CHAPTER 12

Microbial TRP Channels and Their Mechanosensitivity

Yoshiro Saimi, Xinliang Zhou, Stephen H. Loukin, W. John Haynes, and Ching Kung

Laboratory of Molecular Biology, Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706

I. OVERVIEW

The pioneering phenotype-to-gene approach that dissects biological pathways has repeatedly led to TRP channels. Follow-up examinations of these TRPs and their homologues constitute the bulk of current research on this superfamily of fascinating channels, which are central to many aspects of animal physiology. Beyond animals, *TRP* genes can be found in *Paramecium*, *Tetrahymena*, *Dictyostelium*, *Trypanosoma*, *Leishmania*, and other protists, as well as most species of fungi. Thus, these channels and their basic mechanisms have apparently evolved long before the appearance of multicellular animals. Experiments showed that the TRPY1 channel of the budding yeast (*Saccharomyces cerevisiae*) releases Ca^{2+} from the vacuole into the cytoplasm when the yeast cell is subjected to a sudden osmotic upshock.

Current Topics in Membranes, Volume 58 1063-5823/07 $35.00

DOI: 10.1016/S1063-5823(06)58012-3

Under patch clamp, TRPY1 displays a 300-pS cation conductance that rectifies inwardly. Stretch force on the vacuolar membrane, on the order milliNewton per meter, activates TRPY1. TRPY1's mechanosensitivity examined *in vitro* explains the osmotically induced Ca^{2+} release *in vivo*. TRPY2 and TRPY3, homologues of TRPY1 from other yeasts, have similar properties. Unlike most animal TRPs, the yeast TRP channels can be examined directly with patch clamp for their mechanosensitivity. The cloned TPRY genes can also be manipulated with ease, using yeast molecular genetics. The study of microbial TRPs should be of value in further analysis on the molecular basis of mechanosensitivity.

II. A HISTORY TRP-CHANNEL RESEARCH

There must be more than 50 review papers on TRP channels in the last three years, in English alone. One author wrote 19. The great enthusiasm for these channels stems from their novelty (associated with pepper, menthol, garlic, marijuana, and so on), physiological roles (hot, cold, pain, touch, hearing, osmotic senses, and so on), diseases (retinal degeneration, mucolipidosis, polycystic kidney disease, and glomerulosclerosis), and promises (therapy, drugs, and research grants). The following summary on the historical origins of the current bloom of TRP research should be instructive.

The bulk of current research, mostly on mammalian TRPs, is the derivative of some 10 different prospecting adventures. With no known sequence targets to start with, all of these projects independently arrived at different TRP channels. The term TRP, ''transient receptor potential,'' describes the electroretinographic phenotype of a near-blind mutant *Drosophila* isolated in the Pak laboratory in 1975 (Minke *et al.*, 1975), the corresponding gene of which was cloned by Montell *et al.* in 1985 in the Rubin laboratory. This TRP channel is the founding member of TRPC (C for *c*anonical) and is the crux of phototransduction in insects, although how it is activated *in vivo* remains obscure despite intensive research (Minke, 2006). In 1997, TRP channels were ''rediscovered'' in two different contexts. In one, Julius and coworkers used expression cloning to search and found a heat/pain receptor, by using a heat surrogate (the pepper essence capsaicin) as a probe. This vanilloid receptor, VR1, turned out to have clear homology to *Drosophila* TRP and is now called TRPV1 (Caterina *et al.*, 1997). In the other, the Bargmann Laboratory isolated and analyzed mutant worms (*Caenorhabditis elegans*) defective in their avoidance of 4-M fructose. Position cloning of the mutation in one such mutants led to a gene, *osm-9*, which is homologous to TRPV1 (Colbert *et al.*, 1997). Expression cloning using a surrogate of cold (menthol) revealed the receptor CMR1, now a TRPM (McKemy *et al.*, 2002).

In the fly, mutations that cause defects in balance and touch response led to *NOMPC*, now a TRPN (Walker *et al*., 2000); those insensitive to pain to *PAINLESS*, now a TRPA (Tracey *et al*., 2003). In the worm, mutations causing defect in the males' ability to locate the vulvas of hermaphrodites during mating were traced to LOV-1, a homologue of PKD1 that forms channels by associating with PKD2, now TRPP (Barr and Sternberg, 1999). Cloning gene of heritable diseases is the medical equivalent of the phenotype-to-gene forward genetics. Thus polycystic kidney disease was traced to PKD1 and PKD2, now TRPPs (Hughes *et al*., 1995; Mochizuki *et al*., 1996). IV was traced to MCOLN1, now TRPML (Sun *et al*., 2000). In short, each founding member of the TRP subfamily, TRPC, TRPV, TRPN, TRPP, TRPM, or TRPML, was independently discovered by a piece of prospecting research and not through alignment of known TRP sequence. [TRPA was founded by the identification of *PAINLESS* (Tracey *et al*., 2003) and through candidate sequence homology (Story *et al*., 2003) almost simultaneously.] The convergence of these multiple original studies onto the same superfamily of ion channels gives us strong confidence that TRPs are indeed central to many aspects of biology.

Note that expression cloning of genes through probe recognition or position cloning of mutations with biological or clinical phenotypes are both "fishing expeditions," not driven by known sequence targets or concrete preconceived hypotheses. The findings of such "forward-genetic" studies are therefore more objective and original. Once these molecular targets are found, their sequence homologues can be recognized and used in further research. Commonly, mammalian homologues are heterologously expressed in oocytes or cultured cells and examined biophysically or biochemically. Knockout mice are also generated to examine possible phenotypes. These studies are generically referred to as "reverse genetics," and constitute the bulk of the current research, much of which is summarized in the ~50 reviews mentioned above.

