^+ transport. These channels exhibit diverse gating properties, ranging from near constitutive opening to rapid inactivation. We discuss working understanding of DEG/ENaC functions, channel properties, structure/activity correlations and possible evolutionary relationship to other channel classes. *BioEssays* 21:568–578, 1999.

Introduction

What do touch sensation, coordination, gametogenesis, pain perception, and blood pressure regulation have in common? Recent investigations indicate that each of these fundamentally important processes is mediated by members of the DEGenerin/Epithelial Na^+ Channel (DEG/ENaC) superfamily. Members of the DEG/ENaC superfamily have been identified from nematodes, snails, flies, and several vertebrates, including humans, and are expressed in tissues as diverse as kidney epithelia, muscle, and neurons. Although DEG/ENaC channels participate in strikingly diverse biological processes and are activated in response to markedly distinct stimuli, the overall secondary structure of channel subunits encoded by this gene superfamily is highly conserved. DEG/ENaC subunits range from about 500 to 1,000 amino acids in length and share several distinguishing blocks of sequence similarity. Subunit topology (see Fig. 1) is invariable: all members of the DEG/ENaC superfamily have two membrane-spanning domains with Cysteine-rich domains (CRDs, the most conserved is designated CRD3) situated between the transmembrane segments.[1] N- and C-termini project into the intracellular cytoplasm, whereas most of the protein, including the CRDs, is extracellular. DEG/ENaC channels are probably heteromeric, with most published data favoring a tetramer[2–4] (but see also Reference 5). Comparative sequence algorithms define distinct subfamilies that share higher sequence similarity and are known to have different cellular functions and gating properties (see Fig. 2). Subunits, likely of the same subfamily, co-assemble into Na^+-preferring/selective channels that can be almost constitutively active (as the ENaC channels[6]), activated by mechanical stimuli (as postulated for *C. elegans* degenerins[7,8]) or activated by proton binding (as the Acid Sensing Ion Channels, ASICs[9]). Here, we review aspects of

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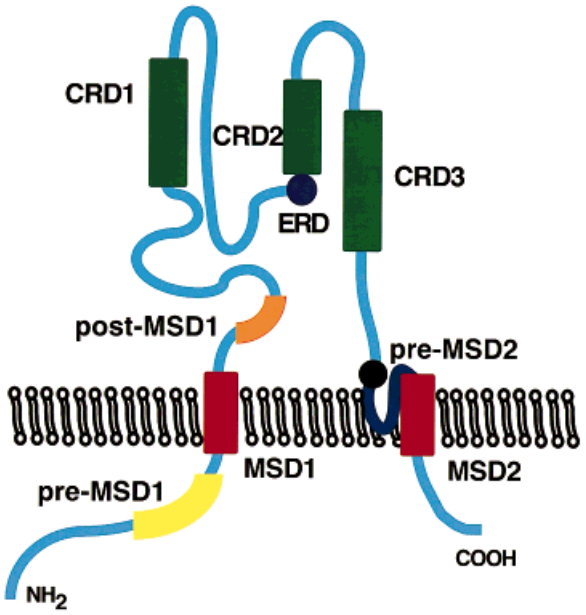

Figure 1. General organization of conserved domains in a DEG/ENaC subunit. A *C. elegans* degenerin subunit is depicted to show domain organization. Top is extracellular; bottom is intracellular. MSD, membrane spanning domain; CRD, cysteine-rich domain; ERD, extracellular regulatory domain. Note that CRD1 and ERD are unique to degenerins, and CRD2 is only partially conserved across subfamilies (see Fig. 2 for domain organization of specific subfamilies). Black-filled circle adjacent to the pre-MSD1 indicates the location of the degeneration-inducing A713V mutation in MEC-4. Domains are not drawn to scale.

the biology of DEG/ENaC channels by discussing the similarities and differences among the subgroups of the DEG/ENaC gene family and analyses of structure/function relations with emphasis on *C. elegans* members of the superfamily. These studies are beginning to provide insight into the mechanisms by which DEG/ENaC channels contribute to touch transduction, proprioception, neurodegeneration, early development, pain sensation, and salt homeostasis and suggest an intriguing evolutionary relationship to other ion channels.

C. elegans degenerin subunits are implicated in mechanotransduction and neurodegeneration

One of the great standing mysteries in sensory transduction (relevant to mechanisms of touch, hearing, balance, and proprioception, i.e., our ability to sense the posture of our body) concerns the identity of the molecules that convert mechanical forces such as stretch, deflection, or pressure, into cellular responses. Genetic and molecular analysis in *C. elegans* has defined prototype mechanotransducing channels postulated to be the primary mediators of touch[7,10,11] and proprioception.[12] The core subunits in these channels are DEG/ENaC family members.[12–14]

