CHAPTER 8

MechanoTRPs and TRPA1

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I. OVERVIEW

Genetic and molecular searches in animals identify two families of ion channels used by specialized mechanosensory cells (Duggan *et al.*, 2000). These are Deg/ENaC channels (reviewed by Corey and García-Añoveros, 1996; García-Añoveros and Corey, 1997; Ernstrom and Chalfie, 2002; and elsewhere in this volume) and TRP channels (reviewed in this and other chapters of this volume). Some of these channels open in response to mechanical forces and/or mediate cellular responses to mechanical stimulation (Table I). TRPA1 is expressed in nociceptive neurons of peripheral ganglia and in the sensory

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TABLE I

TRP Channels Implicated in Mechanical Sensitivity

Channel	Mechanical sensitivity	Evidence	References
TRPY			
TRPY1	Osmotic swelling; pipette pressure	Yeast mutant phenotype	Zhou *et al.*, 2003
TRPV			
TRPV1	Response to bladder filling (bladder stretch)	Mouse mutant phenotype	Birder *et al.*, 2002
TRPV2	Osmotic swelling of cell; pipette pressure on patch	Antisense oligo effect; functional expression	Muraki *et al.*, 2003
TRPV4	Osmotic swelling of cell; touch	Functional expression; mouse mutant phenotype	Liedtke *et al.*, 2000; Strotmann *et al.*, 2000; Mizuno *et al.*, 2003; Suzuki *et al.*, 2003; Vriens *et al.*, 2004
OSM-9	Osmotic shock; touch	Worm mutant phenotype	Colbert *et al.*, 1997
NAN	Auditory	Fly mutant phenotype	Kim *et al.*, 2003
IAV	Auditory	Fly mutant phenotype	Gong *et al.*, 2004
TRPP			
PKD1 and PKD2 (TRPP2)	Fluid flow in epithelia (cilia deflection)	Mouse mutant phenotype	Pennekamp *et al.*, 2002; McGrath *et al.*, 2003; Nauli *et al.*, 2003

TRPC			
TRPC1	Stretch; membrane tension; pipette pressure	Functional expression; siRNA effect	Maroto *et al*., 2005
TRPN			
dmTRPN1	Touch and proprioception (bristle deflection)	Fly mutant phenotype; altered transient receptor currents on deflection	Walker *et al*., 2000
drTRPN1	Auditory and vestibular (hair cells)	Morpholino treatment alters extracellular receptor potentials in zebrafish hair cells	Sidi *et al*., 2003
ceTRPN1 (TRP-4)	Proprioception (body bending)	Worm mutant phenotype	Li *et al*., 2006
TRPA			
Painless	Mechanothermal (heated prodding)	Fly larvae mutant phenotype	Tracey *et al*., 2003
drTRPA1	Lateral line and vestibular (hair cells)	Morpholino treatment alters extracellular receptor potentials in zebrafish hair cells	Corey *et al*., 2004
mmTRPA1	Mechanical nociception	Mouse mutant phenotype; functional expression; expression pattern	Corey *et al*., 2004; Nagata *et al*., 2005; Kwan *et al*., 2006
TRPML			
TRPML3	Hair cells	Mouse mutant is deaf	Di Palma *et al*., 2002

epithelia of the inner ear. In nociceptors, TRPA1 forms chemosensitive channels that mediate the response to exogenous pain-producing chemicals as well as to the endogenous proalgesic bradykinin (BK). More indirect evidence suggests that TRPA1 might also form mechanosensory channels.

II. MECHANOTRP CHANNELS

Some of the TRP channels that mediate mechanical responses are not necessarily mechanically gated. For example, TRPV4 mutant mice have reduced sensitivity to noxious tactile stimulation, and heterologously expressed TRPV4 opens in response to hypotonic solution (which induces cell swelling and thus stretches membranes). However, this response appears to be mediated by lipid second messengers gating TRPV4 (Liedtke *et al.*, 2000; Strotmann *et al.*, 2000; Mizuno *et al.*, 2003; Suzuki *et al.*, 2003; Vriens *et al.*, 2004). In addition, the capsaicin receptor TRPV1 is expressed in bladder epithelium, which is less sensitive to stretch in TRPV1 mutant mice (Birder *et al.*, 2002). However, heterologously expressed TRPV1 and TRPV4 have not been shown to be activated by membrane stretch. Rather, they have been shown to be activated by nonmechanical stimuli like hot temperature and extracellular acidity.

Other TRP channels have been shown to be activated directly by membrane stretch elicited in membrane patches by pipette suction. TRPY1 of yeast has not been heterologously expressed, but TRPY mutants lack a stretch-activated and osmosensitive channel in their vacuoles (Zhou *et al.*, 2003). The vertebrate TRPC1 generates stretch-sensitive channels when heterologously expressed (Maroto *et al.*, 2005). In addition, gene overexpression and inhibition studies suggest it to be the long-sought stretch-activated channel of frog oocytes. Although mechanosensitive cation channels like the one expressed in the oocyte have been implicated in cell-volume regulation, cell locomotion, muscle dystrophy, and cardiac arrhythmias, there is so far no data supporting a sensory function for the mechanosensitivity of TRPC1 channels.

