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Structural mechanisms underlying the function of epithelial sodium channel/acid-sensing ion channel

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Abstract

Purpose of review—The epithelial sodium channel/degenerin family encompasses a group of cation-selective ion channels that are activated or modulated by a variety of extracellular stimuli. This review describes findings that provide new insights into the molecular mechanisms that control the function of these channels.

Recent findings—Epithelial sodium channels facilitate Na⁺ reabsorption in the distal nephron and hence have a role in fluid volume homeostasis and arterial blood pressure regulation. Acid-sensing ion channels are broadly distributed in the nervous system where they contribute to the sensory processes. The atomic structure of acid-sensing ion channel 1 illustrates the complex trimeric architecture of these proteins. Each subunit has two transmembrane spanning helices, a highly organized ectodomain and intracellular N-terminus and C-terminus. Recent findings have begun to elucidate the structural elements that allow these channels to sense and respond to extracellular factors. This review emphasizes the roles of the extracellular domain in sensing changes in the extracellular milieu and of the residues in the extracellular–transmembrane domains interface in coupling extracellular changes to the pore of the channel.

Summary—Epithelial sodium channels and acid-sensing ion channels have evolved to sense extracellular cues. Future research should be directed toward elucidating how changes triggered by extracellular factors translate into pore opening and closing events.

Keywords

acid-sensing ion channel; epithelial sodium channel; gating

Introduction

The epithelial sodium channel/degenerin (ENaC/Deg) family comprises cation-selective ion channels found in animals from hydra and nematodes to vertebrates. Members of this family expressed in mammals are divided into three groups based on the primary structure of the proteins: acid-sensing ion channels (ASICs), brain–liver–intestine sodium channels and human intestine sodium channel (BLINaCs and INaC), and ENaCs. ASICs are expressed in the central and peripheral nervous system where they contribute to sensory processes, such as mechanosensation and nociception [1,2]. BLI-NaC and INaC were originally identified by homology cloning, and their physiological function remains unknown [3]. ENaCs are

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Conflicts of interest

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highly selective Na⁺ channels assembled from three homologous subunits termed α , β , and γ . These channels are expressed at the apical membrane of Na⁺ transporting epithelia where they facilitate Na⁺ reabsorption from the luminal space. ENaC-mediated Na⁺ reabsorption in the distal nephron is important for fluid volume homeostasis and control of arterial blood pressure [4]. In the airways, ENaCs contribute to the maintenance of the airway surface-liquid and mucociliary clearance [5]. This review describes the recent progress in our understanding of ENaC and ASIC function.

Atomic structure of ASIC1

The structure of chicken ASIC1 (cASIC1) in a desensitized-like state, a nonconducting proton insensitive state, was recently resolved [6,7]. cASIC1 has a trimeric quaternary structure with a chalice-like shape (Fig. 1). Each subunit contains two transmembrane spanning helices connected by a large extracellular region with intracellular N-terminus and C-terminus. The extracellular region has well defined domains that have been termed thumb, finger, palm, knuckle, and β -ball (Fig. 1). The core of the protein is constituted by the β -ball and palm domains, whereas the finger, thumb, and knuckle domains are in the periphery. The thumb domain of cASIC1 has a cylindrical organization and contains 10 cysteine residues forming disulfide bridges, which are conserved within ENaC/Deg subunits. This suggests that the overall organization of the thumb domain is similar among members of the ENaC/Deg family. The finger and knuckle domains are poorly conserved among the members of this family, and as discussed below several studies suggest that these domains may be involved in sensing extracellular cues. The pore of cASIC1 has an hourglass-like shape with residues in the TM2 segments forming the ion permeation pathway [7]. Residues in the TM1 segments form a portion of the extracellular vestibule, but are peripheral to the TM2 segments in the rest of the pore. The TM2 helices are tilted by a 50° angle to the membrane normal and their intersection constitutes the desensitization gate. The overall organization of the pore is probably similar in ASIC, ENaC, FMRFamide-gated sodium channel (FaNaCh), and Deg proteins, as suggested by the many residues in common within the TM2 segments.