Touch sensation in C. elegans

C. elegans depends on the function of six structurally unique sensory neurons to detect gentle body touch, such as that delivered by a stroke with an eyelash hair. The processes of the six touch receptor neurons lie in close proximity to the nematode skin (the cuticle) and are surrounded by a specialized extracellular matrix material that appears to "glue" the neuron to the cuticle sensory field. The six touch receptor neurons are also distinguished by intracellular bundles of 15-protofilament microtubules that line their processes.[15] Elegant genetic analysis has identified approximately 15 genes, which, when mutated, specifically disrupt gentle body touch sensation and, therefore, encode candidate mediators of touch sensitivity (these genes were named *mec* genes, because when they are defective, animals are mechanosensory abnormal.[11,15]) Many of the *mec* genes have now been molecularly identified and several encode proteins postulated to make up a touch-transducing complex[7,8] (see Fig. 3A). The core elements of this mechanosensory complex are the DEG/ENaC subunits MEC-4[13,16] and MEC-10,[14] which can interact genetically and physically.[7,14,17] Genetic arguments support that at least two MEC-4 and at least two MEC-10 subunits are assembled in the heteromeric touch-transducing channel.[14,18] The MEC-4 and MEC-10 extracellular domains are postulated to be linked to the touch cell-specific specialized extracellular matrix, perhaps by associating directly with the MEC-5 collagen and/or MEC-9, a large protein rich in interaction domains such as EGF repeats and Kunitz protease inhibitor domains.[19] Channel intracellular domains are hypothesized to be tied to the unique 15-protofilament microtubules, made of the MEC-12 α-tubulin and the MEC-7 β-tubulin,[20,21] by means of the stomatin-related peripheral membrane linker protein MEC-2.[22] The tethering of channel subunits to the extracellular matrix and the intracellular cytoskeleton is postulated to confer channel gating tension. In this model, the minute mechanical deflection produced by light touch causes a conformational change in the channel, which is stretched between two attachment points, directly opening a gate for ion flow.[7,8,22] Although this working model accommodates many genetic observations, it should nevertheless be emphasized that the protein interactions it predicts (except for that between MEC-4 and MEC-10) have not been experimentally demonstrated. In addition, despite recent advances in the electrophysiologic characterization of degenerins,[23,24] direct biophysical evidence for mechanical gating of the MEC-4/MEC-10 touch-transducing channel remains a major challenge in the field.