III. THE MECHANOSENSITIVITY OF ANIMAL TRP CHANNELS

Each subfamily of animal TRP channels (TRPC, TRPV, TRPN, TRPP, TRPM, or TRPML) has been associated with mechanosensitivity. The evidence for this association varies greatly from case to case. At the organismic level, evidence comes from mutant behavioral phenotypes such as deafness (Kim *et al*., 2003; Gong *et al*., 2004), touch-blind (Walker *et al*., 2000), osmotactic failure (Colbert *et al*., 1997), drinking behavior (Liedtke and Friedman, 2003), and so on or from knockout animals' physiological defects such as bladder malfunction (Birder *et al*., 2002), and so on. At the cellular and tissue level, circumstantial evidences include the presence of the TRP

proteins or their mRNAs being in the expected places or at the expected developmental time (Corey *et al.*, 2004). At the molecular level, evidence most commonly comes from experimentation through heterologous expression. Here, mechanosensitivity is commonly indicated by osmotic downshock-induced entry of Ca^{2+} (monitored with a dye) into cultured cells expressing a foreign TRP transgene (Liedtke *et al.*, 2000; Strotmann *et al.*, 2000; Kim *et al.*, 2003; Gong *et al.*, 2004).

More direct electrophysiological examinations of animal TRP channels are rare. Under a current clamp, TRPV1 in isolated magnocellular neurosecretory cells has been found to correlate with hypotonically induced spikes (Naeini *et al.*, 2006). Under patch clamp, in a cell-attached mode, heterologously expressed TRPV4 [previously OTRPC4 (Strotmann *et al.*, 2000) and VR-OAC (Liedtke *et al.*, 2000)] and TRPV2 (Muraki *et al.*, 2003) were shown to be activated by hypotonicity. Maroto *et al.* (2005) provided the most direct and convincing evidence for animal TRP-channel mechanosensitivity. They showed that, when reconstituted into liposome, a TRPC1-rich detergent-solubilized fraction of frog-oocyte membrane correlates with unitary conductances that are activated by direct suction exerted on the bilayer patch. They also showed that human *TRPC1*, expressed in oocytes, correlates with a tenfold increase in stretch-activated current (Maroto *et al.*, 2005).

A part of the difficulty in the analysis of animal TRP channels is that they are often located in specialized cells and strategically located even within those cells. For example, the transducing channels for hearing are located near the tips of stereocilia of vertebrate hair cells (Corey *et al.*, 2004) or the sensory cilia of insect chordotonal organ (Kim *et al.*, 2003; Gong *et al.*, 2004). TRPP is located in the primary cilia of renal epithelial cells (Nauli *et al.*, 2003); TRPML in intracellular endosomes and lysosomes (Di Palma *et al.*, 2002); and others in the compound eyes, taste buds, and Merkel cells, Meissner corpuscle, and so on. These locations are currently nearly inaccessible to the patch-clamp pipette. Since these TRP channels cannot be studied *in situ*, they are therefore expressed heterologously in arenas such as oocytes or culture cells and examined therein. Results from heterologous experiments may include artifacts such as the contributions (or the lack of such contribution) from host subunits or host enzyme modifications. By contrast, the yeast TRPY1 channels can be directly patch clamped and examined for its mechanosensitivity in its natural location (the vacuolar membrane) with relative ease. See in later section.

IV. DISTRIBUTION AND THE UNKNOWN ORIGIN OF TRPs

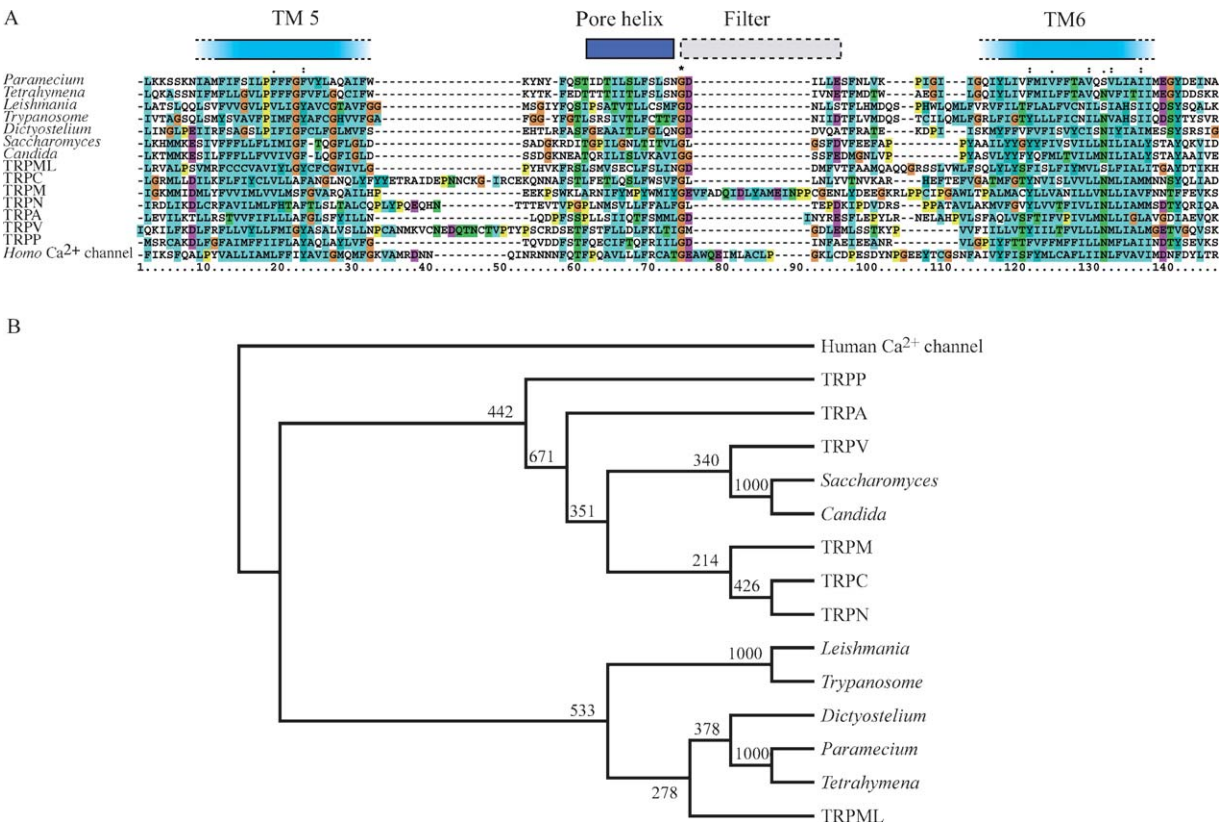
Classification of TRPs, iterated in most review papers, is by primary-sequence comparison and not by biophysical characteristics or biological functions (see below). Primary sequence cannot predict confidently any