Gating of epithelial sodium channels/acid-sensing ion channel channels

ENaC/Deg channels are gated or modulated by diverse stimuli including neuropeptides, proteases, mechanical forces, and extracellular ions [1,8–14]. Some family members are constitutively active, for instance ENaCs. The overall topology of ENaC/Deg channels with a large ectodomain resembles the organization of ligand-gated ion channels and suggests a role in sensing extracellular cues. Proton is the only identified ligand for ASICs. ENaCs were initially characterized in the apical membrane of native epithelia as highly selective for sodium over potassium and blockable by amiloride [15]. Although these channels are constitutively active and gating does not require ligand binding, their open probability (P_o) is modulated by extracellular ions, phosphatidylinositides, ATP, proteases, and mechanical forces [8–11,16,17].

Structural basis for proton sensing

Higher mammals express seven different ASIC subunits: ASIC1a, ASIC1b, ASIC1b2, ASIC2, ASIC2b, ASIC3, and ASIC4 [1,2]. ASIC1b2, ASIC2b, and ASIC4 subunits are not activated by extracellular acidification when expressed in heterologous systems. Proton-sensitive ASICs are constitutively closed, but undergo activation and desensitization following extracellular acidification. Then, these channels reside in at least three different states: a resting nonconducting (closed) state where channels can be activated by extracellular acidification, a conducting open state, and a desensitized nonconducting state where channels are insensitive to protons.

Efforts to identify proton-binding sites combining site-directed mutagenesis and electrophysiology established the contribution of the extracellular region in proton sensing [6,18–20]. Paukert *et al.* [18] systematically screened more than 40 conserved charged amino acids in the ectodomain of ASIC1a for possible contribution to proton gating. Liechti *et al.* [19] combined a computational and functional approach to identify potential proton-binding sites in the extracellular region of ASIC1a. Although both studies identified residues within the extracellular region where single amino acid substitutions decreased the apparent affinity for protons, these mutant channels retained the property of being activated by extracellular acidification. Jasti *et al.* [6] identified a highly negatively charged cavity in the extracellular accessible surface of cASIC1, which they proposed constitutes the primary site for proton sensing. This region, formed by the intrasubunit contacts between the thumb, the β -ball, and the finger, and with residues in the palm of the adjacent subunit, contains unusually close carboxyl–carboxylate interactions between the side chains of aspartate and glutamate residues. Mutations of amino acids forming this putative proton-sensor did not prevent proton activation of ASIC1, suggesting that additional residues participate or contribute to proton sensing [6,19,20]. Interestingly, Sherwood *et al.* [21] found that the tract 323–370 of ASIC1a, which contains residues that form part of the proton-sensor, accounts for the differences in proton sensitivity of ASIC1a and ASIC2a. Broadly, these studies suggest that multiple sites in the extracellular region of ASIC1a contribute to proton sensing.

Whether activation triggers desensitization of ASICs or if these two processes occur independently of each other is unknown. Several studies have defined areas within the extracellular region that contribute or alter the rates of desensitization of these channels [22–24]. Recently, Bargeton and Kellenberger [25] investigated the accessibility to sulfhydryl reactive reagents of engineered Cys in the contact region between the finger, adjacent β -ball, and upper part of the palm. They found that this region undergoes a conformational change that is associated with the inactivation of the channel during desensitization. Additional studies are needed to define the contribution of the finger, thumb, knuckle, β -ball, and palm domains of ASIC to proton-dependent activation and desensitization.

Coupling extracellular cues to pore opening in epithelial sodium channel/acid-sensing ion channels

How does proton binding in the extracellular region trigger pore opening of ASICs? Residues Leu71–Pro73 and Ly423–Glu426 connect TM1 and TM2 to β strands 1 and 12 in the palm domain, respectively (Fig. 1). Trp288, at the base of the thumb, is in close proximity to these residues connecting the extracellular region to the transmembrane segments. Jasti *et al.* [6] proposed that the thumb undergoes conformational changes following proton binding/unbinding to peripheral sites. These conformational changes are transmitted to the pore through noncovalent interactions mediated by Trp288 at the base of the thumb. Nonconservative mutations at Tyr72, located immediately after TM1, and Trp288, at the base of the thumb, disrupt proton-dependent activation of rat ASIC1a [20]. Then, π – π interactions between the aromatic groups of Tyr72 and Trp288 were proposed to provide functional coupling between the extracellular domain and the pore of the channel.

