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Insight into DEG/ENaC Channel Gating from Genetics and Structure

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Abstract

The founding members of the superfamily of DEG/ENaC ion channel proteins are *C. elegans* proteins that form mechanosensitive channels in touch and pain receptors. For more than a decade, the research community has used mutagenesis to identify motifs that regulate gating. This review integrates insight derived from unbiased *in vivo* mutagenesis screens with recent crystal structures to develop new models for activation of mechanically-gated DEGs.

The neurons that detect mechanical stimuli, such as the gentle touch of a loved one, the vibration of a cell phone, or the painful stub of a toe, have been characterized [reviewed in (19)], but the ion channels that translate these stimuli into electrical signals are only beginning to come to light. Established mechanosensitive ion channels in animals include several members of the degenerin and epithelial sodium channel (DEG/ENaC) family expressed in mechanoreceptor neurons [reviewed in (26)]. Here, we review and amalgamate insight derived from touch disrupting channel mutations and high-resolution crystal structures of a DEG/ENaC channel, reinterpreting the former in the context of the latter. Our goal is to explore shared design principles that govern activation of DEG/ENaC channels, focusing on the specializations that enable a subset of these channels to detect touch in mechanoreceptor neurons. Based on this exploration, we develop models for the structural rearrangements that embody DEG/ENaC channel activation. Such heuristic gating models are naturally speculative and will require extensive testing with experiment, additional high-resolution structures, and simulation.

The Physiology of DEG/ENaC Channels

DEG/ENaCs are nonvoltage-gated, sodium-selective, amiloride-sensitive channels with diverse cellular functions assembled from three identical or homologous proteins [reviewed in (42)]. Four major functional subclasses are shown in Figure 1. Three DEG/ENaC proteins are confirmed, pore-forming subunits of mechanosensitive ion channels critical for touch and pain sensation: MEC-4, MEC-10, and DEG-1 (25, 52). They belong to the DEG subfamily that also includes UNC-8 and UNC-105, which are linked to mechanosensation in neurons and muscle [reviewed in (26)]. These DEG channels open in response to both the delivery and removal of mechanical stimulation and close during the stimulus pulse (Fig. 1).

Such phasic response dynamics are a shared feature of five classes of mechanoreceptor neurons in *C. elegans* (26), have been reported in mammalian somatosensory neurons probed *in vitro* (54), and were first described in the 1950s in the vibration sensors of mammals (Pacinian corpuscles) (3, 29). This consensus suggests that many mechanoreceptor neurons detect both the application and withdrawal of mechanical stimulation.

Classical genetic approaches including unbiased chemical screens have uncovered more than 50 missense mutations in the DEG/ENaC proteins that mediate *C. elegans* touch sensation (12, 20, 24, 27, 31-33, 51, 57, 66, 72). Such mutations disrupt behavioral responses to mechanical stimuli either because of a loss of DEG channel function or because of neuronal cell death caused by inappropriate activation of DEG channels. The latter phenotype results from gain-of-function mutations, including those affecting the so-called *d* (for degeneration) position in DEG-1 and MEC-4 (12, 20). In this review insight inferred from analysis of these mutations will be combined with data from other members of the superfamily in building hypotheses about the closed, open, and desensitized states of DEG channels during mechanical gating.

Trimeric DEG/ENaC Channel Structures

All DEG/ENaC proteins share a common topology consisting of two transmembrane helices (TM1, TM2), a large ectodomain, and intracellular N- and C-terminal domains (Fig. 2A). Sequence conservation suggests abundant structural similarities and allows for integration of experimental data across the various family members (Fig. 3). For example, replacing the native, small-volume residue found at the *d* position at the top of TM2 with larger-volume residues increases open probability in most, if not all, DEG/ENaC channels tested.

The two published structures of the proton-activated chicken (c) ASIC1a likely capture the channel in a desensitized state (28, 35). Their ectodomains are identical and are compared to that of a hand (Fig. 2A): a wrist joins the seven-stranded β -sheet palm and disulfide-rich α -helical thumb to the TM domain, while an α -helical knuckle and finger help the palm and thumb grasp a five-stranded β -ball (35). The two structures differ in their resolution and the orientation of their TM helices. The 1.9 Å-resolution 2007 structure is derived from a non-functional channel with kinked TM helices (35), and the 3.0 Å-resolution 2009 structure (Fig. 2B, C) is of a functional cASIC1a channel with symmetrically-oriented helices (28). The higher resolution structure (35) will be used for analysis of the ectodomain, and the subsequent lower resolution structure of a functional channel (28) will be used for analysis of the TM domain.