tertiary or quaternary structures of proteins using current bioinformatics. Thus, without a crystal structure, the commonly cited model of TRP as a tetramer with a funnellike center fitted with a filter is only by analogy to the known crystal structure of K^+ channels (Doyle *et al.*, 1998; Jiang *et al.*, 2002), based on the belief that these cation channels are distantly related (Yu and Catterall, 2004) and will have similar structures. However, primary sequence can predict secondary structures and general topology with some confidence. The sequence of *TRP*-gene product is predicted to have six transmembrane (TM) α-helices with extensive N- and C-terminal domains in the cytoplasm. These cytoplasmic domains contain recognizable regions with proposed functions (e.g., ankyrin repeats, calmodulin-binding sites) or unknown functions ("TRP box," "TRPM homology," and so on) that sort the members found in animals into seven subfamilies (TRPC, TRPV, TRPA, TRPN, TRPM, TRPP, and TRPML). The resemblances between these subfamilies are limited. Most similarities are found in the sequence from the predicted TM5 to slightly beyond the C-terminus of the predicted TM6, a region that comprises the presumed filter and gate (Fig. 1A).

Though the visible animals and plants loom large in our mind, they are in fact a small part of the eukaryotic diversity (Embley and Martin, 2006). Currently, taxonomists divide *Eukarya* into six clusters (Adl *et al.*, 2005), one of which comprises both animals and fungi. Thus, the very diverse kinds of unicells are generally lumped under the nondescript term "protists." Using the above key sequence (TM5 through TM6) as the criterion, searches in the existing databases recognize TRP-channel genes without ambiguity in the genomes of *Paramecium*, *Tetrahymena* (both ciliates), *Dictyostelium* (cellular slime mold), *Trypanosoma* (an agent of African sleeping sickness), and *Leishmania* (leishmaniasis) (Fig. 1A) (Haynes, unpublished results). Fragments of similarities can also be found in the genomes of *Chlamydomonas*, (a green flagellate), *Plasmodium* (malaria), and *Thalassiosira* (diatom), and so on, though full-length TRP-channel genes have not been recognized or assembled from these genomes due to technical difficulties. No experimental work has been reported on these putative TRP homologues in protists at this writing.

The same search criterion revealed a TRP-channel gene in the genome of the budding yeast *S. cerevisiae*, which has been experimentally studied at length (see below) (Palmer *et al.*, 2001; Zhou *et al.*, 2003). This channel, TRPY1, has homologues in some 30 different fungal genomes of fungi. See below for the relatedness of 18 fungal TRPs (Zhou *et al.*, 2005) (Fig. 5). An additional TRPP-like channel gene in the *Schizosaccharomyces pombe* has similarity with the *Drosophila* TRPP (Palmer *et al.*, 2005).

The putative TRP channels in fungal and protist genomes usually do not bear the cytoplasmic features (ankyrin, "TRP box," and so on) used to


A
TM 5
Pore helix
Filter
TM6
Paramecium
Tetrahymena
Leishmania
Trypanosome
Dictyostelium
Saccharomyces
Candida
TRPML
TRPC
TRPM
TRPN
TRPA
TRPV
TRPP
Homo Ca2+ channel
B
Human Ca2+ channel
TRPP
TRPA
TRPV
Saccharomyces
Candida
TRPM
TRPC
TRPN
Leishmania
Trypanosome
Dictyostelium
Paramecium
Tetrahymena
TRPML
442
671
340
1000
351
214
426
1000
533
378
1000
278

distinguish the animal TRP subtypes. This makes it difficult to fit these channels into the official but animal-centric classification system (Montell *et al.*, 2002; Clapham *et al.*, 2003). Blast searches using the strictly defined S5-to-S6 sequences from all vertebrate-defined TRP subfamilies have protist TRPs locally aligned with a greater bit score to TRPML, while fungal sequences align with other TRP subtypes (Haynes, unpublished data). The no-gap bootstrap cladogram (Fig. 1B) drawn from the ClustalW global alignment (Fig. 1A) also shows this same tendency for clustering with different TRP subtypes. Whether this clustering is evolutionarily meaningful cannot be asserted at the moment since available microbial genome sequences are limited and include many reduced genomes of parasites.

The criteria we used have so far failed to identify TRP candidates among bacteria and archaea. Since the relationship among the three domains of life (*Bacteria*, *Archaea*, and *Eukarya*) remains unclear at the moment (Embley and Martin, 2006), it would be presumptuous to speculate that TRPs of ''higher'' animals must have evolved from some prototypes found in bacteria or archaea. One cannot rule out the scenario that the commingled gene pool of primordial cell communities (Embley and Martin, 2006) encoded the first detectors of force and heat, from which the first TRPs were derived.

V. TRPY1: THE TRP CHANNEL OF BUDDING YEAST

Long before the molecular biology of TRPY1, Wada *et al.* (1987) first described an ~300-pS conductance observed with a planar lipid bilayer into which a vacuolar-membrane fraction of yeast had been reconstituted. Others have observed a similar conductance by patch clamping the vacuolar membrane, after releasing the vacuoles from yeast spheroplasts (Minorsky *et al.*, 1989;

FIGURE 1 An alignment and unrooted cladogram of the major family members of TRP channels. (A) A clustalW (Gonnet 250) alignment made using the program ClustalX. Several representative TRP genes found in various protists were aligned with a member of each major family of TRP channel along with a Ca^{2+} channel (conserved in multicellular organisms) for comparison. The protist channels were found by Blast searching genomic sequences currently available for each organism listed. The majority of these protist sequences were predicted by automated annotation procedures at the respective sequencing centers. The *Leishmania* sequence was described (Chennik *et al.*, 2005). (B) A single unrooted bootstrapped cladogram using neighbor joining method (Saitou and Nei) drawn from the clustalW (Gonnet 250) alignment (with all gapped sequence removed) shown above made using the program ClustalX. 1000 possible trees were compared and the numbers represent the number of trees in which the branches shown were present. The Ca^{2+} channel was selected as the outgroup for the purpose of drawing this tree.