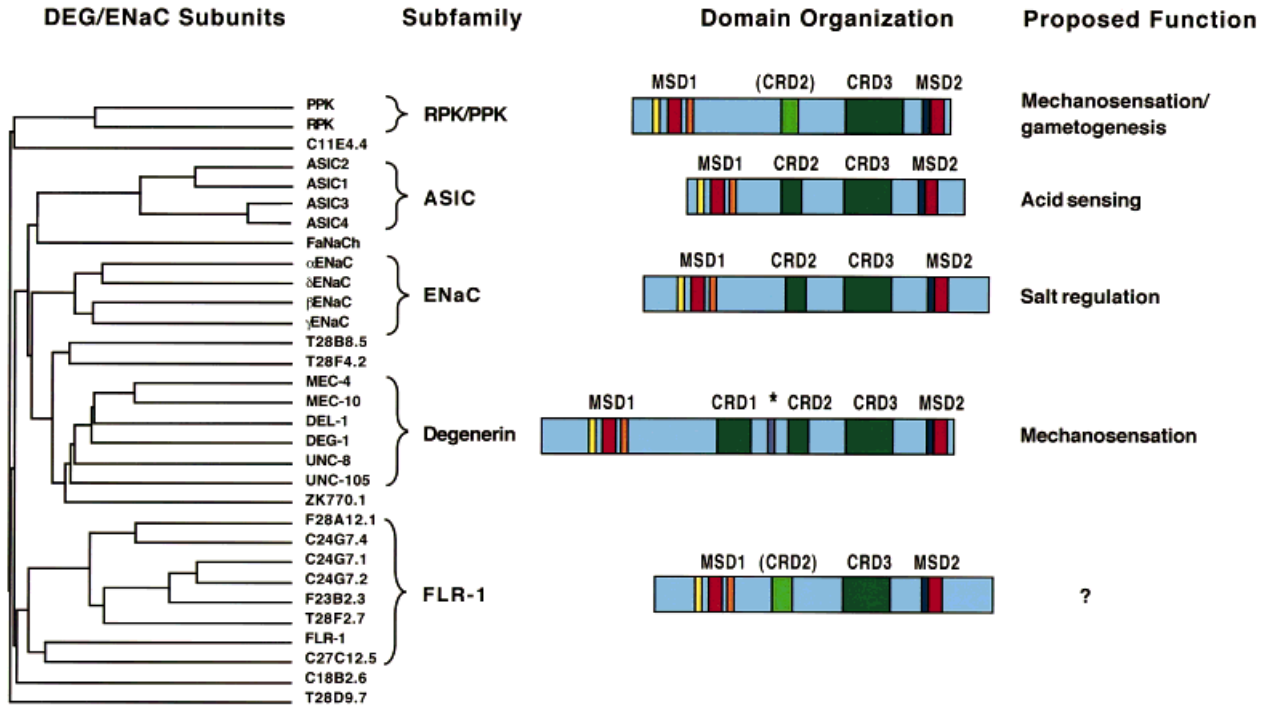

Figure 2. Summary of members of the DEG/ENaC superfamily and their postulated functions. On the left is a dendogram including currently identified members of the DEG/ENaC superfamily, showing their organization into subfamilies of related sequences. Where protein names are not known, the open reading frames predicted by the *C. elegans* sequencing consortium are listed. (All sequences can be retrieved from the NCBI Web site at http://www.ncbi.nlm.nih.gov/Entrez. Sequences were aligned by using the Pileup algorithm of the Genetic Computer Group analysis software package, version 8.) Schematic diagrams to the right of the dendogram show the conserved regions found in each of the subfamilies, color coded as in Figure 1 (*asterisk* in degenerins indicates ERD). In the RPK/PPK and the FLR-1 subfamilies, unique domains related to degenerin's CRD2 (showing only partial conservation of the specific cysteines) are indicated in brackets. Listed on the right is a suggested function common to at least most of the members of the subfamily. We emphasize that the relationships in the dendogram do not accurately reflect evolutionary time. Rather, they indicate relative sequence similarity of currently identified superfamily members.

Proprioception

As of this writing date, 20 members of the DEG/ENaC family have been identified in the *C. elegans* genome (Fig. 2; see more discussion below). The question naturally arises as to whether other related subunits are assembled into channel complexes that might mediate other mechanically dependent functions. Indeed, recent evidence supports the idea that some of the other *C. elegans* DEG/ENaC subunits may contribute to nematode proprioception. Moreover, some proteins proposed to be associated with the touch channel core appear to have homologous counterparts in the proprioception complex.

unc-8 encodes a DEG/ENaC superfamily member expressed in several neuronal classes, including some ventral cord motorneurons that control locomotion.[12] A subclass of these motorneurons exhibits peculiar neuroanatomic features: all synapses the motorneurons make with interneurons or with muscle are situated near the motorneuron cell body, leaving the majority of the motorneuron process synapse-free, much like a stretch-sensory process.[25] In support of a stretch sensory function for these motorneurons is the finding that corresponding motorneurons in the anatomically analogous, but more electrophysiologically accessible, nematode *Ascaris suum* have been shown to be stretch responsive.[26] Because *unc-8* loss-of-function mutations disrupt the normal sinusoidal body wave of the moving worm by reducing the wavelength and amplitude of the body curve, UNC-8 is postulated to function as a subunit of stretch-sensory channels situated in mechanosensitive regions of the motorneurons where they respond to body stretch to potentiate muscle contraction, driving deep localized body bends. In other words, UNC-8 contributes to nematode proprioception by providing feedback information on body posture.

The UNC-8 subunit is likely to associate with other members of the degenerin subfamily in different cell types (Fig. 3B). At least one other channel subfamily member, *del-1*, is co-expressed with *unc-8* nearly exclusively in the

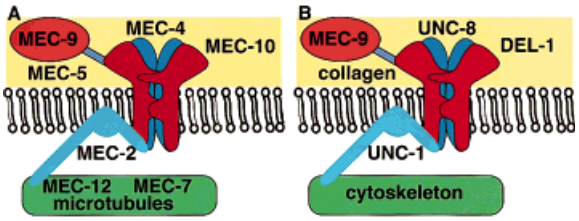

Figure 3. Mechanotransducing complexes in *C. elegans* are thought to have similar molecular compositions. A: A working model of a touch-transducing complex. Touch transduction is thought to be mediated by a degenerin ion channel made up of at least two MEC-4 (*red*) and two MEC-10 (*blue*) subunits. The large extracellular domains of the subunits project into a specialized extracellular matrix (*yellow*) called the mantle. This matrix likely includes the MEC-5 collagen (*purple*) and the MEC-9 protein (*orange*; rich in protein interaction domains such as epidermal growth factor domains and Kunitz protease inhibitor domains), which may associate with the channel. Inside the touch receptor neuron are special microtubules (*green*) made up of the MEC-12 α-tubulin and the MEC-7 α-tubulin. The microtubules are proposed to be linked to the channel by means of stomatin-related MEC-2 (*light blue*), a peripheral membrane protein. Proteins tethered to the extracellular and intracellular sides are thought to exert pressure on the channel, conferring gating tension when the channel is stretched between these two contact points. B: A working model for a stretch-sensitive complex that mediates proprioception. A degenerin channel made up of UNC-8 (*red*) and most likely DEL-1 (*blue*) is assembled in motorneurons and is required for the normal sinusoidal body wave characteristic of *C. elegans* locomotion. Extracellular domains of this channel could associate with an alternative splice variant of MEC-9 (*orange*) in the extracellular matrix (*yellow*). Because collagen-channel interactions have been implicated in function of other degenerin channels, a yet-to-be-identified collagen may also interact with the UNC-8/DEL-1 channel. UNC-1 (*light blue*), a stomatin-like protein, genetically interacts with the UNC-8 channel and is postulated to contact the channel directly. The link to other cytoskeletal proteins is hypothetical and based on potential homology to the touch receptor channel. Note that physical associations shown in A and B are proposed on the basis of genetic observations, but direct protein-protein interactions have not been shown.