A Dynamic Rule of Thumb

The thumb is made up of two α -helices and several structured loops (35) (Fig. 2). Five highly conserved disulfide bridges stabilize its architecture and likely lock it into a rigid rod that hinges where it connects to the base of the palm. The proximity of the thumb's hinge to the top of the TM domain led to the proposal that this region is important for transferring gating motions from the ectodomain to the TM domain (35). Experiment supports this view (47, 64). Genetic screens identified several mutations in the thumb that disrupt touch

sensation in *C. elegans*; many cluster at the tip, but none localize to the hinge (Fig. 2B, C). Thus, while experiments with ASICs (47) and ENaCs (64) suggest that residues in the hinge are crucial for gating, genetic screens have not recovered mutations in this region in DEGs. Instead, key regions highlighted by mutation cluster on the face of $\alpha 5$ that is adjacent to the finger and on the surface of the $\alpha 4$ - $\alpha 5$ linker that is in contact with the β -ball (Fig. 2B).

Among the mutations that map onto the thumb domain, only one is a gain-of function mutation presumed to induce channel hyperactivation. This residue, UNC-8 A586T, is predicted to be at the tip of $\alpha 5$ (66) (Fig. 2B, 3). Gessmann *et al.* (27) proposed that the increase in steric bulk or the presence of a novel hydrogen-bonding partner might account for the change in function induced by this mutation. Mutations at the tip of $\alpha 5$ in cASIC1a also affect channel gating: cASIC1a P338A displays decreased proton sensitivity, a change attributed to disruptions of a favorable hydrophobic interaction between the thumb and the finger (70). A screen for suppression of hyperactivation caused by the MEC-4 *d* mutation (57) identified a mutation one turn down $\alpha 5$, MEC-4 R619L (Fig. 2B, 3), that suppresses degeneration. This finding suggests that this residue plays an important role in the open state. Neighboring this residue is a histidine linked to zinc potentiation of rat (r) ASIC2a-containing channels (6). The next two turns down $\alpha 5$ contain aspartates critical to proton sensitivity in cASIC1a, D346 and D350 (35, 70) (Fig. 3). These two aspartates are likely involved in proton-dependent carboxyl-carboxylate interactions with residues in the finger (35).

Three touch-disrupting mutations (MEC-4 G598E, D599N, and R601C) (32) are found in the structured loop between $\alpha 4$ and $\alpha 5$ that packs against the β -ball (35) (Fig. 2B, 3). This interface likely regulates the open state of the channel since these three mutations also suppress degeneration caused by a hyperactivating mutation at the *d* position (32). When the residue aligned with MEC-4 R601 in cASIC1a (H328) (Fig. 3) is mutated to alanine, proton sensitivity is decreased (70). Yang *et al.* (70) argued that this effect was caused by a decrease in a favorable hydrophobic interaction. A mutation at the bottom of $\alpha 4$ in UNC-8 (E552K) that disrupts locomotion (27) further supports the importance of the thumb- β -ball interface since this mutation is found at the other major contact point between these two domains (Fig. 2B, 3). Thus, channel gating may involve important interactions not only between the thumb and finger but also between the thumb and β -ball.

The two last thumb mutations to discuss may affect the disulfide bridges knitting the thumb together. The first is at a conserved proline (MEC-4 P643L) (57) adjacent to a disulfide bridge connecting the structured loops at the base of the thumb (Fig. 2B, 3). This mutation suppresses degeneration caused by mutation of the *d* position. Perhaps it reorients the disulfide bridge, preventing the thumb from adopting its open-state conformation. The second (MEC-4 C595Y) (32) is predicted to eliminate the disulfide bridge connecting $\alpha 4$ with $\alpha 5$ (Figs 2B, 3). Yet, the equivalent cysteine is not required for rENaC constitutive activity (23). Perhaps the rigid association between $\alpha 4$ and $\alpha 5$ provided by this particular disulfide bridge is critical for the gating movements of the thumb during mechanical activation but not for the constitutive activity of ENaCs.