Bertl and Slayman, 1990; Saimi *et al.*, 1992) (Fig. 2A). It rectifies inwardly, that is, from the vacuole into the cytoplasm (Fig. 2B). It is cation selective, $P_{Na^+} = P_{K^+} \gg P_{Cl^-}$. It also passes divalent cations, $P_{Ca^{2+}} = P_{Ba^{2+}} > P_{Mg^{2+}}$ (Zhou, unpublished result), and it passes the physiologically important Ca^{2+} even when it is the sole cation (Palmer *et al.*, 2001). Vacuolar Ca^{2+} (mM) or low pH (<5, vacuolar or cytoplasmic) inhibits its activity. More importantly, cytoplasmic Ca^{2+} (μM) enhances its activity, allowing a positive-feedback loop in the process of Ca^{2+}-induced Ca^{2+} release (CICR; see below) (Zhou *et al.*, 2003).

In 1996, the genome of *S. cerevisiae* was completely sequenced, the first among eukarayotes (Goffeau *et al.*, 1996). A search in this genome revealed

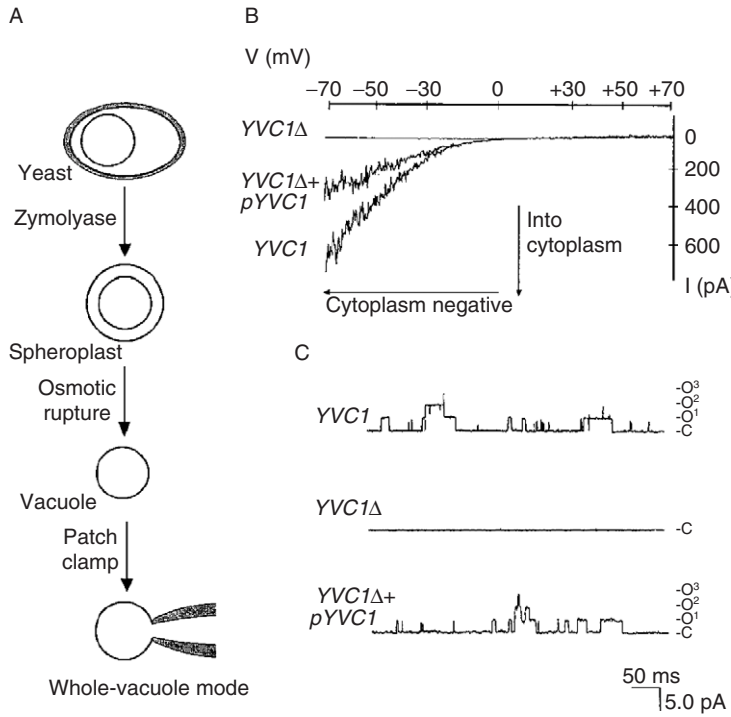


FIGURE 2 Absence and restoration of the yeast vacuolar conductance are correlated with the deletion and expression of the *TRPY1* gene, formerly *YVC1*. (A) Diagrammatic representation of the method of vacuole preparation and patch clamping. (B) Whole-vacuole macroscopic currents on applying a voltage ramp from +70 to −70 mV (bath voltage, cytoplasmic side) from each of the three strains: *YVC*, wild type; *YVC1*Δ, knockout; *YVC1*Δ + *pYCV1*, reexpression from a plasmid. (C) Sample traces from whole vacuoles held at +10 mV. C, closed level; O^1, O^2, O^3, open levels. From Palmer *et al.* (2001).

an open reading frame that corresponds to known TRP-channel amino acid sequences. Palmer *et al.* (2005) found the full-length gene to be necessary for the above cation conductance by using a combination of gene deletion, reexpression, and direct patch clamping on the yeast vacuolar membrane (Fig. 2C). The observation was confirmed by others (Bihler *et al.*, 2005) and this gene product was first named Yvc1, for yeast vacuolar channel (Palmer *et al.*, 2001; Zhou *et al.*, 2003), and later assigned as TRPY1 (Zhou *et al.*, 2005) since it is a TRP homologue. This identification of TRPY1 took this vacuolar channel beyond biophysical description into the realm of cell and molecular biology.

The first finding on TRPY1 of cell-biological significance was made in the Cyert laboratory (Denis and Cyert, 2002). Previously, Batiza *et al.* (1996) showed that a sudden hyperosmotic shock causes a release of Ca^{2+} from the vacuole into the cytoplasm in live yeast cells. Using transgene-produced aequorin as a Ca^{2+} reporter in luminometry, Denis and Cyert (2002) found that this pulse of Ca^{2+} is missing in the *TRPY1*-deleted strain, leading to the conclusion that the TRPY1 channel is the conduit for this Ca^{2+} passage. This work implicates TRPY1 as the link between the osmotic stimulus and Ca^{2+}-release response (Fig. 3).