Finally, a large number of TRP channels, primarily in flies and worms, are required for the specialized sensory neurons that mediate auditory, proprioceptive, tactile, and nociceptive mechanotransduction. One of these genes encodes TRPN1 (originally called NompC), which is expressed in the bristle organs of flies, in the lateral line hair cells of zebrafish, as well as in ciliated dopaminergic mechanosensory neurons (ADEs and CEPs) and in stretch-activated neurons (DVA) of worms (Walker *et al.*, 2000; Sidi *et al.*, 2003; Li *et al.*, 2006). When the TRPN1 gene is mutated (in flies and worms), the mechanoreceptor currents of these sensory cells are eliminated or reduced. Furthermore, a point mutation in the fly TRPN1 alters the speed of mechanoreceptor current adaptation,

strongly suggesting that TRPN1 is the mechanically gated channel in these cells. In zebrafish, TRPN1 mRNA was detected in hair cells of the inner ear and its inhibition with morpholinos produced deaf and unbalanced fish whose hair cells failed to generate mechanoreceptor potentials. A very similar effect was obtained by morpholinos against a structurally analogous protein, TRPA1 (Corey *et al*., 2004). It is likely that TRPN1, perhaps in association with other proteins like TRPA1, contributes to the mechanosensitive channel in zebrafish hair cells. However, mammals have hair cells but their genomes do not appear to have an orthologue of TRPN1. Other proteins must constitute mechanosensory channels in mammals. A search for TRP channel expression in specialized mechanosensory organs revealed several candidate genes expressed in dorsal root ganglia, trigeminal ganglia, or inner ear. One of them is TRPA1 (also known as ANKTM1 and P120), which is absent from (or very weakly expressed in) most organs yet is expressed in both somatosensory neurons and sensory epithelia of inner ear. Although mice with mutations in TRPA1 have no auditory or vestibular defects reported, they do have nociceptive phenotypes, some of which may be attributed to defective mechanotransduction.

III. CHARACTERISTICS OF TRPA1 GENE AND PROTEIN

TRPA1 genes in mammals are large, occupy around 50 kb of chromosomal DNA, and are encoded by at least 27 exons. In humans, the TRPA1 gene is located on chromosome 8q13 (contig NT_008183.18; June, 2006). In mice, the TRPA1 gene is located on chromosome 1A3. TRPA1 homologues have been found in many species including human, rat, mouse, zebrafish, pufferfish, sea squirt, fly, mosquito, and nematode, which suggests an evolutionary conservation of TRPA1 function. TRPA1 protein is predicted to have canonical TRP structure in its pore-forming region with six transmembrane domains (S1–S6), a putative pore loop between S5 and S6, together with cytoplasmic N- and C-termini. The most distinguishing feature of TRPA1 is a long N-terminal region containing up to 18 predicted ankyrin repeat motifs (the exact number depending on the consensus definition used by the computer prediction). Ankyrin repeats help to cluster and organize ion channels and receptors at specialized regions of cells (Bennett and Chen, 2001; Lee *et al*., 2006); however, ankyrin repeat domains may do more than clustering and subcellular organization. Force measurements demonstrate that ankyrin repeats are elastic and can constitute molecular springs (Lee *et al*., 2006), supporting the hypothesis that ankyrin repeats of TRPN1 and other TRP channels form mechanical gating springs (Howard and Bechstedt, 2004; Sotomayor *et al*., 2005). Eighteen is the greatest number of ankyrin repeats known in a mammalian TRP channel and helps to explain TRPA1's predicted

molecular weight of 127.4 kD. Other TRPs possess between 0 and 8 ankyrin repeats, with the notable exception of TRPN1 (NompC) which has 29 predicted ankyrin repeats and is found in flies (Walker *et al.*, 2000), nematodes (Walker *et al.*, 2000; Li *et al.*, 2006), and fish (Sidi *et al.*, 2003). Intriguingly, mammalian genomes do not appear to encode a homologue of TRPN1, begging the question of which mammalian protein may play the mechanosensory roles that TRPN1 has in nematodes, flies, and fish. TRPA1 has been proposed as a candidate because of its structural similarity to TRPN1 and its expression pattern in mechanosensory organs (Corey *et al.*, 2004; Nagata *et al.*, 2005).

IV. TRPA1 EXPRESSION IN MECHANOSENSORY ORGANS

Initial Northern blot analysis showed TRPA1 mRNA present at very low levels in human fibroblasts but this expression was lost on oncogenic transformation (Jaquemar *et al.*, 1999). Thus far, the only tissues expressing detectable TRPA1 mRNA and protein are those specialized in sensation: somatosensory neurons in the peripheral nervous system and mechanosensory epithelia of the inner ear. Northern blot and *in situ* hybridization did not detect TRPA1 mRNA in most organs of late embryonic or adult mice including brain, heart, liver, kidney, skeletal muscle, lung, spleen, testis, whisker pad skin, and superior cervical ganglia (Jaquemar *et al.*, 1999; Nagata *et al.*, 2005). Lack of detection suggests lack of TRPA1 expression; however, these findings do not rule out the possibility that TRPA1 mRNA is present below detection levels. For example, TRPA1 could be expressed in restricted regions of the brain. In support of this localized or low-level expression, five cDNA clones of mouse TRPA1 were derived from mesencephalon (see the UniGene cluster Mm.186329).

A. Somatosensory Neurons

In dorsal root, trigeminal, and nodose ganglia of mouse and rat, *in situ* hybridization detected TRPA1 mRNA exclusively in small diameter neurons, most of which are nociceptors. Neurons expressing TRPA1 constitute a sizeable percentage (between 20% and 56%) of all neurons in a ganglia (Jaquemar *et al.*, 1999; Kobayashi *et al.*, 2005; Nagata *et al.*, 2005). Precise estimates on the proportion of TRPA1-expressing neurons per ganglion vary from study to study, although this might largely be attributed to differences in species, strain, age, type of ganglia, sensitivity, and perhaps the nociceptive history of the ganglion. Prior nociception may affect the proportion of

neurons that express TRPA1 (Obata *et al.*, 2005). TRPA1-expressing peripheral neurons do not coexpress neurofilament 200 or the growth factor receptors TrkC and TrkB, but do express the NGF receptor TrkA, a marker of nociceptive neurons (Bautista *et al.*, 2005; Nagata *et al.*, 2005). Western blots confirm TRPA1 protein expression in peripheral ganglia and their nerves (i.e., sciatic nerve; Nagata *et al.*, 2005). Using antibodies raised against either the mouse N-terminus (Nagata *et al.*, 2005) or the rat C-terminus (Bautista *et al.*, 2005), most TRPA1-expressing neurons are reported to also express other markers of nociceptive neurons, including the capsaicin receptor (TRPV1), calcitonin gene-related peptide (CGRP), substance P (SP), and peripherin (Bautista *et al.*, 2005; Kobayashi *et al.*, 2005; Nagata *et al.*, 2005). These immunoreactivities also demonstrate the presence of TRPA1 protein in the peripheral nerve endings of target organs, such as the bladder epithelium and the cornea, where sensation initially occurs. All these experiments confirm that small diameter C-fiber nociceptors, not large diameter A-fiber innocuous mechanoreceptor neurons, express TRPA1.