stretch-sensory motorneurons.[12] Interestingly, *unc-1*, which encodes a stomatin homolog,[27] is expressed in nearly the same set of neurons as *unc-8*, and shows genetic interactions with *unc-8* (S. Sabnis, P. Morgan, and M. Sedensky, Case Western Reserve University and University Hospitals of Cleveland; personal communication), once again implicating an interaction between a *C. elegans* DEG/ENaC channel and a stomatin-like peripheral membrane protein. Thus, an association with stomatin-related proteins is an emerging theme for members of this channel subclass. Additional candidate *unc-8* interacting genes that have been identified in genetic screens remain to be characterized.[28,29] The genetic screens also suggest that the UNC-8 channel may be regulated by a second messenger signal transduction pathway involving phospholipase A_2 and lipoxigenase.

Yet another member of the *C. elegans* degenerin subfamily may contribute to proprioception by monitoring muscle stretch. Semidominant mutations of the *unc-105* DEG/ENaC gene create constitutively activated channels that cause muscle hypercontraction.[30,31] Interestingly, this phenotype can be reversed by specific mutations in the *let-2* type IV collagen gene. In conjunction with studies of the MEC-4/MEC-10 touch receptor channel that implicate the MEC-5 collagen in touch transduction,[19] these data suggest that association with extracellular matrix collagens may also be a common feature of *C. elegans* degenerin channel complexes. UNC-105 is the only *C. elegans* family member that participates in a channel that has been characterized electrophysiologically.[24] Although homomeric UNC-105 channels expressed in heterologous systems are inactive, activated mutant variants exhibit an amiloride-sensitive nonselective cation current. Stretch-activated gating and physical association with other subunits remain to be established.

Neurodegeneration

Several of the *C. elegans* channel subunits implicated in mechanotransduction have a second, unusual property: specific amino acid substitutions in these proteins result in aberrant channels that induce the swelling and subsequent necrotic death of the cells in which they are expressed. This pathologic property is the reason that proteins of this subfamily were originally called degenerins.

Toxic or cell-crippling degenerin mutant subunits have been reported for MEC-4, MEC-10, UNC-8, UNC-105, and DEG-1 (another family member identified by an unusual dominant mutation that causes neuronal degeneration[32]). Toxic substitutions can affect either of three sites: (1) a region immediately after the first membrane spanning domain;[31] (2) a stretch of amino acids in the extracellular loop that might be part of the channel gate,[12,33] and (3) a single small side chain residue in the vicinity of the channel pore, corresponding to MEC-4 Ala713.[13,14,16] All three classes of substitutions are thought to generate hyperactive channels that allow increased ion influx. Interestingly, the cell death that occurs appears to involve more than the bursting of a cell in response to osmotic imbalance.[34] Rather, it appears that the necrotic cell death induced by these channels may activate a death program that is similar in several respects to that associated with the excitotoxic cell death that occurs in higher organisms in response to injury in stroke. Thus, the toxic degenerin mutations provide the means with which to examine the molecular genetics of injury-induced cell death in a highly manipulable experimental organism.

Additional DEG/ENaC family members are encoded by the C. elegans *genome*

With sequence analysis of the *C. elegans* genome complete, it is possible to survey the entire gene family within one organism (there do not appear to be any readily definable members of the DEG/ENaC superfamily encoded in the fully sequenced genome of the yeast *Saccharomyces cerevisiae*). ~20 members of the DEG/ENaC superfamily have been identified in the *C. elegans* genome. The degenerins make up one six-member subfamily, which features two conserved extracellular cysteine-rich domains (CRD1 and CRD2) in addition to CRD3. Other related genes are probably best categorized into multiple subfamilies, but one subfamily (eight distant members) can be defined by the presence of a conserved region in the extracellular domain related to, but distinct from, degenerin CRD2. The only genetically characterized member of this group is the *flr-1* gene.[35] *flr-1* mutations confer fluoride resistance, slow growth, and altered defecation cycles. How the FLR-1 channel affects these phenotypes is unknown.[36,37] The recent development of practical techniques for gene knock-outs in *C. elegans* should enable other members of the *C. elegans* superfamily to be genetically characterized in the near future.