Finger Tuning Channel Function

The finger is the region of lowest sequence conservation and highest variability between DEG/ENaC subclasses. Thus, this region may underlie much of the functional specificity between proteins within the superfamily. In the cASIC1a crystal structure (35), the finger is made up of three α -helices and several well-structured loops. The structures of DEG and ENaC fingers likely differ considerably since large cysteine-rich insertions are found in the mechanosensitive DEG protein fingers, while smaller insertions are found in the fingers of the constitutively active ENaCs (Fig. 3). The DEG insertions include a short stretch of residues called the extracellular regulatory domain (ERD), which can mutate to cause channel hyperactivation (24, 66). [The mutations found through genetic screens were DEG-1 A393T and UNC-8 G387E (24, 66) (Fig. 3).] Touch-disrupting mutations are spread throughout the finger of the MEC-4 protein (G230E, A321T, P395L, E397K, A420V, and E445K) (32) (Fig. 3), underscoring the importance of the entire domain on protein function.

The finger is involved in a wide range of functions in ASICs. The finger's association with the thumb (35), role in zinc potentiation (6), and importance for proton sensitivity (35) were mentioned above. Residues in the thumb of ASICs regulate the proton dependence of activation and steady-state inactivation (5), the level of channel expression (70), and binding of the ASIC1a inhibiting toxin psalmotoxin-1 (55).

The finger is important for FaNaC and ENaC activation as well. Residues in the finger of FaNaCs that align with $\alpha 1$ in cASIC1a determine the effective concentration of FMRFamide (16). In ENaCs the finger has been functionally linked to both shear stress sensitivity (63) and proteolytic regulation (10, 34, 36). ENaC α and γ subunits are proteolytically cleaved at two sites in the finger domain, resulting in the removal of dozens of residues and an increase in open probability (10, 34). If the deleted ENaC peptides are applied to the mouse (m) ENaC α channel, they inhibit activity through an interaction with both the finger and the tip of the thumb (36). Residues implicated in this interaction include mENaC α E470 and D473, which align near the hyperactivating UNC-8 A586T thumb mutation (66) discussed above (36) (Fig. 3). Kashlan et al. (36) proposed that the intact finger inhibits the thumb through a physical interaction between the two and that proteolysis relieves this intramolecular inhibition. ASIC1 can also be proteolytically cleaved at a site in the finger *in vitro*, but in this case cleavage results in decreased channel function (14, 69).

The Importance of the Palm

The core of the ectodomain is comprised of a network of β -sheets that come together to form an internal solvent-filled central cavity in the ASIC1a crystal structure (35). Mutagenesis screens identified an acidic residue in UNC-105 (E677) located on $\beta 12$ that can mutate to cause channel hyperactivation and is predicted to extend into this central cavity (51) (Fig. 2B, 3). The corresponding residue in rASIC3 is accessible to small molecule derivatives of 2-guanidine-4-methylquinazoline (GMQ) in the closed state (71) and binding of GMQ leads to channel activation. Furthermore, this residue likely packs against a glutamate in $\beta 1$ that is also accessible to derivitizing reagents when the channel is closed but not once it is desensitized (18, 71). Mutation of this latter residue to alanine speeds

desensitization but has no effect on activation (18). [In cASIC1a the homologous residues are E417 and E80 (Fig. 3)]. The inaccessibility of the derivitizing reagents during desensitization agrees with the isolated central cavity seen in the cASIC1a structure (35), while the accessibility in the closed state argues for a means of entry to this central cavity in other channel states. An intersubunit widening of the lower portion of the palm could allow reagents into the central cavity through lateral fenestrations. A widening could also account for the hyperactivating phenotype of UNC-105 E667K since the larger cavity volume might minimize clash caused by the mutant lysine. This model parallels what is expected of the P2X channel family (30, 38, 39, 59) and was first proposed by Gonzales *et al.* (28).

The other palm channel-hyperactivating DEG mutations, UNC-105 P134S/T (51) and MEC-4 A149V (72) (Fig. 2B, 3), are located nearby in the region linking the $\beta 1$ and $\beta 2$ sheets. Hyperactivation by the latter only occurs in the presence of the MEC-10 *d* mutation (72). The $\beta 1$ - $\beta 2$ linker is also associated with tuning the kinetics of proton-gated currents carried by ASICs (15, 48, 50). For example, interchanging residues in this domain between channels with differing desensitization rates swaps their rates of desensitization (15, 48, 50). The nearby $\beta 11$ - $\beta 12$ linker was shown to have a similar impact on channel gating (46). These linkers may participate in the intersubunit widening proposed to occur during activation.