B. Inner Ear

Analyses of mRNA by RT-PCR and *in situ* hybridization as well as analyses of protein by Western blot and immunohistochemistry also detected TRPA1 mRNA in vestibular and auditory sensory epithelia (Corey *et al.*, 2004; Nagata *et al.*, 2005). In neonatal organ of Corti, *in situ* hybridization revealed clear expression in supporting cells which is confirmed by immunohistochemistry with antibodies to both the N-terminus (Nagata *et al.*, 2005; our unpublished results) and C-terminus (Corey *et al.*, 2004) of mouse TRPA1. Weak immunoreactivities were also detected in the sensory hair cells apically at the cuticular plates and in the mechanosensory stereocilia. Important functional evidence that TRPA1 is present in hair cells was provided with a report that TRPA1-agonists allyl isothiocyanate (AITC) and icilin (AG 3-5) activate currents in hair cells (Stepanyan *et al.*, 2006). Although we are not sure how or if these agonists specifically activate TRPA1, at present we know no other ion channel activated by both AITC and AG 3-5.

V. FUNCTION OF TRPA1

A. Nociception

Mice with a deletion that eliminates the pore domain of TRPA1 have impaired nociception. TRPA1 mutant mice show reduced or eliminated nocifensive responses to pain-producing TRPA1-agonists like mustard oil,

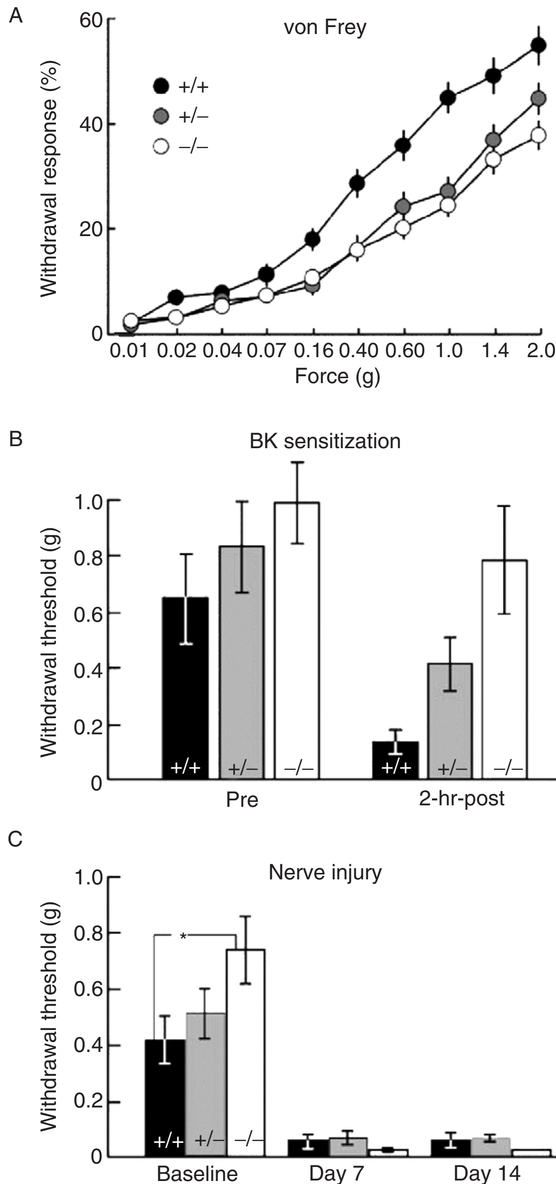

FIGURE 1 TRPA1$^{-/-}$ and TRPA1$^{+/-}$ mice have reduced responses to mechanical stimuli. Loss of TRPA1 differentially protects from development of hypersensitivity. (A) Plantar withdrawal response measured with calibrated von Frey hairs for 10 presentations at each force. Fourteen animals (seven males and seven females) of each genotype were tested; there was no significant difference between sexes, so results were pooled. Mean ± SEM. (B) Plantar

allicin, and acrolein (and their trigeminal neurons, in culture, do not uptake Ca^{2+} in response to these agonists), whereas these mutant animals do respond nocifensively (and their trigeminal neurons uptake Ca^{2+}) to capsaicin, a pungent that is not a TRPA1 agonist (Bautista *et al*., 2006; Kwan *et al*., 2006). Clearly TRPA1 mediates the response of nociceptors to certain pungent chemicals. These compounds, however, are not endogenous and are not normally in contact with most TRPA1-expressing nociceptive neurons. Typically, we encounter these chemicals when we eat them and they mainly contact the nerves innervating the epithelia lining the buccal cavity. Although it is possible that there are similar substances endogenously produced by tissue damage or inflammation, these have not been reported. It is also possible that these compounds are agonists that bear no structural relationship with the nociceptive signals that under normal physiological conditions activate TRPA1, like BK.

TRPA1 is also a mediator of BK proalgesic effects (Bandell *et al*., 2004; Bautista *et al*., 2006). Not only do TRPA1 channels open if coexpressed with the BK receptor and exposed to BK, but TRPA1 mutant mice do not develop hyperalgesia when exposed to BK. As the BK receptor activates phospholipase C, and this enzyme also mediates the effects of other proalgesics like ATP, monoamines, and neurotrophins, TRPA1 channels may also mediate their effects. Surprisingly, induction of hyperalgesia by BK requires both TRPA1 and TRPV1 (Bautista *et al*., 2006). Therefore, TRPV1 has two different roles: (1) to mediate BK-induced hyperalgesia in concert with TRPA1 and (2) to transduce noxious thermal and acidic stimuli. By analogy, TRPA1 might also play a role as a primary sensory transducer in addition to mediating the action of proalgesics.