Mutations of these conserved aromatic residues at the base of the thumb and the TM1- β 1 loop of ENaC subunits had modest effects on macroscopic activity, the Na^+ self-inhibition response, or the activation by laminar shear stress (LSS) [20,26]. This may indicate that the molecular mechanisms that mediate pore opening and closing events differ between ENaCs and ASICs. ENaC activity is also modulated by proteases [11]. Furin, a proprotein convertase expressed in the trans-Golgi network, cleaves the α and γ ENaC subunits in the extracellular region [27]. The regions that encompass the furin cleavage sites in the α and γ subunits align with the finger domain of cASIC1. Mutations of the furin cleavage sites in the

α subunit dramatically reduce channel Po and enhance Na⁺ self-inhibition [28,29]. Interestingly, mutation of a conserved aromatic residue at the base of the α -subunit thumb domain restored the activity of channels lacking furin cleavage sites in the α subunit [26]. Although conserved aromatic residues at the base of the thumb are not required to maintain ENaC constitutive activity, the study of Shi *et al.* [26] denotes a functional interaction between the base of the thumb domain of the α subunit and the channel gate.

In the distal nephron, rates of Na⁺ reabsorption and K⁺ secretion are tightly regulated by intraluminal flow rates [10]. Studies performed in heterologous expression systems revealed that LSS increases ENaC's Po [30,31]. Higher vertebrates express a δ -ENaC subunit that is structurally related to α . The magnitude and time course of the response to LSS of $\alpha\beta\gamma$ and $\delta\beta\gamma$ channels is markedly different [32]. As both $\alpha\beta\gamma$ and $\delta\beta\gamma$ channels respond to LSS, structural differences between the α and γ subunits must alter the way that changes triggered by LSS are transmitted to the pore. Abi-Antoun *et al.* [32] generated a series of α - δ chimera to define structural elements that influence the response to LSS. Specific residues in the region preceding TM2 (pre-TM2) and in TM2 itself account for the differences in the response to LSS observed between $\alpha\beta\gamma$ and $\delta\beta\gamma$ channels. The molecular mechanism that allows ENaC to sense changes in flow rates remains unknown. The transmembrane helices apparently do not have a role in sensing LSS [33]. The work of Abi-Antoun *et al.* [32] suggests that the pre-TM2 region has a role coupling changes triggered by LSS in the extracellular region to the pore of the channel [32].

Opening the pore of ASIC1

To allow ion permeation following extracellular acidification, the transmembrane segments must reorganize. Passero *et al.* [34] using voltage clamp fluorometry detected a conformational rearrangement in the pre-TM2 region of ASIC1a that occurred concurrently with pore opening. Tolino *et al.* [35^{**}] found that in the absence of extracellular acidification, channels bearing a G428C mutation were activated by positively charged methanethiosulfonate (MTS) reagents. Gly428 localizes to the outer vestibule of the pore one helical turn above the degenerin site, a conserved position in the pore of ENaC/Deg channels where bulky substitutions increase Po. Mutational studies suggested that steric repulsion between the covalently modified G428C and nearby Tyr424 in the pre-TM2 region triggers a conformational change that opens the ion pore. Interestingly, Y424C–G428C subunits were associated by intersubunit disulfide bonds formed at the outer vestibule of the pore between Cys residues at positions 424 and 428. These channels were insensitive to thiol-modifying reagents, but were activated by extracellular acidification. Although disulfide bonds between adjacent subunits should largely restrict radial tilting of the transmembrane helices, Y424C–G428C subunits appear to gate competently. It is unknown whether movements on TM2 elicited by extracellular acidification are triggered by residues in TM1 or by other mechanisms. Tolino *et al.* [35^{**}] proposed that pore opening of Y424C–G428C could be accomplished by a concerted rotation of the TM2 helices in the clockwise direction.