This region carries another mutation that suppresses necrosis caused by mutation of the *d* position: MEC-4 T701I (57) (Fig. 2B, 3). Though it is close to UNC-105 E667, MEC-4 T701 does not extend into the central cavity. Instead it projects into the extracellular space in the cASIC1a structure (35). Data in ASICs suggest that the intersubunit interactions seen in this region of the cASIC1a crystal structure (35) are important for channel function (6, 18). Perhaps mutation of MEC-4 T701 disrupts similar intersubunit interactions that occur in the open state, explaining its suppression of hyperactivation phenotype.

The upper portion of the palm contains five mutations that disrupt touch sensitivity, MEC-4 G533S, G539E, S542F, and UNC-8 T664I (32, 66) (Fig. 2B, 3). The first three MEC-4 mutations and one other, MEC-4 P655L, suppress degeneration caused by the MEC-4 *d* mutation (32, 57). Therefore, these residues are likely important for the channel's open state. The cASIC1a residues that correspond to MEC-4 G533, S542, and P655 pack in a ring where the β -sheets come together at the top of the central cavity. When analyzing their MEC-4 homology model, Gessmann *et al.* (27) proposed that these mutations introduce deformations and break hydrogen bonds important for this region's β -sheet structure. The palm is littered with conserved glycines marking tight turns and β -sheets. Two of the residues identified by mutation, MEC-4 G533 and G539, initiate the β -ball's $\beta 8$ and the palm's $\beta 9$ sheets, respectively (Fig. 2C). MEC-4 S542F affects a conserved residue in $\beta 9$ (Fig. 2C) that projects towards $\beta 8$ in the β -ball. Thus, distortions in this region could disrupt intra and intersubunit interfaces important for channel gating.

In the cASIC1a structure (35), the residues corresponding to MEC-4 G539 and UNC-8 T664 cluster at the tip of the palm, just below the knuckle. Also aligning in this cluster is a human (h) ENaCa residue linked to cystic fibrosis and increased ENaC activity (56) (corresponding

to mENaCα W520 in Fig. 3). Perhaps these residues properly orient the knuckle with respect to the rest of the channel.

The Pore-Lining GxxxG Motif

Any discussion of the mutations identified through genetic screens in the TM domain must begin with the degeneration-causing mutations that gave the DEG family its name. These mutations, DEG-1 A707V (12) and MEC-4 A713V/T (20), affect a residue at the top of TM2 called the *d* position (Fig. 2B, 3). The *d* position is occupied by a glycine, alanine, or serine in most DEG/ENaC proteins, and introducing larger residues results in channel hyperactivity regardless of the channel's normal mode of activation. In some ENaCs (61, 65) and ASICs (67), nearby residues behave similarly when modified with derivitizing reagents. Single-channel *in vitro* recording of MEC-4 channels mutated at the *d* position determined that increasing side chain volume at this site stabilizes the open state of the channel (9). The *d* position is buried in the pore occlusion in the desensitized cASIC1a structure (28); it packs between the side-chain of the *d*+1 aspartate and the *d*+3 and +4 glycines. Derivitizing reagents modified the *d* position of hASIC2 in open but not closed states (2), while no change in current was seen in a similar test performed on ENaCs (65). Yet, in the closed state of a non-desensitizing ASIC mutant, the *d* position is accessible to derivitizing reagents applied externally (49). This last study suggests that exposure of the *d* position in the closed state may be subtype specific.

The *d* position is part of a conserved GxxxG motif, a common helix-packing motif (58) that is also found in the pore-lining helices of the bacterial mechanosensitive channels MscS and MscL (7, 44). Mutations found at all of the GxxxG glycines are known to alter DEG channel function *in vivo* (12, 20, 24, 32, 33, 57). Genetic screens determined that mutating the *d*+3 glycine (MEC-4 G716D, MEC-10 G676R, and DEG-1 G710R) disrupts touch-sensitivity but does not induce degeneration (24, 32, 33) (Fig. 2B, 3). Mutation to glutamate in MEC-4 also suppresses degeneration caused by mutation at the *d* position (57). The same suppression phenotype is seen when the glycines at *d*+4 (MEC-4 G717D/S) and *d*+7 (MEC-4 G720D) are mutated (57). Analysis of *in vivo* mechanoreceptor currents shows that these glycines, as well as those at *d*+7 and *d*+11 (MEC-10 G680E, G684R), are required for sodium selectivity but not for mechanical gating (4, 25, 52). The change in selectivity is expected to decrease responses to touch stimulation by decreasing the driving force for mechanoreceptor currents, an effect that accounts for both the behavioral deficits and suppression of degeneration.