Indeed, one of the reports on TRPA1 knockout mice reveals that they have a reduced response to punctate mechanical stimulation. Knockout mice and heterozygous mice display significantly lower withdrawal responses and elevated withdrawal thresholds as compared to wild-type mice (Kwan *et al*., 2006; Fig. 1). This impairment of behavioral response to mechanonociceptive stimuli is consistent with a role of TRPA1 in mechanical transduction, although further tests will be necessary to determine this. For example, nerve recordings showing a reduction in mechanically induced firing rates from nociceptors of TRPA1 mutant mice would further the argument that TRPA1

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withdrawal threshold measured before and 2 h after injection of BK into footpad. Bradykinin increased the sensitivity of wild-type mice to von Frey stimulation, reducing thresholds by fivefold, but in knockout mice, thresholds were not significantly reduced. Thirteen animals of each genotype were tested. (C) Plantar withdrawal thresholds measured before and at 7 and 14 days after spared nerve injury. Knockout mice showed significantly higher thresholds than wild type (*, $p = 0.03$) before nerve injury. Figure and legend adapted from Kwan *et al.* 2006. Copyright 2006 by Elsevier.

is acting in these cells as a mechanotransducer. This is especially important given the potential for TRPA1 expression in the CNS previously mentioned. Still, to demonstrate that TRPA1 channels are mechanically gated we would need (1) recreation of mechanical gating of TRPA1 in heterologous cells and (2) demonstration of impaired mechanoreceptive currents in TRPA1 knockout neurons. Neither experiment has been reported yet, largely because of the prevailing thought that, although some mechanically gated channels can be opened by direct pressure applied through the recording pipette, those channels used by specialized mechanosensory cells are part of macromolecular complexes where the channel physically interacts with structural components that transmit the gating force (García-Añoveros and Corey, 1997). In this context, receptor potentials would have to be recorded from the extremely fine free nerve endings of nociceptors embedded in other tissues, something never performed. Heterologous expression would necessitate other proteins, which are presently unknown.

B. Auditory and Vestibular

To date, there are no reports of mechanical activation of heterologously expressed TRPA1, although there are reasons to suspect that mechanical activation might occur in the appropriate cellular context. Mice with a deletion of the pore domain of TRPA1 have no overt vestibular or auditory defect, as determined by behavioral observation, auditory brainstem response thresholds, and distortion product otoacoustic emissions (Bautista *et al.*, 2006; Kwan *et al.*, 2006). In addition, the hair cell transduction currents in wild-type mice and mice with a deletion of the TRPA1 pore are indistinguishable (Kwan *et al.*, 2006). Importantly, both strains of knockout mice delete a small part of the TRPA1 gene, so it will be critical to determine if they are functional nulls. If not, these mice could make a truncated TRPA1 protein bearing the N-terminal ankyrin repeats and the initial transmembrane domains. Such a truncated protein would not form a channel by itself, but it could function as part of a multimeric complex. Data obtained prior to knockout studies indicated that acute inhibition of TRPA1 mRNA using viral-mediated siRNA in mouse utricular hair cells and morpholinos in zebrafish hair cells reduced but did not eliminate the mechanotransduction currents of these cells (Corey *et al.*, 2004). These apparently contradictory results could be reconciled if TRPA1 participates in, but its pore domain is not essential for, hair cell mechanotransduction complexes. An alternative explanation is that TRPA1 plays no role in hair cells but rather functions in support cells, where its expression is most prominent (our unpublished data). Presently, the role of TRPA1 in the inner ear is unclear.

C. Channel Similarities Between Heterologously Expressed TRPA1 and Endogenous Mechanotransducers

Without a clear mechanical stimulus paradigm for heterologously expressed TRPA1, these channels have primarily been studied by activation with agonists like AITC (Nagata *et al*., 2005). This permitted a comparison of TRPA1 with other mechanosensory channels, primarily those of hair cells (unfortunately, for reasons stated above, there is little information regarding the mechanotransducer channels in nociceptors). Although data obtained from two knockout analyses of TRPA1 suggests that TRPA1 does not play a crucial role in hair cell mechanotransduction, there is pharmacological evidence that the pore formed by TRPA1 is similar to the pore of mechanotransduction channels, including the one in hair cells. While numerous pain-producing chemicals act as TRPA1 agonists (Bandell *et al*., 2004; Jordt *et al*., 2004; Bautista *et al*., 2005, 2006; Nagata *et al*., 2005), four antagonists have also been studied: gentamycin, ruthenium red, gadolinium (Gd^{3+}), and amiloride (Nagata *et al*., 2005; Table II). Although each of these antagonists blocks other types of channels, block by all four is characteristic of mechanosensory channels from various cell types, including hair cells (Hamill and McBride, 1996). All four antagonists show similar Hill coefficients of block between heterologously expressed TRPA1 and the hair cell mechanotransducer. This suggests a shared mechanism of action on TRPA1 channels and the hair cell mechanotransducer. Further, IC_{50} values for gentamycin and ruthenium red (both simple pore blockers) are also indistinguishable between heterologously expressed TRPA1 and the hair cell mechanotransducer. However, the IC_{50} values for amiloride and Gd^{3+} differ by factors of 10 and 100, respectively. On the basis of these data, Gd^{3+} is a more potent blocker of heterologously expressed TRPA1 than of any other channel, suggesting a potential analgesic use (Nagata *et al*., 2005).

Other studies of heterologously expressed TRPA1 revealed that these channels are permeable to both monovalent and divalent cations, although the ionic selectivity has not been calculated in detail. The estimated conductance of a single TRPA1 channel in the negative voltage range was 100 pS, but traces with estimated values as low as 38 pS were observed. In fact, single-channel current amplitude varied widely, even within one sweep of single-channel openings, suggesting that TRPA1 channels have a range of conductance levels. For example, addition of external Ca^{2+} decreased the amplitude of single TRPA1 channel inward current to 54% of the level in the absence of Ca^{2+} (Nagata *et al*., 2005). Curiously, the conductance of the hair cell transducer is similar in magnitude (although it also varies, as it ranges tonotopically along the cochlea) and it is reduced by Ca^{2+} to the same extent (Table II).