The observation by Zhou *et al.* (2003) that this channel is in fact mechanosensitive connects the osmotic-upshock-induced Ca^{2+} response *in vivo* and the TRPY1-channel activities *in vitro*. Whether examined in the whole-vacuole mode or the excised cytoplasmic-side-out mode, application of

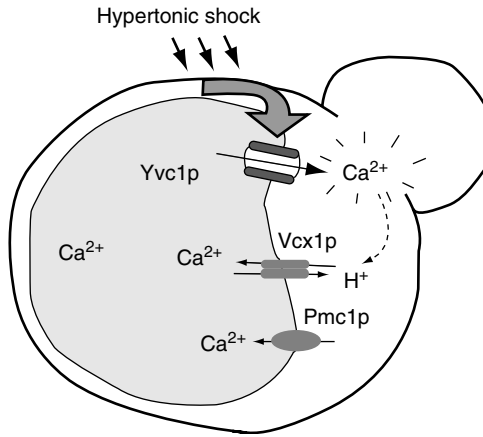


FIGURE 3 A cell-biological model showing that a sudden hypertonic shock leads to the activation of the TRPY1 (Yvc1p) channel, which releases Ca^{2+} into the cytoplasm (Vcx1p and Pmc1p are for Ca^{2+} reuptake). From Denis and Cyert (2002).

pressure on the order of a few milli-Newton per meter through the patch-clamp pipette activates the TRPY1 unitary conductances. Such conductances are always observed regardless of whether the *TRPY1* gene resides in the chromosome or on a plasmid but are never observed in cells from a *TRPY1*-knockout strain (Fig. 4, left). Technical ease demands that a positive pressure be applied, inflating the vacuole. Suctions (negative pressure) through the pipette usually break the gigaseal or confound the recording modes. Nonetheless, direct application of osmoticum to the bath, which visibly shrinks the vacuole on the pipette, can be shown to activate *TPRY1* (Zhou *et al.*, 2003). This observation echoes the upshock-induced Ca^{2+} response *in vivo*. It also shows that the stretch force, generated by membrane deformation through inflation or shrinkage, can activate this channel. The cell-biological and the biophysical observations together led to a model that explains mechanistically

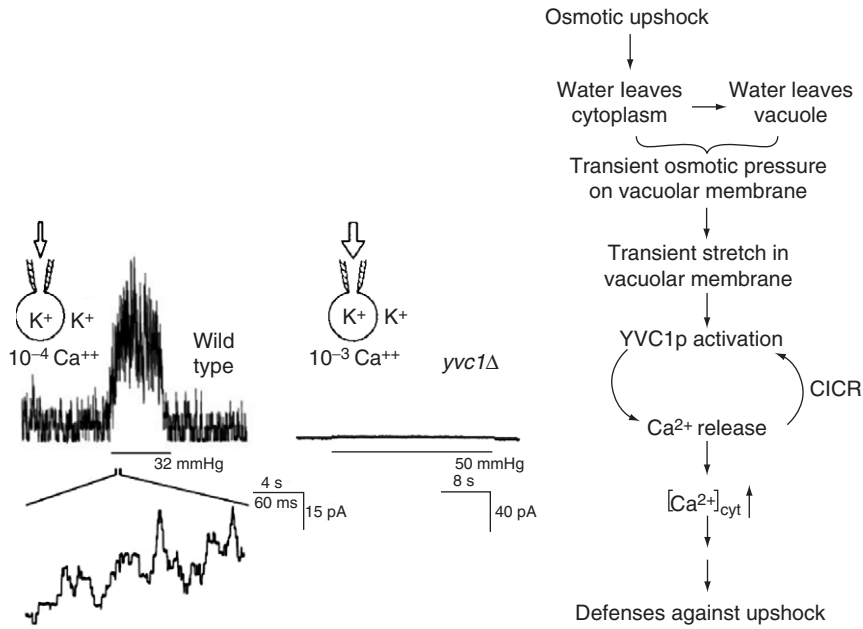


FIGURE 4 The mechanosensitivity of TRPY1 and an extended cell-biological model. Left: channel activities recorded in whole-vacuole mode. Pressure pulse (in mmHg) activates unitary conductances in the wild-type vacuole but not in the knockout mutant. The TPRY1 is also activated by Ca^{2+} (Palmer *et al.*, 2001). The comparison is made here showing that even at a higher concentration of Ca^{2+}, no mechanosensitive conductance can be elicited from the knockout. Right: an extension of the model shown in Fig. 3. Here the mechanosensitivity of TRPY1 explains the linkage between the mechanical stress of osmotic upshock and the Ca^{2+} flux. From Zhou *et al.* (2003).

how an osmotic upshock causes the rise of cytoplasmic free Ca^{2+}, a presumed defensive response (Fig. 4, right).

VI. OTHER FUNGAL TRP HOMOLOGUES

The TRPY1 channel of the budding yeast *S. cerevisiae* has homologues in some 30 different genomes of fungi spanning the two major mycological divisions: the ascomycetes (molds, yeasts, truffles, lichens, and so on) and basidiomyces (smuts, mushrooms, and so on) (Zhou *et al.*, 2005). The relatedness of 18 TRPY homologues are shown in Fig. 5. A more distant homologue in the *Schizosaccharomyces pombe* (fission yeast), with some similarity with TRPP, appears essential and relates to cell wall synthesis (Palmer *et al.*, 2005).

TRPY2 from *Kluyveromyces lactis* and TRPY3 from *Candida albicans*, the infectious yeast, have been studied recently. They were examined by expressing their corresponding genes, borne on a plasmid, in *S. cerevisiae* cells from which the native *TRPY1* has been deleted. Patch-clamp examination of these fungal TRP channels in this heterologous setting showed that their unitary conductance, ion selectivity, rectification, and Ca^{2+} sensitivity are similar to TRPY1. Most importantly, their mechanosensitivity is preserved in such a setting. This was demonstrated by following the hyperosmotically induced Ca^{2+} release from the vacuole to the cytoplasm *in vivo* (Fig. 6, top panels) as well as by following channels response to direct pressure applied through the patch-clamp

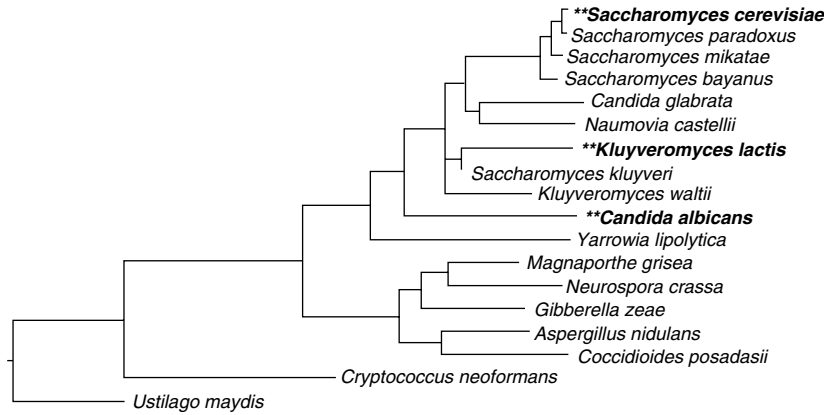


FIGURE 5 A dendrogram showing the relatedness in amino acid sequence of 18 TRPY-channel genes. Bold with asterisks are the three TRPYs that have been experimentally shown to be mechanosensitive. From Zhou *et al.* (2005).