Epithelial Na^+ channel (ENaC) regulates salt reabsorption

There is little question that salt homeostasis is fundamentally important to the survival of all organisms. In humans, elevated Na^+ levels increase blood pressure and potentiate circulatory and renal system failure, whereas Na^+ deficits cause potentially lethal dehydration and metabolic acidosis. ENaCs mediate the rate-limiting step in sodium reabsorption in distal kidney, colon, and lung epithelia and, thus, play a central role in Na^+ homeostasis in higher organisms. Not unexpectedly, these channels also can contribute to hereditary Na^+-balance disorders.[38,39] Elegant expression cloning studies established that the core of this channel is usually composed of the DEG/ENaC family subunits α- β-, and γ-ENaC,[40–42] and occasionally of α- β-, and δ-ENaC.[43]

The ENaC channel is highly selective for Na^+ and Li^+. This channel is unusual among the members of the DEG/ENaC superfamily in that it exhibits a high open probability and long open channel times.[6] Despite this high activity level, tight regulation over ENaC function is maintained. Hormonal control of channel activity is mediated by aldosterone, vasopressin and insulin[44,45] (at least some of this regulation is at the transcriptional level). In addition, strict control over the number of channels assembled in the apical membrane is accomplished in part by regulation of subunit turnover. α- and β-ENaC subunits have proline-rich motifs in their cytoplasmic C-termini that target subunits for rapid degradation by the ubiquitin pathway.[46,47] The importance of the degradation system is underscored by the finding that mutations that disrupt the proline-rich sites cause the hypertensive disorder Liddle's syndrome: lack of subunit turnover increases the number of functional complexes assembled at the apical membrane, which in turn increases Na^+ uptake and chronically elevates blood pressure.[38,48–51]

Most ENaC channel complexes at the cell surface are inactive at a given time,[51] but channels can be activated by means of a variety of regulatory mechanisms, including the action of PKA/cAMP, PKC, Ca^{2+}, and G-proteins (reviewed in Reference 6). Interestingly, ENaC activity can be down-regulated by the activity of Cl^- transporter CFTR.[52,53] The nature of this interaction is a matter of controversy. Down-regulation of ENaC may also occur by means of an interaction with extracellular proteins, an inhibition that can be relieved by an extracellular serine protease that cleaves an unknown protein.[54,55] As is true for the *C. elegans* degenerins, several proteins appear to associate with the ENaC channel complex in vivo (see Reference 6). At present, known ENaC-associated proteins are distinct from those thought to be associated with *C. elegans* mechanotransducing degenerin complexes.

In addition to roles in renal Na^+ reabsorption, subunits of the ENaC channel contribute to several other aspects of salt/fluid regulation. A critical role in neonatal lung is exemplified by the phenotype of the αENaC knockout mouse: newborns fail to clear lung fluid and consequently suffocate.[56] ENaC subunits are expressed in epithelia of sweat ducts, where they likely modulate salt excretion, and in taste buds, where they may play a role in taste transduction. The δ subunit, most closely related to α, is mainly expressed in brain, testis, ovary, and pancreas, and has an unknown function.[43] The ENaC subfamily is likely to contain additional members that may hetero-multimerize to create channels that participate in a range of biological activities related to Na^+ homeostasis.

ASICs: a mammalian DEG/ENaC subfamily gated by protons

Chronic inflammatory conditions are accompanied by tissue acidosis, a localized drop in pH. Such pH changes activate proton-gated channels in nociceptors that contribute to pain sensation. Recent data suggest that DEG/ENaC family members may play a role in this process. Four mammalian DEG/ENaC channel subunit genes make up a single subfamily, named ASICs according to a recently suggested nomenclature.[9] ASICs participate in proton-sensitive gating and are expressed extensively in neurons: ASIC1 (also known as BNaC2[57,58]) is widely expressed in the brain and peripheral nervous system and has an alternative splice variant expressed specifically in sensory neurons;[59] ASIC2 (previously named MDEG1/BNC1/BNaC1[57,60,61]) is expressed widely in the central and peripheral nervous system and in taste buds[62] (an alternatively spliced variant, ASIC2a/MDEG2,

encodes a different N-terminal region[63]); ASIC3 (previously named DRASIC, for dorsal root ganglion acid-sensing channel) is specifically expressed in peripheral sensory neurons,[64] and the htNaC/ASIC4 subunit is highly expressed in human testis.[65] Several ASIC channels expressed in *Xenopus* oocytes show rapid gating by protons: exposure to very low pH induces a rapidly inactivating cation current.[58,63] ASIC3 channels respond to dramatic pH changes by activating a biphasic Na^+ current, which is more characteristic of acid-responsive currents in nociceptors.[64] Heteromeric ASIC1+ASIC2 and ASIC2/2a + ASIC3 channels have properties distinct from homomeric channels,[63,66] and thus subfamily members are likely to heteromultimerize in vivo to create channels of many different properties. Given the extremely low pH needed for the activation of these channels (which is in the range of the pK_a of the acidic side chain of aspartate and glutamate), it is tempting to speculate that channel opening could involve protonation of acidic residues at the extracellular face of the channel.

Although members of this subfamily are strong candidates for nociceptors in the peripheral nervous system, their function elsewhere in the nervous system is an interesting open question. Low pH environments are known only for limited populations of acid-sensitive neurons, yet ASIC1, ASIC2, and possibly ASIC4, all of which require very low pH conditions to gate the channel in oocyte expression systems, are expressed throughout the brain and other tissues. One possibility is that localized transient pH changes are more important in synaptic signaling than currently appreciated. Alternatively, heteromeric channels assembled in vivo may require moderate proton concentrations for activation.