TABLE II

Properties of Heterologous TRPA1 Channels Compared to Mechanotransduction Channels of Hair Cells

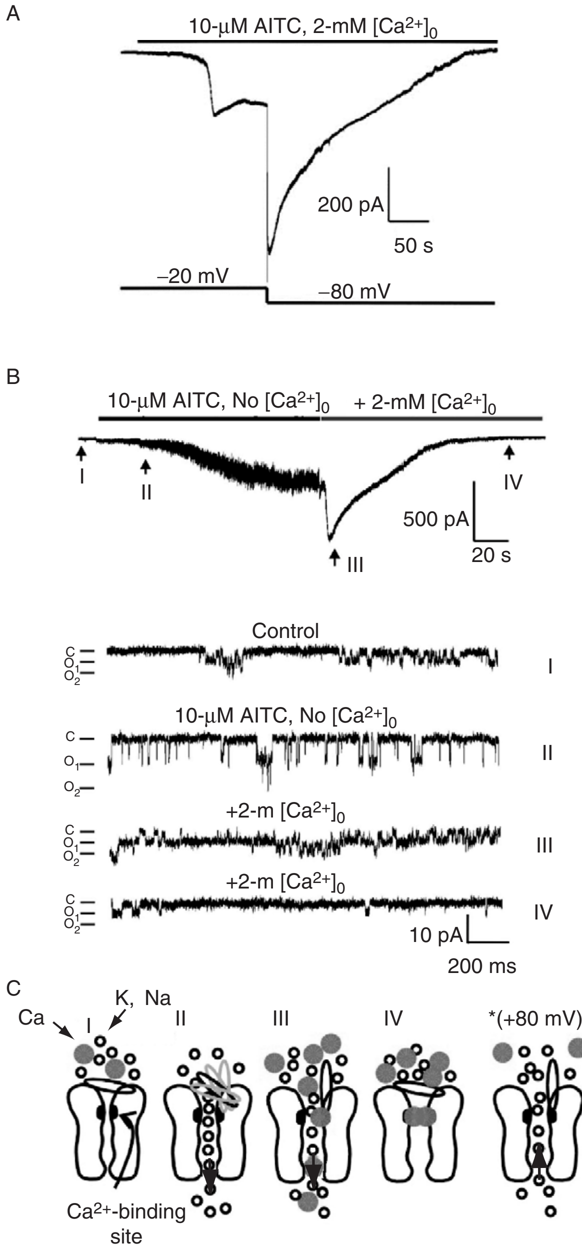
Channel property	Heterologous TRPA1 (Nagata *et al.*, 2005)	Hair cell transducers	References for hair cell transducers
Gentamycin block (IC_{50})	$6.7 \pm 0.7\ \mu M$	$7.6\ \mu M$	Kroese *et al.*, 1989
Gentamycin block (Hill coefficient)	1.2	1.2	Kroese *et al.*, 1989
Ruthenium red block (IC_{50})	$3.4 \pm 0.1\ \mu M$	$3.6 \pm 0.3\ \mu M$	Farris *et al.*, 2004
Ruthenium red block (Hill coefficient)	1.1 ± 0.03	1.4 ± 0.2	Farris *et al.*, 2004
Gd^{3+} block (IC_{50})	$0.1 \pm 0.02\ \mu M$	$10.1\ \mu M$	Kimitsuki *et al.*, 1996
Gd^{3+} block (Hill coefficient)	1.2 ± 0.25	1.1	Kimitsuki *et al.*, 1996
Amiloride block (IC_{50})	$511 \pm 12\ \mu M$	$50\ \mu M$	Jorgensen and Ohmori, 1988; Rusch *et al.*, 1994
Amiloride block (Hill coefficient)	2.4 ± 0.1	2.2 ± 0.1	Ricci, 2002
Ca^{2+}-induced potentiation	Yes	Yes	Ricci *et al.*, 2003
Ca^{2+}-induced closure (adaptation)	Yes ($\tau = 3.5$ s)	Yes ($\tau =$ few millisecond)	Howard and Hudspeth, 1988; Ricci *et al.*, 1998; Kennedy *et al.*, 2003
Reopening by depolarization after Ca^{2+}-induced closure	Yes	Yes	Ricci *et al.*, 2000
Reduction of unitary conductance by Ca^{2+}	$53.7 \pm 2.0\%$	54%	Ricci *et al.*, 2003

Heterologous TRPA1 channels were expressed in HEK293 cells and opened by AITC. Hair cell transducers were opened by mechanical force.

Indeed, Ca^{2+} exerts similar pronounced effects on the gating of both TRPA1 and the mechanotransducer of hair cells (Table II). Whole-cell recordings of AITC-activated currents in HEK293 cells transiently expressing TRPA1, voltage-clamped at −80 mV, and in the presence of physiological external Ca^{2+} reveal a multiphasic inward current. This current initially turns on slowly, but potentiates further before inactivating. In the absence of external Ca^{2+}, the slowly developing inward currents do not potentiate or inactivate as they do in the presence of Ca^{2+}. However, if Ca^{2+} is suddenly added to the bath solution when the channels are already opened by AITC, the currents potentiate and then inactivate (Fig. 2). The Ca^{2+}-induced phenomena are also voltage dependent. At more depolarized potentials (−20 mV in Fig. 2A), inactivation of channels (activated in the continuous presence of 2 mM external Ca^{2+}) does not occur as it does at −80 mV. At +80 mV, the potentiation and inactivation induced by sudden exposure to external Ca^{2+} at −80 mV are absent or much reduced. In addition, channels inactivated at −80 mV will reopen if held at +80 mV. It appears that Ca^{2+} must enter the cell to exert its effects on TRPA1 channels. However, these effects occurred whether the cytoplasm of the cells was loaded with Ca^{2+} (up to 3 mM) or with Ca^{2+} chelators (EGTA or BAPTA). Therefore, it is unlikely that Ca^{2+} is exerting these effects through the cytoplasm.