The *d*+11 glycine is also part of a conserved GxS sequence linked to channel function through genetic screens in *C. elegans* (24, 31, 33). Such screens recovered mutations replacing the native residues at *d*+11 and *d*+13 with phenylalanines: MEC-4 G726F and DEG-1 S720F (Fig. 2B, 3). Mutating the GxS motif in ENaCs alters ion selectivity and surface expression (41, 45, 60, 65). Replacing *d*+13 serine with larger residues decreases the relative sodium permeability such that the larger the side chain, the less selective the channel becomes (40). Cysteine-derivitization accessibility studies in ENaCs and ASICs are consistent with a constriction at this conserved GxS sequence in the open state (49, 65). In this scenario mutations to the GxS sequence alter the proper formation of this sodium-

selective constriction in the open state. Since the GxS motif is not associated with a constriction in existing cASIC1a crystal structures (28), Li et al. (49) proposed that opening is likely required for its formation. The crystal structures of the closed and open P2X₄ receptors suggest how this may occur (30). It is interesting to note that opening of the P2X₄ receptor involves a kink in TM2 at a glycine that by alignment neighbors the GxS sequence (28, 30).

Beyond the Glycines in the Pore

Six other mutations have been identified in TM2. MEC-10 L679R (33) affects a conserved leucine at *d*+6 (Fig. 2C), disrupts touch sensation, and decreases sodium selectivity *in vivo* (4). This *d*+6 site is also regulates lateral shear stress sensitivity of ENaCs (1). Two additional touch-disrupting mutations follow the GxS motif and affect conserved residues (Fig. 2C): MEC-4 T729I and E732K (31). The corresponding residues in mENaCa are needed for normal sodium selectivity of the mENaCa $\beta\gamma$ channel *in vitro* (60, 62). Three non-conserved residues also affect touch sensation by suppressing degeneration caused by mutation of the *d* position: MEC-4 C723Y falls just prior to the GxS motif at *d*+10, while MEC-4 L728F and L738E follow the GxS motif (57). Their proximity to the GxS sequence (Fig. 3) suggests that they may be important for refining the selectivity filter, though this idea awaits experimental confirmation.

The Intracellular Domain

A cluster of touch-disrupting mutations that includes MEC-4 T91I, MEC-4 S92F and the homologous MEC-10 S105F, UNC-8 H114Y, and MEC-4 G95E (32, 33, 66) highlights the importance of N-terminal domain (Fig. 3). This domain contains an invariant HG sequence that can mutate to cause not only touch-insensitivity in *C. elegans* but also pseudohypoaldosteronism type I in humans (13). Both MEC-4 S92F and MEC-10 S105F decrease sodium selectivity *in vivo*, and MEC-10 S105F requires slightly more force than wild type for channel activation (4). The impact of these intracellular mutations on selectivity implies that they may shape the pore.

A Proposed Gating Model

Having covered DEG channel structure and function from top to bottom, what does this analysis suggest about channel gating? We propose a model in which activation involves a shift in the relative orientation of the thumb, finger, and palm (Fig. 4A, B). These motions coincide with conformational changes in the lower palm domains, perhaps an intersubunit widening similar to the structural changes predicted for the P2X receptors (30) (Fig. 4A, B). A widening and rotation of the TM helices releases the pore occlusion, freeing the *d* position and potentially forcing the formation of a constriction further down the pore-lining TM2 (Fig. 4A, B). The extent of this rotation and expansion may be constrained by interactions between adjacent subunits, as inferred from the introduction of intersubunit disulfide bonds (67). The palm rearranges upon desensitization, reforming the occlusion seen in the closed state (Fig. 4B, C).