Modulation of epithelial sodium channel open probability by proteolysis

ENaC α and γ subunits undergo limited proteolytic processing during channel maturation [27,29,36]. This mechanism of regulation has important physiologic implications that have been discussed in detail elsewhere [11]. Furin processes ENaC extracellular regions at two sites in the α subunit and one site in the γ subunit [27]. A number of proteases selectively cleave the γ subunit at sites that are distal to the furin cleavage site [11]. Double cleavage of the α or γ ENaC subunits releases inhibitory fragments and increases the Po of the channel [29,36]. Noncleaved channels are almost electrically silent with extremely low Po. Channels

lacking the α inhibitory fragment have an intermediate P_o , whereas channels lacking the γ inhibitory tract are practically constitutively open. Synthetic peptides corresponding to these released α and γ fragments are inhibitory [29,36]. These inhibitory tracts align with the finger domain of cASIC1, a region poorly conserved among the members of the ENaC/Deg family. To elucidate the mechanism of activation of ENaC by proteases, Kashlan *et al.* [37^{**}, 38] performed Trp-scanning mutagenesis in large portions of the predicted periphery of the α subunit and measured the inhibition by an 8-mer peptide derived from the α subunit. A large number of mutations in the finger and thumb domains of the α subunit reduced 8-mer inhibition. Mutant cycle analyses identified a number of residues that may have a role in peptide binding. A homology model of the α -subunit extracellular region was generated based on the resolved structure of cASIC1 and a large set of functional data from mutations that attenuated 8-mer inhibition [37^{**}]. On the basis of this model, the authors proposed that the presence of this α inhibitory tract in noncleaved channels reduces P_o by constraining motions within the thumb and finger domains [37^{**}].

Na⁺ self-inhibition

Na⁺ self-inhibition refers to the mechanism by which extracellular Na⁺ reduces ENaC's P_o [39]. Na⁺ self-inhibition is generally assessed by rapidly replacing an extracellular solution containing a low [Na⁺] concentration with one containing high [Na⁺] [8,40]. Studies in heterologous expression systems suggest that extracellular [Na⁺] influences channel P_o by altering the conformational state of the protein [28,40–42]. Conversely, mutations that alter channel P_o also have an effect on Na⁺ self-inhibition [17,28,42]. Whereas the Na⁺-binding site has not been identified for ENaC, mutations at specific sites within the extracellular region of the α , β , and γ subunits modify the Na⁺ self-inhibition response [17,28,37^{**},40–42]. Recently, Winarski *et al.* [43] identified a stretch of 22 residues in the finger domain of the γ subunit that contributes to the response to extracellular Na⁺. The authors proposed that this region, which is not present on ASICs, functions as an extracellular allosteric regulatory domain to specifically modulate the response of the channel to extracellular Na⁺. Kashlan *et al.* [37^{**}] found that specific residues in the finger and thumb domains of the α subunit altered the Na⁺ self-inhibition response, suggesting that these regions also provide allosteric regulation of ENaC function. These studies indicate that each ENaC subunit contributes differentially to the Na⁺ self-inhibition process, while also highlighting the importance of the extracellular region in the modulation of channel gating.

Contribution of the N-terminus and TM1 to epithelial sodium channel/acid-sensing ion channel gating

ASIC3 participates in acid and pain sensation [1]. This channel generates a sustained current under moderate extracellular acidification, in addition to the transient current characteristic of ASICs following extracellular acidification. Salinas *et al.* [44] studied a series of chimeric ASIC1a–ASIC3 channels to define the structural elements that allow ASIC3 to generate sustained currents. Their work suggests that the intracellular N-terminus and TM1 domain of ASIC3 stabilize the open conformation and consequently promote the generation of sustained currents. The stabilization of the open state may reflect an inability to accomplish desensitization-mediated pore closing. Residues in the TM1 helices contact residues in the TM2 helices of both their own and neighboring subunits. The study of Salinas *et al.* [44] emphasizes the importance of the interactions between residues in the TM1 and TM2 segments in the modulation of channel gating [44]. Residues in the TM1 segment are poorly conserved within ENaC/Deg family members, with the exception of a Trp residue at the base of the TM1 segment. A recent study from Pochynyuk *et al.* [45] suggests that these Trp residues anchor the TM1 domains of the ENaC subunits to the plasma membrane and prevent allosteric inhibition by intracellular Na⁺ and membrane voltage.