Single channel recordings from outside-out patches of cells heterologously expressing TRPA1 further reveal the effects of Ca^{2+} on channel conductance and open probability (P_o). In the presence of AITC, but no external Ca^{2+}, heterologous TRPA1 channels adopt a high conductance (5.7 ± 0.4 pA) and low P_o state (II of Fig. 2B). On external Ca^{2+} addition, the channel conductance reduces (3.1 ± 0.4 pA) but P_o increases (potentiation; III in Fig. 2B) for several seconds before the P_o drops essentially to zero (inactivation; IV in Fig. 2B).

It seems that entering Ca^{2+} binds to the channel and causes a brief potentiation (rise in P_o) followed by closure (adaptation or inactivation; Fig. 2B and C). Interestingly, similar effects of Ca^{2+} have been reported for the mechanotransduction channel of hair cells, albeit at a rate 1000× faster (Table II). This quantitative difference may be accounted for by a difference in affinity of the Ca^{2+}-binding site, which could result from small molecular differences in the channel proteins. Nonetheless, the same mechanistic model, presented on Fig. 2C, accounts for the behavior of both TRPA1 and hair cell mechanotransduction channels. Although TRPA1 is not essential for hair cell transduction, the above-mentioned similarities suggest that (1) the mechanotransducer of hair cells is similar to TRPA1 and (2) TRPA1 may form a mechanosensory channel in other cells where it is expressed, namely supporting cells of inner ear and somatosensory nociceptors.


A
10-μM AITC, 2-mM [Ca2+]0
200 pA
50 s
–20 mV
–80 mV
B
10-μM AITC, No [Ca2+]0
+ 2-mM [Ca2+]0
I
II
III
IV
500 pA
20 s
Control
C
O1
O2
10-μM AITC, No [Ca2+]0
+2-m [Ca2+]0
+2-m [Ca2+]0
10 pA
200 ms
C
Ca
K, Na
I
II
III
IV
*(+80 mV)
Ca2+-binding
site

VI. PROPOSED BIOLOGICAL ROLES FOR TRPA1

The highly restricted expression of TRPA1 in nociceptive neurons of peripheral ganglia and in mechanosensory epithelia of the inner ear strongly suggests sensory roles. The activation of heterologously expressed TRPA1 by various pain-producing chemicals (Bandell *et al.*, 2004; Jordt *et al.*, 2004; Bautista *et al.*, 2005; Nagata *et al.*, 2005; Bautista *et al.*, 2006), together with the impaired nociception of mice bearing engineered deletions in TRPA1, demonstrate a role in nociception. The voltage sensitivity of TRPA1's Ca^{2+}-dependent inactivation renders it well suited for a role in pain (Nagata *et al.*, 2005). In a sensory terminal insufficiently depolarized by low threshold (i.e., innocuous) stimulation, any TRPA1 channels that open would quickly inactivate. But with stimulation above threshold (i.e., noxious), enough TRPA1 channels would open and depolarize the terminal. Under conditions of sustained depolarization, TRPA1 channels inactivate incompletely and slowly compared to conditions of weak or no depolarization. Thus, TRPA1 may have the inherent abilities to (1) distinguish between innocuous (even if persistent) stimuli and noxious stimuli and (2) respond continuously to prolonged noxious stimulation by failing to desensitize. These two abilities are characteristic of nociceptive sensation.

In nociceptors, TRPA1 responds to pain-producing chemicals, mediating both their acute noxious effects as well as the sensitization that these chemicals generate. These compounds are either synthetic or plant derived and they are not known to be produced endogenously. TRPA1 is also responsive to the proalgesic BK, which activates TRPA1 via its G-protein–coupled receptor.

FIGURE 2 Biophysical properties of heterologously expressed TRPA1 channels. (A) When opened by AITC in the presence of external Ca^{2+}, TRPA1 channels inactivate completely at strongly hyperpolarized (−80 mV) holding potentials but not at more depolarized (−20 mV) holding potentials. (B) Recapitulation of TRPA1 slow activation, Ca^{2+}-induced potentiation, and subsequent inactivation at the single-channel level recorded from outside-out patches (at a holding potential of −80 mV). In the absence of Ca^{2+}, AITC activates a large conductance, flickery channel (II, activation). On exposure to extracellular Ca^{2+}, this channel transitions to a low conductance but high P_o state (III, potentiation) followed by a low P_o state (IV, inactivation). The upper trace is a whole-cell record, obtained just before patch formation, indicating the general times that correspond to the single-channel traces. (C) A model for how Ca^{2+} and voltage affects TRPA1 channels, which can also describe similar behaviors of hair cell transducers. (I) At rest, the channel opens spontaneously. (II) AITC-activated channels, with no external Ca^{2+}, have a high unitary conductance, but a low P_o. (III) AITC-activated channels in the presence of external Ca^{2+} have a low unitary conductance, but a high P_o. (IV) Prolonged exposure to external Ca^{2+} causes channels to enter very low P_o state. (*) This inactivation can be relieved by strong depolarization (+80 mV) by an unknown mechanism. Figure and legend adapted from Nagata *et al.*, 2005. Copyright 2005 by Society for Neuroscience.

Clearly, a biological role for TRPA1 is to mediate the proalgesic effects of BK, and perhaps of other proalgesics, in conjunction with TRPV1. The question remains whether TRPA1 has, like TRPV1, a role as a sensory transducer of other pain stimuli, like extreme temperature or physical deformation.

Taken together, (1) the similar pharmacology and pore properties between heterologously expressed TRPA1 and hair cell mechanotransducers, (2) the structural or phylogenetic similarity of TRPA1 with mechanosensory channels from invertebrates (*TRPN1* and *Painless*), (3) the wide distribution of TRPA1 mRNA among nociceptors (many of which are mechanosensory), and (4) the elevated withdrawal thresholds to punctate mechanical stimulation of one of the TRPA1 knockout mouse lines suggest a role for TRPA1 in mechanonociception. However, it should be noted that the elevated withdrawal thresholds were not detected in both strains of TRPA1 knockout mice. Further experimentation will be necessary.