Fly family members: activity in sensory neurons and gametogenesis

Two *Drosophila* DEG/ENaC members, Pickpocket (PPK/dmdNaC1[67,68]) and Ripped-pocket (RPK/dGNaC1[67,69]), are distinguished by yet another unique sequence motif in the extracellular domain. It is intriguing that PPK is expressed in some candidate mechanosensory neurons in late stage embryogenesis and early larval development. However, the PPK mutant phenotype remains to be characterized and the question of whether any isolated *Drosophila* mechanosensory mutants[70] actually harbor mutations in fly DEG/ENaC genes remains to be answered. Efforts to record currents mediated by PPK channels expressed in *Xenopus* oocytes have not yet been successful, although the PPK subunit can associate with a mammalian ASIC subunit.[67]

Amiloride-sensitive Na^+ channels have been implicated in some of the earliest events in development, including immediate block of secondary sperm entry in *Xenopus* fertilization and generation of the fluid-filled blastocoel space in vertebrates. The RPK/dGNaC subunit links a DEG/ENaC superfamily member to gametogenesis and early postfertilization events. RPK/dGNaC1 transcripts are present exclusively in fly ovary (nurse cells, oocytes, and follicle cells with peak expression at stage 10) and testis (in male genital tract, cysts harboring primary spermatocytes or meiotic cells) and are maternally deposited into the embryo, where they persist until late gastrulation. This expression pattern suggests a specialized role for RPK/dGNaC1-containing channels in early development, perhaps in hydration or gamete-loading events in oogenesis and spermatogenesis.[67,69] Although flies lack a blastocoel in early development, it is intriguing that the electrophysiologic properties of the fly RPK/dGNaC1 channel in oocytes (equally permissive for Li^+ or Na^+; nonpermissive for K^+; amiloride IC_{50} = 9–24 μM) are similar to those described for an amiloride-sensitive channel in rabbit embryos that contributes to the formation of the blastocoel.[71] Interestingly, although RPK/dGNaC1 is expressed in *Xenopus* oocytes, its activity cannot be detected upon transfection into mammalian cells, suggesting that RPK/dGNaC1 activity may be modulated by an oocyte-specific factor, possibly another subunit.[69] Perhaps members of the branch of the superfamily defined by RPK/dGNaC1 play conserved roles in fluid distribution and cell volume regulation in gametogenesis and early development, a hypothesis that can begin to be addressed by determining the mutant phenotype in the fly and by characterizing mammalian orthologs.

FaNaCh: a peptide-gated channel

Yet another example of diversity in the DEG/ENaC superfamily comes from a distant member (named FaNaCh) cloned from the snail *Helix aspersa*.[72] *Xenopus* oocytes expressing FaNaCh exhibit an amiloride-sensitive Na^+ current in response to application of the neuropeptide FMRF-amide. Although the direct FMRFamide gating of this channel is somewhat surprising (in light of the prevailing view associating neuropeptides with activation of G-protein coupled receptors), data support the idea that FaNaCh is the first cloned ion channel to be directly gated by a peptide and suggests that mammalian counterparts, still to be discovered, could be gated by peptide binding.

Structure-function relations in DEG/ENaCs

Although DEG/ENaC superfamily members participate in a remarkable range of biological activities and are gated/regulated by means of diverse mechanisms, a striking degree of sequence conservation holds for the group. Conserved domains most likely identify key functional elements of this channel class. Likewise, blocks of sequence conserved uniquely within each subfamily may help identify regions that contribute to subfamily specific properties.

Intracellular N-terminus

The intracellular N-termini of DEG/ENaC subunits can be divided into two subregions: (1) a variable domain of un-

known function (but see Reference 73) present at the beginning of the amino terminus, which is highly divergent even within subfamilies (except for the *C. elegans flr-1* subfamily); and (2) the pre-MSD1 region, a highly conserved region of ~15 amino acids, positioned ~10 residues before the first hydrophobic domain (see Fig. 1).

As predicted, the conserved pre-MSD1 domain is critical for the function of several DEG/ENaC subclasses. Some loss-of-function mutations in the *mec-4, mec-10,* and *unc-8* degenerins disrupt this region.[12,14,74] Moreover, a point mutation specifying change G37S in human αENaC causes pseudohypoaldosteronism (PHA) type I,[75] a severe neonatal salt-wasting syndrome associated with a dramatic reduction in Na^+ reabsorption. Studies suggest that alteration of this conserved Gly residue causes a reduction of channel open probability,[75] implicating the pre-MSD1 region in channel gating.