Epithelial sodium channel/acid-sensing ion channel gating, chloride and the trimeric architecture of epithelial sodium channel

The resolved atomic structure of cASIC1 revealed the existence of Cl^- bound between the two helices ($\alpha 4$ and $\alpha 5$) of the thumb domain (Fig. 1) [6]. Extracellular Cl^- did not affect pH sensitivity or selectivity of ASIC1a, but reduced desensitization rates. Mutations at the Cl^- coordination sites (Lys211, Arg309, and Glu313) abolished the effect of Cl^- [46]. The Cl^- coordination site is located in the thumb domain approximately 10 Å below Asp350, one of the residues in the putative proton-sensor. The study of Kusama *et al.* indicates that extracellular Cl^- does not interfere with the conformational rearrangements associated with proton-dependent activation. The results also suggest that the thumb influences channel desensitization.

Extracellular Cl^- at concentrations within the range found in the kidney collecting duct decreases ENaC's P_o [9]. Cl^- inhibition of ENaC occurs through two different mechanisms, one dependent and another independent on extracellular Na^+ [9,17]. The sites for Cl^- coordination are formed by the residues at the interface of the α subunit thumb and the β subunit palm and the β subunit thumb and the γ subunit palm. Mutations at homologous sites in the α , β , and γ subunits have differential effects on the inhibition by Cl^- and on the dependence of Cl^- on Na^+ self-inhibition. ENaCs are heterotrimers that could potentially be assembled in a clockwise $\alpha\beta\gamma$ or $\alpha\gamma\beta$ arrangement when viewed from the extracellular side. Collier and Snyder [47**] used the differential sensitivity to extracellular anions and Na^+ of these mutants to elucidate the trimeric channel architecture of ENaC. Their data suggest that ENaCs are assembled in an $\alpha\gamma\beta$ clockwise orientation when viewed from the extracellular side. The α , β , and γ subunits contribute unequally to gating and ion permeation [48,49]. The findings of Collier and Snyder provide a structural basis to understanding the asymmetric contribution of each subunits to ENaC function.

Conclusion

Extracellular factors gate or modulate the activity of ENaC/ASIC. Recent findings defined restricted regions within the ectodomain that allow these channels to sense and respond to extracellular cues. The region connecting the transmembrane helices to the ectodomain has an essential role coupling conformational changes that occur in the extracellular region to the pore of the channel. Additional studies are needed to define the sequence of events triggered by extracellular factors that lastly allow or facilitate ion permeation on ENaC/ASIC.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 571).