What exactly TRPA1 does in the inner ear is a very intriguing question. Although not stated on current publications, the expression of TRPA1 is weak in hair cells and more prominent in the adjacent supporting cells. It is possible that TRPA1 has a role in supporting cells and that its low-level presence in hair cells is an accident of their location and developmental history (both hair and support cells come from the same precursors). Alternatively, TRPA1 may play a nontransduction role in hair cells that is not essential to hearing. A role in mechanotransduction is not completely eliminated by the current data, although one would have to assume that TRPA1 is acting redundantly. Hair cell transduction channels appear functionally heterogeneous, with tens to hundreds of conductance levels arranged tonotopically (Ricci *et al*., 2003). We think that molecular diversity may underlie this functional diversity. Heterogeneity could be accomplished by multimerization of several channel subunits, perhaps including TRPA1 and/or other TRP proteins that we and others find in hair cells. An interesting candidate in this regard is TRPML3, which is also present in hair cell stereocilia. Mutations in TRPML3 produce stereocilia malformations and deafness in mice (Di Palma *et al*., 2002). Unfortunately, these mutations act in a dominant fashion, so they do not clarify the function of TRPML3 in hair cells. It will be interesting to determine if conditionally induced loss of function mutations in TRPML3 specifically impair hair cell mechanotransduction.

References

Bandell, M., Story, G. M., Hwang, S. W., Viswanath, V., Eid, S. R., Petrus, M. J., Earley, T. J., and Patapoutian, A. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* **41,** 849–857.

Bautista, D. M., Movahed, P., Hinman, A., Axelsson, H. E., Sterner, O., Hogestatt, E. D., Julius, D., Jordt, S. E., and Zygmunt, P. M. (2005). Pungent products from garlic activate the sensory ion channel TRPA1. *Proc. Natl. Acad. Sci. USA* **102,** 12248–12252.

Bautista, D. M., Jordt, S. E., Nikai, T., Tsuruda, P. R., Read, A. J., Poblete, J., Yamoah, E. N., Basbaum, A. I., and Julius, D. (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* **124,** 1269–1282.

Bennett, V., and Chen, L. (2001). Ankyrins and cellular targeting of diverse membrane proteins to physiological sites. *Curr. Opin. Cell Biol.* **13,** 61–67.

Birder, L. A., Nakamura, Y., Kiss, S., Nealen, M. L., Barrick, S., Kanai, A. J., Wang, E., Ruiz, G., De Groat, W. C., Apodaca, G., Watkins, S., and Caterina, M. J. (2002). Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nat. Neurosci.* **5,** 856–860.

Colbert, H. A., Smith, T. L., and Bargmann, C. I. (1997). OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *J. Neurosci.* **17,** 8259–8269.

Corey, D. P., and García-Añoveros, J. (1996). Mechanosensation and the DEG/ENaC ion channels. *Science* **273,** 323–324.

Corey, D. P., García-Añoveros, J., Holt, J. R., Kwan, K. Y., Lin, S. Y., Vollrath, M. A., Amalfitano, A., Cheung, E. L., Derfler, B. H., Duggan, A., Geleoc, G. S., Gray, P. A., *et al.* (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* **432,** 723–730.

Di Palma, F., Belyantseva, I. A., Kim, H. J., Vogt, T. F., Kachar, B., and Noben-Trauth, K. (2002). Mutations in Mcoln3 associated with deafness and pigmentation defects in varitint-waddler (Va) mice. *Proc. Natl. Acad. Sci. USA* **99,** 14994–14999.

Duggan, A., García-Añoveros, J., and Corey, D. P. (2000). Insect mechanoreception: What a long, strange TRP it's been. *Curr. Biol.* **10,** R384–R387.

Ernstrom, G. G., and Chalfie, M. (2002). Genetics of sensory mechanotransduction. *Annu. Rev. Genet.* **36,** 411–453.

Farris, H. E., LeBlanc, C. L., Goswami, J., and Ricci, A. J. (2004). Probing the pore of the auditory hair cell mechanotransducer channel in turtle. *J. Physiol.* **558,** 769–792.

García-Añoveros, J., and Corey, D. P. (1997). The molecules of mechanosensation. *Annu. Rev. Neurosci.* **20,** 567–594.

Gong, Z., Son, W., Chung, Y. D., Kim, J., Shin, D. W., McClung, C. A., Lee, Y., Lee, H. W., Chang, D. J., Kaang, B. K., Cho, H., Oh, U., *et al.* (2004). Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in *Drosophila*. *J. Neurosci.* **24,** 9059–9066.

Hamill, O. P., and McBride, D. W., Jr. (1996). The pharmacology of mechanogated membrane ion channels. *Pharmacol. Rev.* **48,** 231–252.

Howard, J., and Bechstedt, S. (2004). Hypothesis: A helix of ankyrin repeats of the NOMPC-TRP ion channel is the gating spring of mechanoreceptors. *Curr. Biol.* **14,** R224–R226.

Howard, J., and Hudspeth, A. J. (1988). Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog's saccular hair cell. *Neuron* **1,** 189–199.

Jaquemar, D., Schenker, T., and Trueb, B. (1999). An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. *J. Biol. Chem.* **274,** 7325–7333.

Jordt, S. E., Bautista, D. M., Chuang, H. H., McKemy, D. D., Zygmunt, P. M., Hogestatt, E. D., Meng, I. D., and Julius, D. (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* **427,** 260–265.

Jorgensen, F., and Ohmori, H. (1988). Amiloride blocks the mechano-electrical transduction channel of hair cells of the chick. *J. Physiol.* **403,** 577–588.

Kennedy, H. J., Evans, M. G., Crawford, A. C., and Fettiplace, R. (2003). Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells. *Nat. Neurosci.* **6,** 832–836.

Kim, J., Chung, Y. D., Park, D. Y., Choi, S., Shin, D. W., Soh, H., Lee, H. W., Son, W., Yim, J., Park, C. S., Kernan, M. J., and Kim, C. (2003). A TRPV family ion channel required for hearing in *Drosophila. Nature* **424,** 81–84.