MSD1

The contribution of MSD1 to DEG/ENaC function has not been tested extensively. In one study, a channel made of chimeric subunits in which the *C. elegans* MEC-4 MSD1 was substituted for that of rat αENaC was assayed electrophysiologically.[23] The chimeric channel exhibits properties similar to the homomeric αENaC channel in *Xenopus* oocytes, except for a decline in open channel probability. The specific amino acids responsible for this change were not identified, and it is unclear whether residues within the transmembrane domain directly participate in the gate or whether the MSD1 substitution confers a subtle conformational change that influences gating. Acknowledging the paucity of data on this region, we speculate that MSD1 contributes to channel activity in a structural rather than catalytic manner. Perhaps hydrophobic MSD1 domains contributed by several members of the multimeric channel encircle the pore-lining MSD2 helix (see below) to buffer/support its positioning in the membrane.

Extracellular domains

The extracellular domains of DEG/ENaC family members include both variable and conserved regions. Highly conserved among all superfamily members are a short region about 20 to 30 amino acids after MSD1 (referred to as post-MSD1), CRD2, and CRD3. Little experimental data address the functions of these domains. In *C. elegans* UNC-105, amino acid change P134S/T situated adjacent to conserved post-MSD1, causes channel hyperactivity,[24,31] suggesting that this region might prevent inappropriate channel opening. A human αENaC loss-of-function mutation causative for the disorder PHA disrupts a splice site affecting the post-MSD1 region, producing two alternative transcripts: one encoding a truncated polypeptide, and another encoding substitution KYS→N, which most likely creates a nonfunctional subunit.[76] Clearly, more experimental manipulation of this region must be conducted before any model for its role in channel function can be constructed.

How do CRD2 and CRD3 contribute to channel function? The strict conservation of cysteine residues suggests that disulfide bonds may provide the backbone for an extracellular structure essential for channel function. Mutations identified in *C. elegans mec-4* and *unc-8* indicate that single amino acid substitutions that disrupt channel function cluster near and within CRD3 and do, in fact, affect some of the conserved cysteine residues.[12,74] Interestingly, in *mec-4*, some substitutions that knock out normal channel function are not able to fully override the toxicity of the channel-activating substitution A713V, suggesting the CRD3 residues are not needed for channel activity if the channel is forced open by abnormal means. Finally, we note that, although several residues are highly conserved among all family members, domains CRD2 and CRD3 are even more conserved within each subfamily. Perhaps these regions contribute both to conserved and specialized functions of the channel class.

A particularly striking example of the extended conservation within a subfamily is that of the *flr-1 C. elegans* subfamily, which has a highly conserved, but novel, extracellular region related to CRD2. It is not known how this domain contributes to channel function. In *C. elegans* degenerins, one subfamily-specific conserved domain that has been experimentally analyzed is the Extracellular Regulatory Domain (ERD), situated before CRD2 (see Fig. 1), where mutations cause swelling and often cell degeneration.[12,33] It has been proposed that this region contributes to an extracellular gate whose normal function is to keep the channel closed unless stimulated to open.

MSD2 region contributes to the channel pore

The second hydrophobic domain of DEG/ENaC family members is strikingly conserved and includes residues that contribute to the channel pore. The conserved region consists of a short loop that dips into the membrane from the extracellular side (the pre-MSD2 loop) and a transmembrane span (MSD2). Several lines of evidence suggest that the pre-MSD2 region is partially embedded in the membrane. The hydrophobic pre-MSD2 region of αENaC is protected from proteolysis from either side of the membrane.[1,77] Experiments in which a Ca^{2+} binding site (G522D) was introduced into the αENaC subunit allowed manipulation of a voltage-dependent channel block that indicates αG522 is buried about 15% into the membrane.[78] Mutagenesis of the cognate positions in the α and β subunits, as well as of the residue situated three amino acids further into the hydrophobic domain, established that these amino acids influence ion permeation. The emerging working model for the channel predicts that αβ, and γ subunits are arranged around a central conducting pore and that the pre-MSD2 region of each subunit is situated at the outer mouth of the hydrophilic pore.

The pre-MSD2 region also includes a key amino acid position, which, when altered, has the most pronounced effect in several hyperactive DEG/ENaC channels. This site, which corresponds to MEC-4 A713, was first identified in the *C. elegans* degenerins. Small side chain amino acids are found at the cognate position in all DEG/ENaC family members. Large side-chain substitutions at this position in DEG-1, MEC-4, MEC-10, ASIC1, ASIC2, and RPK (but not any of the ENaC subfamily) cause channel hyperactivity.[13,14,23,32,61,66,67] Further insight into the role of this site in channel gating is brought by the recent observations suggesting that proton binding and channel opening in ASIC2 cause a conformational change exposing this site to the extracellular side of the membrane.[79] Mutations that substitute a large residue in this site lock the channel in a low conductance open state and increase channel sensitivity for protons, allowing full opening at pH values close to 7.[80] Although the exact mechanism by which substitutions activate the channel is unknown, mutagenesis data are consistent with a model in which the small side chain amino acid serves as a ''hinge'' site that enables the channel to switch between open and closed conformations; a large amino acid residue at this position forces the channel into an at least partially open conformation and may also reduce the barrier for full opening. Hyperactive effects have also been observed for other substitutions near, but more N-terminal to, the analogous site in UNC-105 and in αENaC,[31,81] supporting the idea that this region is an important determinant in channel gating.