1. Lingueglia E, Deval E, Lazdunski M. FMRFamide-gated sodium channel and ASIC channels: a new class of ionotropic receptors for FMRFamide and related peptides. *Peptides*. 2006; 27:1138–1152. [PubMed: 16516345]
2. Wemmie JA, Price MP, Welsh MJ. Acid-sensing ion channels: advances, questions and therapeutic opportunities. *Trends Neurosci*. 2006; 29:578–586. [PubMed: 16891000]
3. Sakai H, Lingueglia E, Champigny G, et al. Cloning and functional expression of a novel degenerin-like Na⁺ channel gene in mammals. *J Physiol*. 1999; 519 (Pt 2):323–333. [PubMed: 10457052]
4. Kellenberger S, Schild L. Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. *Physiol Rev*. 2002; 82:735–767. [PubMed: 12087134]
5. Mall M, Grubb BR, Harkema JR, et al. Increased airway epithelial Na⁺ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med*. 2004; 10:487–493. [PubMed: 15077107]
6. Jasti J, Furukawa H, Gonzales EB, Gouaux E. Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature*. 2007; 449:316–323. [PubMed: 17882215]
7. Gonzales EB, Kawate T, Gouaux E. Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. *Nature*. 2009; 460:599–604. [PubMed: 19641589]
8. Chraïbi A, Horisberger JD. Na self inhibition of human epithelial Na channel: temperature dependence and effect of extracellular proteases. *J Gen Physiol*. 2002; 120:133–145. [PubMed: 12149276]
9. Collier DM, Snyder PM. Extracellular chloride regulates the epithelial sodium channel. *J Biol Chem*. 2009; 284:29320–29325. [PubMed: 19713212]
10. Satlin LM, Carattino MD, Liu W, Kleyman TR. Regulation of cation transport in the distal nephron by mechanical forces. *Am J Physiol Renal Physiol*. 2006; 291:F923–F931. [PubMed: 16849691]
11. Kleyman TR, Carattino MD, Hughey RP. ENaC at the cutting edge: regulation of epithelial sodium channels by proteases. *J Biol Chem*. 2009; 284:20447–20451. [PubMed: 19401469]
12. Askwith CC, Cheng C, Ikuma M, et al. Neuropeptide FF and FMRFamide potentiate acid-evoked currents from sensory neurons and proton-gated DEG/ENaC channels. *Neuron*. 2000; 26:133–141. [PubMed: 10798398]
13. Sherwood TW, Askwith CC. Endogenous arginine–phenylalanine–amide-related peptides alter steady-state desensitization of ASIC1a. *J Biol Chem*. 2008; 283:1818–1830. [PubMed: 17984098]
14. Sherwood TW, Askwith CC. Dynorphin opioid peptides enhance acid-sensing ion channel 1a activity and acidosis-induced neuronal death. *J Neurosci*. 2009; 29:14371–14380. [PubMed: 19906984]
15. Palmer LG, Frindt G. Amiloride-sensitive Na channels from the apical membrane of the rat cortical collecting tubule. *Proc Natl Acad Sci U S A*. 1986; 83:2767–2770. [PubMed: 2422661]
16. Pochynyuk O, Bugaj V, Stockand JD. Physiologic regulation of the epithelial sodium channel by phosphatidylinositides. *Curr Opin Nephrol Hypertens*. 2008; 17:533–540. [PubMed: 18695396]
17. Collier DM, Snyder PM. Extracellular protons regulate human ENaC by modulating Na⁺ self-inhibition. *J Biol Chem*. 2009; 284:792–798. [PubMed: 18990692]
18. Paukert M, Chen X, Pollechtner G, et al. Candidate amino acids involved in H⁺ gating of acid-sensing ion channel 1a. *J Biol Chem*. 2008; 283:572–581. [PubMed: 17981796]
19. Liechti LA, Bernèche S, Bargeton B, et al. A combined computational and functional approach identifies new residues involved in pH-dependent gating of ASIC1a. *J Biol Chem*. 2010; 285:16315–16329. [PubMed: 20299463]
20. Li T, Yang Y, Canessa CM. Interaction of the aromatics Tyr-72/Trp-288 in the interface of the extracellular and transmembrane domains is essential for proton gating of acid-sensing ion channels. *J Biol Chem*. 2009; 284:4689–4694. [PubMed: 19074149]
21. Sherwood T, Franke R, Conneely S, et al. Identification of protein domains that control proton and calcium sensitivity of ASIC1a. *J Biol Chem*. 2009; 284:27899–27907. [PubMed: 19654327]
22. Cushman KA, Marsh-Haffner J, Adelman JP, McCleskey EW. A conformation change in the extracellular domain that accompanies desensitization of acid-sensing ion channel (ASIC) 3. *J Gen Physiol*. 2007; 129:345–350. [PubMed: 17389250]