Kimitsuki, T., Nakagawa, T., Hisashi, K., Komune, S., and Komiyama, S. (1996). Gadolinium blocks mechano-electric transducer current in chick cochlear hair cells. *Hear. Res.* **101,** 75–80.

Kobayashi, K., Fukuoka, T., Obata, K., Yamanaka, H., Dai, Y., Tokunaga, A., and Noguchi, K. (2005). Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. *J. Comp. Neurol.* **493,** 596–606.

Kroese, A. B., Das, A., and Hudspeth, A. J. (1989). Blockage of the transduction channels of hair cells in the bullfrog's sacculus by aminoglycoside antibiotics. *Hear. Res.* **37,** 203–217.

Kwan, K. Y., Allchorne, A. J., Vollrath, M. A., Christensen, A. P., Zhang, D. S., Woolf, C. J., and Corey, D. P. (2006). TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* **50,** 277–289.

Lee, G., Abdi, K., Jiang, Y., Michaely, P., Bennett, V., and Marszalek, P. E. (2006). Nanospring behaviour of ankyrin repeats. *Nature* **440,** 246–249.

Li, W., Feng, Z., Sternberg, P. W., and Xu, X. Z. (2006). A *C. elegans* stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. *Nature* **440,** 684–687.

Liedtke, W., Choe, Y., Marti-Renom, M. A., Bell, A. M., Denis, C. S., Sali, A., Hudspeth, A. J., Friedman, J. M., and Heller, S. (2000). Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* **103,** 525–535.

Maroto, R., Raso, A., Wood, T. G., Kurosky, A., Martinac, B., and Hamill, O. P. (2005). TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat. Cell Biol.* **7,** 179–185.

McGrath, J., Somlo, S., Makova, S., Tian, X., and Brueckner, M. (2003). Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell* **114,** 61–73.

Mizuno, A., Matsumoto, N., Imai, M., and Suzuki, M. (2003). Impaired osmotic sensation in mice lacking TRPV4. *Am. J. Physiol. Cell Physiol.* **285,** C96–C101.

Muraki, K., Iwata, Y., Katanosaka, Y., Ito, T., Ohya, S., Shigekawa, M., and Imaizumi, Y. (2003). TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circ. Res.* **93,** 829–838.

Nagata, K., Duggan, A., Kumar, G., and García-Añoveros, J. (2005). Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. *J. Neurosci.* **25,** 4052–4061.

Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A. E., Lu, W., Brown, E. M., Quinn, S. J., Ingber, D. E., Zhou, J., *et al.* (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **33,** 129–137.

Obata, K., Katsura, H., Mizushima, T., Yamanaka, H., Kobayashi, K., Dai, Y., Fukuoka, T., Tokunaga, A., Tominaga, M., and Noguchi, K. (2005). TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. *J. Clin. Invest.* **115,** 2393–2401.

Pennekamp, P., Karcher, C., Fischer, A., Schweickert, A., Skryabin, B., Horst, J., Blum, M., and Dworniczak, B. (2002). The ion channel polycystin-2 is required for left-right axis determination in mice. *Curr. Biol.* **12,** 938–943.

Ricci, A. (2002). Differences in mechano-transducer channel kinetics underlie tonotopic distribution of fast adaptation in auditory hair cells. *J. Neurophysiol.* **87,** 1738–1748.

Ricci, A. J., Wu, Y. C., and Fettiplace, R. (1998). The endogenous calcium buffer and the time course of transducer adaptation in auditory hair cells. *J. Neurosci.* **18,** 8261–8277.

Ricci, A. J., Crawford, A. C., and Fettiplace, R. (2000). Active hair bundle motion linked to fast transducer adaptation in auditory hair cells. *J. Neurosci.* **20,** 7131–7142.
Ricci, A. J., Crawford, A. C., and Fettiplace, R. (2003). Tonotopic variation in the conductance of the hair cell mechanotransducer channel. *Neuron* **40,** 983–990.
Rusch, A., Kros, C. J., and Richardson, G. P. (1994). Block by amiloride and its derivatives of mechano-electrical transduction in outer hair cells of mouse cochlear cultures. *J. Physiol.* **474,** 75–86.
Sidi, S., Friedrich, R. W., and Nicolson, T. (2003). NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. *Science* **301,** 96–99.
Sotomayor, M., Corey, D. P., and Schulten, K. (2005). In search of the hair-cell gating spring elastic properties of ankyrin and cadherin repeats. *Structure* **13,** 669–682.
Stepanyan, R. B. E., Friedman, T. B., and Frolenkov, G. L. (2006). TRPA1, a hair cell channel with unknown function? *In* "29th ARO Midwinter Meeting," pp. 211–212. Baltimore, MD.
Strotmann, R., Harteneck, C., Nunnenmacher, K., Schultz, G., and Plant, T. D. (2000). OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat. Cell Biol.* **2,** 695–702.
Suzuki, M., Mizuno, A., Kodaira, K., and Imai, M. (2003). Impaired pressure sensation in mice lacking TRPV4. *J. Biol. Chem.* **278,** 22664–22668.
Tracey, W. D., Jr., Wilson, R. I., Laurent, G., and Benzer, S. (2003). Painless, a *Drosophila* gene essential for nociception. *Cell* **113,** 261–273.
Vriens, J., Watanabe, H., Janssens, A., Droogmans, G., Voets, T., and Nilius, B. (2004). Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. *Proc. Natl. Acad. Sci. USA* **101,** 396–401.
Walker, R. G., Willingham, A. T., and Zuker, C. S. (2000). A *Drosophila* mechanosensory transduction channel. *Science* **287,** 2229–2234.
Zhou, X. L., Batiza, A. F., Loukin, S. H., Palmer, C. P., Kung, C., and Saimi, Y. (2003). The transient receptor potential channel on the yeast vacuole is mechanosensitive. *Proc. Natl. Acad. Sci. USA* **100,** 7105–7110.