The transmembrane MSD2 also harbors residues that influence ion conductance and, therefore, is also thought to contribute to the channel pore. Channels made of chimeric subunits in which *C. elegans* MEC-4 MSD2 replaces rat αENaC MSD2 exhibit differences in unitary channel conductance, ion selectivity, and open probability.[18,23] Two amino acids, rat αENaC S589 and S593, are primarily responsible for the differences in channel properties. These residues are proposed to be situated on a transmembrane α-helix that contributes to the channel pore. Consistent with this model are studies of *C. elegans* MEC-4, MEC-10, and DEG-1 in which clustered loss-of-function mutations affect the highly conserved hydrophilic face of the predicted transmembrane α helix.[14,33,74] Affected residues within MEC-4 MSD2 appear essential for ion influx.

Although a working model of the DEG/ENaC channel pore is emerging, more detailed investigation of the pore will be required to clarify some experimental mysteries. For example, small and polar residues within MSD2 confer single-channel conductances that are smaller than bulky hydrophobic residues.[18,23] Also, hydrophobic substitution αENaC S593I, currently thought to lie deep within the channel, induces Ca^{2+} binding, contrary to what would be expected if this residue is located in the narrow constriction site of the inner pore.[23]

C-terminus may mediate different functions in DEG/ENaCs subfamilies

In general, the intracellular C-termini of DEG/ENaCs vary in length and in sequence. Nonetheless, this region can play critical roles in channel function and regulation. In ENaCs, conserved proline-rich motifs in the C-terminal region are essential both for localizing the channel to the apical membrane (α subunit[82]) and for regulating channel stability (β and γ[38,46–51]). Little is known about functions of C-termini of other family members, most of which lack proline-rich repeats identical to those in ENaCs. The *C. elegans flr-1* subfamily has long and conserved C-termini, but characterization of the function of this domain must await the analysis of the more general roles this subfamily plays in *C. elegans* physiology.

Amiloride binding

Members of the DEG/ENaC superfamily that have been characterized electrophysiologically are inhibited by the diuretic amiloride. Where does amiloride bind? Anti-amiloride antibodies were used to identify a potential binding site at position 278–283 in the extracellular domain of rat αENaC (WYRFHY). This site is fairly well conserved in α and β subunits across species and, when altered, dramatically changes amiloride sensitivity.[81] Other experiments indicate that residues in pre-MSD2 and MSD2 influence amiloride binding and suggest that amiloride blocks by interacting at the outer mouth of the channel[4,78] (binding is not equal to all subunits). Perhaps several regions contribute to amiloride binding with a portion of the extracellular loop in close proximity to the pore entrance.

Perspectives

In recent years, it has become clear that DEG/ENaC channels mediate diverse biological functions and are gated by means of several different mechanisms. Beyond this diversity, however, a clear image of a highly conserved subunit structure emerges. This strong conservation across species suggests that DEG/ENaC family members shared a common ancestor early in evolution. The basic subunit structure may have been adapted to fit a range of biological needs by the addition/modification of domains that are uniquely conserved within the subfamilies, a hypothesis that can be tested in future structure/function analyses.

Although much remains to be learned about channel functional domains, it is interesting that the basic design of DEG/ENaC subunits appears strikingly reminiscent of the basic design of voltage-gated K^+ channels, as suggested by Jan and Jan.[83] The pre-MSD1 region implicated in gating, the pre-MSD2 loop that contributes to the channel pore, and tetrameric stoichiometry are features of both DEG/ENaC and K^+ channels that may indicate a common and ancient origin. Interestingly, however, unlike K^+ channels, which are also found in plants and in yeast, DEG/ENaC channels have so far been only identified in the animal kingdom.

Another question of interest is to what extent specific channel functions are conserved across species. For example, do degenerin-like subunits mediate touch sensation in vertebrates?[84] Is salt homeostasis regulated by DEG/ENaCs in *C. elegans*? Given the large number of *C. elegans* DEG/ENaC family members, it is plausible that hundreds of yet-to-be characterized subunits are encoded in mammalian genomes, some of which might participate in mechanotransduction. On the other hand, the *C. elegans* genome sequence is complete, and clearly defined nematode members of the ASIC, *Drosophila* PPK/RPK, and ENaC subfamilies have not been identified. This supports the idea that each subclass could be specialized for distinct functions in different organisms. Clarification of this issue awaits characterization of additional superfamily members; studies that will be fueled by sequencing of the human genome.

Other important questions that remain as yet unanswered concern the fine architecture of the channel pore, the nature of interactions of each subunit with other proteins, the modulation of channel activity by signaling pathways, and the functions of newly identified families. While the field investigates these areas, we can expect that DEG/ENaC family members will be faithfully watching our salt.

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Note added in proof

Two recent structure/function studies of DEG/ENaC channels may be of interest to readers.[85–87]

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