23. Coric T, Zhang P, Todorovic N, Canessa CM. The extracellular domain determines the kinetics of desensitization in acid-sensitive ion channel 1. *J Biol Chem*. 2003; 278:45240–45247. [PubMed: 12947112]
24. Li T, Yang Y, Canessa CM. Asn415 in the beta11–beta12 linker decreases proton-dependent desensitization of ASIC1. *J Biol Chem*. 2010; 285:31285–31291. [PubMed: 20675379]
25. Bargeton B, Kellenberger S. The contact region between three domains of the extracellular loop of ASIC1a is critical for channel function. *J Biol Chem*. 2010; 285:13816–13826. [PubMed: 20215117]
26. Shi H, Ghosh DD, Okumura S, et al. Base of the thumb domain modulates epithelial sodium channel gating. *J Biol Chem*. 2011; 286:14753–14761. [PubMed: 21367859]
27. Hughey RP, Bruns JB, Kinlough CL, et al. Epithelial sodium channels are activated by furin-dependent proteolysis. *J Biol Chem*. 2004; 279:18111–18114. [PubMed: 15007080]
28. Sheng S, Carattino MD, Bruns JB, et al. Furin cleavage activates the epithelial Na⁺ channels by relieving Na⁺ self-inhibition. *Am J Physiol Renal Physiol*. 2006; 290:F1488–F1496. [PubMed: 16449353]
29. Carattino MD, Sheng S, Bruns JB, et al. The epithelial Na⁺ channel is inhibited by a peptide derived from proteolytic processing of its alpha subunit. *J Biol Chem*. 2006; 281:18901–18907. [PubMed: 16690613]
30. Carattino MD, Sheng S, Kleyman TR. Epithelial Na⁺ channels are activated by laminar shear stress. *J Biol Chem*. 2004; 279:4120–4126. [PubMed: 14625286]
31. Althaus M, Bogdan R, Clauss WG, Fronius M. Mechano-sensitivity of epithelial sodium channels (ENaCs): laminar shear stress increases ion channel open probability. *FASEB J*. 2007; 21:2389–2399. [PubMed: 17426066]
32. Abi-Antoun T, Shi S, Tolino LA, et al. Second transmembrane domain modulates epithelial sodium channel gating in response to shear stress. *Am J Physiol Renal Physiol*. 2011; 300:F1089–F1095. [PubMed: 21307123]
33. Carattino MD, Liu W, Hill WG, et al. Lack of a role of membrane-protein interactions in flow-dependent activation of ENaC. *Am J Physiol Renal Physiol*. 2007; 293:F316–F324. [PubMed: 17459954]
34. Passero CJ, Okumura S, Carattino MD. Conformational changes associated with proton-dependent gating of ASIC1a. *J Biol Chem*. 2009; 284:36473–36481. [PubMed: 19858190]
- 35•. Tolino LA, Okumura S, Kashlan OB, Carattino MD. Insights into the mechanism of pore opening of acid-sensing ion channel 1A. *J Biol Chem*. 2011; 286:16297–16307. The permeation pathway of ASIC1a is constituted by the residues in the TM2 segments. To allow ion permeation the TM2 segments need to rotate, tilt, or both. Y424C–G428C subunits were associated by intersubunit disulfide bonds formed at the outer vestibule of the pore. These double Cys mutant channels transit between the resting, open, and desensitized states following extracellular acidification. The results of this study indicate that a concerted rotation in the clockwise direction of the TM2 helices could increase the diameter of the pore of Y424C–G428C allowing ion permeation. [PubMed: 21388961]
36. Bruns JB, Carattino MD, Sheng S, et al. Epithelial Na⁺ channels are fully activated by furin- and prostasin-dependent release of an inhibitory peptide from the gamma-subunit. *J Biol Chem*. 2007; 282:6153–6160. [PubMed: 17199078]
- 37•. Kashlan OB, Boyd CR, Argyropoulos C, et al. Allosteric inhibition of the epithelial Na⁺ channel through peptide binding at peripheral finger and thumb domains. *J Biol Chem*. 2010; 285:35216–35223. Proteolytic processing of the α and γ ENaC subunits releases inhibitory peptides and increases Po. Mutations in the α subunit finger–thumb interface affect apparent affinity of an 8-mer inhibitory peptide. This study suggests that the α inhibitory tract in noncleaved channels reduces Po by constraining motions within the thumb and finger domains. [PubMed: 20817728]
38. Kashlan OB, Adelman JL, Okumura S, et al. Constraint-based, homology model of the extracellular domain of the epithelial Na⁺ channel alpha subunit reveals a mechanism of channel activation by proteases. *J Biol Chem*. 2011; 286:649–660. [PubMed: 20974852]