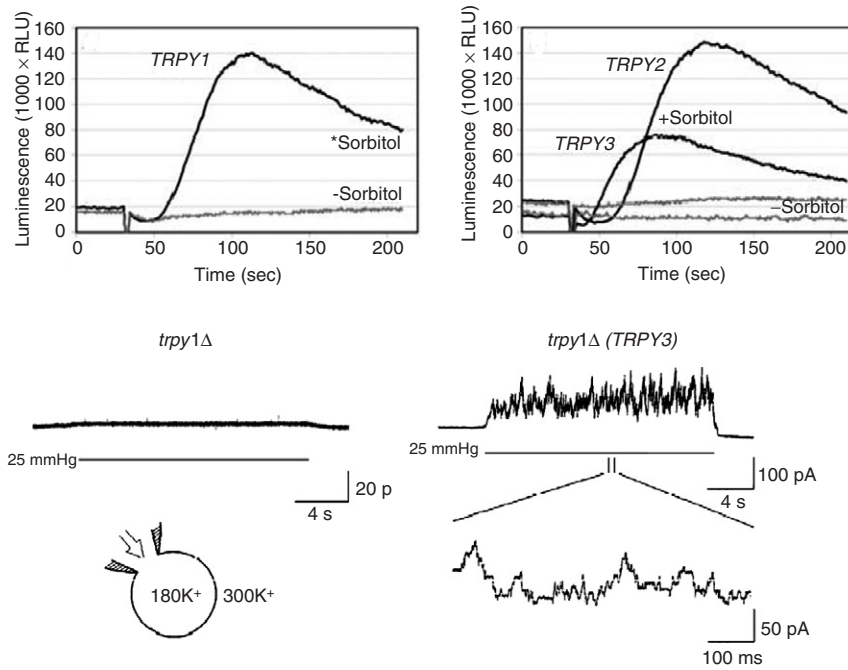


FIGURE 6 TRPY2 from *K. lactis* and TRPY3 from *C. albicans* function as mechnaosensitive channels when heterologously expressed in the vacuoles of *S. cerevisiae*. Top: Ca^{2+} responses to osmotic upshock from live cells, registered as luminescence from trangenically expressed aequorin. Addition of 3-M sorbitol leads to robust responses from the host (*TPRY1*, left) as well as from the guests (*TPRY2* and *TRPY3*, in *trpy1*Δ cells, right). Bottom: *TRPY3* is mechanosensitive in the vacuole of *S. cerevisiae* deleted of *TRPY1*. In whole-vacuole mode, no currents can be evoked with pressure from the *trpy1*Δ vacuole (left) but clearly from one expressing the *TRPY3* gene (right). From Zhou *et al.* (2005).

pipette (Fig. 6, bottom traces). Thus, the mechanosensitivity of TRPY2 and TRPY3 does not require their native membranes.

VII. SEQUENCE INFORMATION DOES NOT EXPLAIN TRP MECHANOSENSITIVITY

As reviewed above, TRP channels and their genes are found in diverse eukaryotes, including many microbes (Fig. 1). In the few cases where the microbial TRPs are examined, namely the several fungal channels, these channels are mechanosensitive (Figs. 2, 4, and 6). The universal presence of mechanosensitive channels should not be surprising since water is crucial to all life forms and life-threatening sudden de- or overhydration can be detected as

changes in the osmotic force exerted on the membrane (Kung, 2005). The presumed primordial origin of such a water-sensing device argues for a basic and evolutionarily preserved molecular mechanism. Can this mechanism be divined from the primary sequence of TRPs?

Unlike in K^+ channels, where the voltage sensors can easily be recognized from sequence, TRP-channel sequences are not useful in predicting the biophysical properties or the physiological functions of TRPs. The various cytoplasmic domains (ankyrin, calmodulin-binding domain, "TRP box,"and so on) that are used to divide TRPs into the subfamilies do not correspond to any gating principles. Mechanosensitivity, variously evidenced, has been reported in all eight subfamilies: TRPC (Strotmann *et al.*, 2000; Chen and Barritt, 2003; Maroto *et al.*, 2005), TRPV (Birder *et al.*, 2002; Liedtke and Friedman, 2003; Liedtke *et al.*, 2003; Mizuno *et al.*, 2003; Suzuki *et al.*, 2003; Gong *et al.*, 2004), TRPA (Walker *et al.*, 2000; Corey *et al.*, 2004), TRPP (Nauli *et al.*, 2003), TRPN (Walker *et al.*, 2000; Sidi *et al.*, 2003; Li *et al.*, 2006), TRPM (Grimm *et al.*, 2003), TRPML (Di Palma *et al.*, 2002), and now TRPY (Zhou *et al.*, 2003). Furthermore, where investigated thoroughly, TRPs are polymodal. TRPV1, the original vanilloid receptor, for example, is activated by heat (Caterina *et al.*, 1997), acidic pH (Tominaga *et al.*, 1998), and inhibited by PIP_2 (Prescott and Julius, 2003). At the same time, there are evidences that TRPV1 (Naeini *et al.*, 2006) or its spicing variants (Birder *et al.*, 2002) are also used as osmoreceptors. Thus, at least some TRPs may integrate several stimuli (e.g., stretch, heat, and ligands) into a single Ca^{2+} flux into the receptor cell and one meaning in the central nervous system (e.g., pain). In addition, the same TRP channels may serve different physiological functions in separate tissues in organisms that differentiate tissues. Readers should be wary of labels, such as thermo-TRPs, mechano-TRPs, and so on, which refer to the history of their discovery and context for their continued investigation and not necessarily their true or only biological roles.