It is straightforward to imagine how binding of protons to the acidic pocket in ASICs (35) or peptides to the finger in FaNaCs (17) could trigger the requisite movements hypothesized for channel opening. Yet, how does a mechanical strain initiate the gating pathway? Current models for mechanical gating of DEG channels involve either tethering of the ectodomain of the channel to the extracellular matrix (Fig. 4A, spring on the left side) or sensitivity of the channel to the lipid bilayer (Fig. 4A, spring on the right side) (11). In these models mechanical strain on the matrix or on the membrane would then translate into channel opening. Potential extracellular matrix tethers for MEC-4-dependent MeT channels include MEC-1 and MEC-9, Kunitz-domain containing proteins required for touch sensitivity (21, 22). These proteins are attractive candidates since a Kunitz domain-containing snake toxin was recently found to activate rASIC1 (8). These tethers could activate DEGs in a top-down fashion, similar to the direction of motion expected for the gating of ASICs by protons and this snake toxin.

Alternatively, if strain in the membrane gates the channel then the gating pathway could begin in the TM domain and culminate in the reorientation of the ectodomain (Fig. 4B). The hyperactivation that results from mutation of the *d* position sets the precedent that gating can occur in this direction. Perhaps mechanical strain results in a thinning or curving of the membrane and destabilizes the occlusion around the *d* position, which drives the constriction downwards and initiates the conformational changes that occur in the ectodomain. In this hypothetical model, the grasp of the finger on the thumb could tune how much mechanical strain on the membrane is needed to open the channel. Consistent with the idea that changes in the lipid bilayer can initiate channel activation, MscL and MscS are known to be gated by membrane tension and to be sensitive to the shape and thickness of the lipid bilayer (53, 68). In these bacterial channels, changes in membrane curvature induce a rotation and tilt of the TM helices along the plane of the membrane that widens and opens the pore (53, 68). If correct, this model implies that specific properties of the native membrane are needed for DEG channel mechanical gating since these channels are impervious to membrane stretch when expressed in heterologous cells.

Conclusion

The current collection of DEG channel data supports a model in which the TM domain and the ectodomain work in concert to regulate DEG channel gating. How the initial mechanical stimulus is applied to the channel and what conformational changes occur during the gating of the channel are still open questions. Further work is needed to test the gating models presented here and to determine exactly how mechanosensitive DEG/ENaC channels operate. Ultimately, a thorough understanding of how these channels function will provide much needed insight into the molecular details of touch sensation and guide future studies on the molecular and physiological origins of touch sensation in animals.

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Figure 1.

DEG/ENaC channels can be activated by both mechanical and chemical stimuli. **A.** DEGs are gated by mechanical loads at both the onset and offset of stimulus. **B.** ENaCs are constitutively active and can be modulated by shear stress. **C.** ASICs are gated by protons. **D.** FaNaCs are gated by the FMRFamide-like peptides.

Figure 2.

Structure and sequence conservation link DEG/ENaC proteins. **A.** Topographical map of a single DEG/ENaC subunit [inspired by (37)]. The TM helices (red), palm (yellow), β -ball (orange), knuckle (blue), thumb (green), and finger (purple) are colored as in (35). The N- and C-termini, wrist, DEG-specific finger insertions (dotted), and ERD (thick dotted) are also identified. **B.** Ribbon diagram of one subunit of the cASIC1a channel color coded according to domain (see Fig. 2A). Residues shown in space-filling representation indicate positions that can mutate to disrupt function. Residues in red can mutate to cause channel hyperactivation and those in blue disrupt touch sensation. **C.** Ribbon diagram of a trimeric cASIC1a channel showing the interaction between adjacent subunits. One subunit is color-coded by domain; the other two are black (rear) or gray (right).

Panels B and C were prepared using VMD and PDB 3HGC.

Figure 3.

Sequence alignment of the DEG/ENaC channels discussed in the text. This alignment was made using ClustalW and manually adjusted to match published alignments (35, 43, 66). The secondary structure derived from cASIC1a structures is indicated above and is color-coded according to domain. Touch-disrupting (blue) and channel-activating (red) DEG mutations, DEG-specific insertions (gray), ERD (pink), and ENaC and ASIC mutations mentioned in the text (black) are highlighted with a colored background.

Figure 4.

Hypothetical gating movements during DEG channel gating. In this scenario mechanical stimuli are delivered by an extracellular tether (left spring), through changes in bilayer tension (right spring), or both. **A.** In the closed state, the DEG channel pore is occluded. **B.** Opening is accompanied by rearrangements in both the ectodomain and the transmembrane domain. **C.** Under constant stimulation the DEG channel may desensitize and adopt the conformation seen in crystal structures of desensitized ASIC1a channels (28, 35). Additional biophysical and structural studies are needed to test these models of mechanical gating.