39. Li JH, Lindemann B. Competitive blocking of epithelial sodium channels by organic cations: the relationship between macroscopic and microscopic inhibition constants. *J Membr Biol.* 1983; 76:235–251. [PubMed: 6100864]
40. Sheng S, Bruns JB, Kleyman TR. Extracellular histidine residues crucial for Na^+ self-inhibition of epithelial Na^+ channels. *J Biol Chem.* 2004; 279:9743–9749. [PubMed: 14701851]
41. Sheng S, Maarouf AB, Bruns JB, et al. Functional role of extracellular loop cysteine residues of the epithelial Na^+ channel in Na^+ self-inhibition. *J Biol Chem.* 2007; 282:20180–20190. [PubMed: 17522058]
42. Maarouf AB, Sheng N, Chen J, et al. Novel determinants of epithelial sodium channel gating within extracellular thumb domains. *J Biol Chem.* 2009; 284:7756–7765. [PubMed: 19158091]
43. Winarski KL, Sheng N, Chen J, et al. Extracellular allosteric regulatory subdomain within the gamma subunit of the epithelial Na^+ channel. *J Biol Chem.* 2010; 285:26088–26096. [PubMed: 20587418]
44. Salinas M, Lazdunski M, Lingueglia E. Structural elements for the generation of sustained currents by the acid pain sensor ASIC3. *J Biol Chem.* 2009; 284:31851–31859. [PubMed: 19778905]
45. Pochynyuk O, Kucher V, Boiko N, et al. Intrinsic voltage dependence of the epithelial Na^+ channel is masked by a conserved transmembrane domain tryptophan. *J Biol Chem.* 2009; 284:25512–25521. [PubMed: 19620245]
46. Kusama N, Harding AM, Benson CJ. Extracellular chloride modulates the desensitization kinetics of acid-sensing ion channel 1a (ASIC1a). *J Biol Chem.* 2010; 285:17425–17431. [PubMed: 20385551]
- 47••. Collier DM, Snyder PM. Identification of epithelial Na^+ channel (ENaC) intersubunit Cl^- inhibitory residues suggests a trimeric alpha gamma beta channel architecture. *J Biol Chem.* 2011; 286:6027–6032. Cl^- binds to two sites situated at the α - β and β - γ ENaC subunits interfaces and inhibits channel activity. Cl^- -binding to these sites reduced channel activity by two different mechanisms, one dependent and another independent of extracellular Na^+ . Functional studies of channels bearing mutations at the Cl^- -binding sites suggest that ENaC subunits are arranged in an $\alpha\gamma\beta$ clockwise orientation when viewed from the extracellular side. [PubMed: 21149458]
48. Carattino MD, Hughey RP, Kleyman TR. Proteolytic processing of the epithelial sodium channel gamma subunit has a dominant role in channel activation. *J Biol Chem.* 2008; 283:25290–25295. [PubMed: 18650438]
49. Li J, Sheng S, Perry CJ, Kleyman TR. Asymmetric organization of the pore region of the epithelial sodium channel. *J Biol Chem.* 2003; 278:13867–13874. [PubMed: 12576470]

Key points

- The region connecting the transmembrane helices to the ectodomain has an essential role coupling conformational changes that occur in the extracellular region to the pore of the channel.
- Multiple sites in the extracellular region of ASIC1a contribute to proton sensing.
- Restricted regions within the ectodomain allow ENaC/ASIC to sense and respond to extracellular cues.
- Pore opening of ENaC/ASIC could be accomplished by a concerted rotation of the TM2 helices.

Figure 1. Architecture of acid-sensing ion channel 1

The model corresponds to the resolved structure of chicken ASIC1 in the desensitized state. The palm and β -ball domains constitute the core of the protein. The finger, thumb, and knuckle domains are in the periphery of the protein. Cl^- binds between the two helices ($\alpha 4$ and $\alpha 5$) of the thumb domain (red ball). Disulfide bridges in the thumb and palm domains are shown in pink.