Since only the sequence from TM5 to just beyond TM6 is conserved significantly among all TRPs (Fig. 1), the central mechanism for force-to-flux transduction should therefore lie within this region, which is largely buried in the membrane. By analogy to K^+ channel, this region covers the filter and the gate. How stretch force, from the lipid bilayer (Kung, 2005) or from cytoskeleton (Sukharev and Corey, 2004), is transmitted to the gate is the crux of mechanosensitivity. This puzzle remains to be solved.

VIII. CONCLUSIONS

The similarities of the invisible molecules continually surprise the mind, which has evolved to distinguish only visible size, shape, and movement. Yet, man, chimp, redwood, and *Escherichia coli*, however different in size,

shape, and movement, are made of the same clay. Very few macromolecules were invented since the appearance of multicellular animals, relative new comers in our evolutionary history. Students of modern biology are familiar with bacterial cytochromes, rhodopsins, ribosomes and yeast actin, tubulin, G-protein coupled receptors, and so on. The presence of TRP channels in protists and fungi, summarized in this chapter, should therefore not be surprising.

If all TRPs have the same origin and the conserved TM5–TM6 region holds the secret, then any TRP channel should be equally appealing as the subject of investigation. Given our innate interests in human and possible medical benefits, it seems natural to gravitate toward studying human TRPs or their mammalian equivalents. Why study microbial TRPs then? Microbes offer tremendous experimental advantages in understanding molecular mechanisms, as evidenced from the last 50 years' revolution in biology. DNA replication, transcription, and translation were largely solved through the study of bacteria and their phages. Central metabolism was understood through the study of mitochondria and yeast. These studies exploit the short generation time, massive clonal culture, relative simplicity, and the ease in genetic manipulations offered by microbes. Ion-channel study is traditionally about the cell biology and biophysics of animal neurons. Molecular research of channels is relatively recent. Nonetheless, massive bacterial and archaeal cultures have led to the crystal structures of K^+ channels and provided great insights into ion filtration and gating mechanisms. The study of bacterial MscL and MscS clearly showed that these mechanosensitive channels receive their gating force from the lipid bilayer (Sukharev *et al.*, 1997; Kung, 2005). Crystallography, genetics, spectroscopy, and molecular dynamics simulations have made MscL and MscS the concrete models for investigating mechanosensitivity at the molecular and atomic level. It remains to be seen whether the research on microbial TRP channels will be as fruitful.

Acknowledgment

Supported by NIH GM054867 (Y.S.), GM047856 (C.K.), and the Vilas Trust of the University of Wisconsin, Madison.

References

Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., *et al.* (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protests. *J. Eukaryot. Microbiol.* **52,** 399–451.

Barr, M. M., and Sternberg, P. W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans* [see comment]. *Nature* **401,** 386–389.

Batiza, A. F., Schulz, T., and Masson, P. H. (1996). Yeast respond to hypotonic shock with a calcium pulse. *J. Biol. Chem.* **271,** 23357–23362.

Bertl, A., and Slayman, C. L. (1990). Cation-selective channels in the vacuolar membrane of Saccharomyces; Dependence on calcium, redox state, and voltage. *Proc. Natl. Acad. Sci. USA* **87,** 7824–7828.

Bihler, H., Eing, C., Hebeisen, S., Roller, A., Czempinski, K., and Bertl, A. (2005). TPK1 is a vacuolar ion channel different from the slow-vacuolar cation channel. *Plant Physiol.* **139,** 417–424.

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.

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., and Julius, D. (1997). The capsaicin receptor: A heat-activated ion channel in the pain pathway [see comment]. *Nature* **389,** 816–824.

Chen, J., and Barritt, G. J. (2003). Evidence that TRPC1 (transient receptor potential canonical 1) forms a Ca(2+)-permeable channel linked to the regulation of cell volume in liver cells obtained using small interfering RNA targeted against TRPC1. *Biochem. J.* **373,** 327–336.

Chennik, M., Douagi, F., Achour, Y. B., Khalef, N. B., Ouakad, M., Louzir, H., and Dellagi, K. (2005). Characterization of two different mucolipin-like genes from Leishmania major. *Parasitol. Res.* **98,** 5–13.

Clapham, D. E., Montell, C., Schultz, G., Julius, D., and International Union of, P. (2003). International Union of Pharmacology. XLIII. Compendium of voltage-gated ion channels: Transient receptor potential channels. *Pharmacol. Rev.* **55,** 591–596.

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

Denis, V., and Cyert, M. S. (2002). Internal Ca(2+) release in yeast is triggered by hypertonic shock and mediated by a TRP channel homologue. *J. Cell Biol.* **156,** 29–34.

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 [see comment]. *Proc. Natl. Acad. Sci. USA* **99,** 14994–14999.

Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998). The structure of the potassium channel: Molecular basis of K+ conduction and selectivity. *Science* **280,** 69–77.

Embley, T. M., and Martin, W. (2006). Eukaryotic evolution, changes and challenges. *Nature* **440,** 623–630.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., *et al.* (1996). Life with 6000 genes [see comment]. *Science* **274,** 546.

Gong, S., 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 chanel subunits, inactive and nanchung, mediate hearing in Drosophila. *J. Neurosci.* **24,** 9059–9066.

Grimm, C., Kraft, R., Sauerbruch, S., Schultz, G., and Harteneck, C. (2003). Molecular and functional characterization of the melastatin-related cation channel TRPM3. *J. Biol. Chem.* **278,** 21493–21501.

Hughes, J., Ward, C. J., Peral, B., Aspinwall, R., Clark, K., San Millan, J. L., Gamble, V., and Harris, P. C. (1995). The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nat. Genet.* **10,** 151–160.

Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R. (2002). The open pore conformation of potassium channels. *Nature* **417,** 523–526.

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* [see comment]. *Nature* **424,** 81–84.

Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature* **436,** 647–654.

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

Liedtke, W., and Friedman, J. M. (2003). Abnormal osmotic regulation in trpv4-/-mice. *Proc. Natl. Acad. Sci. USA* **100,** 13698–13703.

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.

Liedtke, W., Tobin, D. M., Bargmann, C. I., and Friedman, J. M. (2003). Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **100**(Suppl. 2), 14531–14536.

Maroto, R., Raso, A., Wood, T. G., Kurosky, A., Martinac, B., and Hamill, O. P. (2005). The role of TRPC1 in forming the mechanosensitive cation channel in frog oocytes. *Nat. Cell Biol.* **7,** 179–185.

McKemy, D. D., Neuhausser, W. M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* **416,** 52–58.

Minke, B., and Parnas, M. (2006). Insight on TRP channels from *in vivo* studies in *Drosophila*. *Annu. Rev. Physiol.* **68,** 649–684.

Minke, B., Wu, C.-F., and Pak, W. L. (1975). Induction of photoreceptor voltage noise in the dark in *Drosophila* mutant. *Nature* **258,** 84–87.

Minorsky, P., Zhou, X. L., Culbertson, M., and Kung, C. (1989). A patch-clamp analysis of a cation-current in the vacuolar membrane of the yeast Saccharomyces. *Plant Physiol.* **89,** 882.

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.

Mochizuki, T., Wu, G., Hayashi, T., Xenophontos, S. L., Veldhuisen, B., Saris, J. J., Reynolds, D. M., Cai, Y., Gabow, P. A., Pierides, A., Kimberling, W. J., Breuning, M. H., *et al.* (1996). PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272,** 1339–1342.

Montell, C., Jones, K., Hafen, E., and Rubin, G. (1985). Rescue of the *Drosophila* phototransduction mutation trp by germline transformation. *Science* **230,** 1040–1043.

Montell, C., Birnbaumer, L., Flockerzi, V., Bindels, R. J., Bruford, E. A., Caterina, M. J., Clapham, D. E., Harteneck, C., Heller, S., Julius, D., Kojima, I., Mori, Y., *et al.* (2002). A unified nomenclature for the superfamily of TRP cation channels. *Mol. Cell* **9,** 229–231.

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.

Naeini, R. S., Witty, M. F., Seguela, P., and Bourque, C. W. (2006). An N-terminal variant of Trpv1 channel is required for osmosensory transduction. *Nat. Neurosci.* **9,** 93–98.

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., and Zhou, J. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **6,** 6.

Palmer, C. P., Zhou, X. L., Lin, J., Loukin, S. H., Kung, C., and Saimi, Y. (2001). A TRP homolog in *Saccharomyces cerevisiae* forms an intracellular Ca(2+)-permeable channel in the yeast vacuolar membrane. *Proc. Natl. Acad. Sci. USA* **98,** 7801–7805.

Palmer, C. P., Aydar, E., and Djamgoz, M. B. (2005). A microbial TRP-like polycystic-kidney-disease-related ion channel gene. *Biochem. J.* **387,** 211–219.

Prescott, E. D., and Julius, D. (2003). A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science* **300,** 1284–1288.

Saimi, Y., Martinac, B., Delcour, A. H., Minorsky, P. V., Gustin, M. C., Culbertson, M. R., Adler, J., and Kung, C. (1992). Patch clamp studies of microbial ion channels. *Methods Enzymol.* **207,** 681–691.

Sidi, S., Friedrich, R. W., and Nicolson, T. (2003). NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. *Science* **301,** 96–99.

Story, G. M., Peier, A. M., Reeve, A. J., Eid, S. R., Mosbacher, J., Hricik, T. R., Earley, T. J., Hergarden, A. C., Andersson, D. A., Hwang, S. W., McIntyre, P., Jegla, T., *et al.* (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* **112,** 819–829.

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.

Sukharev, S., and Corey, D. P. (2004). Mechanosensitive channels: Multiplicity of families and gating paradigms. *Sci. STKE* 2004, re4.

Sukharev, S. I., Blount, P., Martinac, B., and Kung, C. (1997). Mechanosensitive channels of Escherichia coli: The MscL gene, protein, and activities. *Annu. Rev. Physiol.* **59,** 633–657.

Sun, M., Goldin, E., Stahl, S., Falardeau, J. L., Kennedy, J. C., Acierno, J. S., Jr., Bove, C., Kaneski, C. R., Nagle, J., Bromley, M. C., Colman, M., Schiffmann, R., *et al.* (2000). Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. *Hum. Mol. Genet.* **9,** 2471–2478.

Suzuki, M., Mizuno, A., Kodaira, K., and Imai, M. (2003). Impaired pressure sensation in mice lacking TRPV4. *J. Biol. Chem.* **278,** 22664–22668.

Tominaga, M., Caterina, M. J., Malmberg, A. B., Rosen, T. A., Gilbert, H., Skinner, K., Raumann, B. E., Basbaum, A. I., and Julius, D. (1998). The cloned capsaicin receptor integrates multiple pain-producing stimuli [see comment]. *Neuron* **21,** 531–543.

Tracey, W. D., Jr., Wilson, R. I., Laurent, G., and Benzer, S. (2003). Painless, a *Drosophila* gene essential for nociception. *Cell* **113,** 261–273.

Wada, Y., Ohsumi, Y., Tanifuji, M., Kasai, M., and Anraku, Y. (1987). Vacuolar ion channel of the yeast, *Saccharomyces cerevisiae*. *J. Biol. Chem.* **262,** 17260–17263.

Walker, R. G., Willingham, A. T., and Zuker, C. S. (2000). A *Drosophila* mechanosensory transduction channel. *Science* **287,** 2229–2234.

Yu, F. H., and Catterall, W. A. (2004). The VGL-chanome: A protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci. STKE* 2004, re15.

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.

Zhou, X.-L., Loukin, S. H., Coria, R., Kung, C., and Saimi, Y. (2005). Heterologously expressed fungal transient receptor potential channels retain mechanosensitivity *in vitro* and osmotic response *in vivo*. *Eur. Biophys. J.* E-pub ahead of print.