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Review

Functionality of the TRPV subfamily of TRP ion channels: add mechano-TRP and osmo-TRP to the lexicon!

W. Liedtke[a] and C. Kim[b,*]

[a] Duke University Medical Center, Center for Translational Neuroscience, Department of Neurobiology, Department of Medicine/Division of Neurology, Bryan Research Building, Room 201G, Durham North Carolina 27710 (USA), e-mail: wolfgang@neuro.duke.edu
[b] Hormone Research Center, School of Biological Sciences, Chonnam National University, Yongbong-Dong, Buk-Gu, KwangJu-Si (South Korea), e-mail: Changgk@chonnam.ac.kr

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Abstract. Transient receptor potential (TRP) ion channels have been identified as cellular sensors responding to diverse external and internal stimuli. This review will cover the TRPV subfamily that comprises vertebrate and invertebrate members. The six mammalian TRPV channels were demonstrated to function in thermosensation, mechanosensation, osmosensation and Ca^{2+} uptake. Invertebrate TRPV channels, five in *Caenorhabditis elegans* and two in *Drosophila*, have been shown to play a role in mechanosensation, such as hearing and proprioception in *Drosophila* and nose touch in *C. elegans*, and in the response to osmotic and chemical stimuli in *C. elegans*. We will focus here on the role that TRPV ion channels play in mechanosensation and a related sensory (sub-)modality, osmosensation.

Key words. TRP; TRPV; mechano-TRP; osmo-TRP; *C. elegans; Drosophila*; TRPV4, OSM-9, OCR-2, NAN, IAV.

Introduction

The transient receptor potential (TRP) ion channel was first described in *Drosophila*, deriving its name from the mutant phenotype of a transient receptor potential in response to prolonged light stimulation [1–4]. Thereafter, many TRP-related channels have been identified in invertebrates and vertebrates, one in yeast, none in bacteria or plants. In genetically tractable model organisms such as *Drosophila* and *Caenorhabditis elegans*, genetic screening led to the discovery of additional TRP channels involved in signal transduction in response to mechanical, chemical, osmotic and thermal stimuli. These TRP channels include *Drosophila* NOMPC [5], Nanchung (NAN) [6], Painless [7], and Pyrexia [8] and *C. elegans* OSM-9 [9], OCR-2 [10] and LOV-1 [11]. In mammals, expression cloning led to the discovery of new TRP channels. The finding of a novel TRP channel responding to the ingredient of hot peppers, capsaicin, first named vanilloid receptor 1 (VR1), later TRPV1, represented another landmark [12]. TRPV1 was found to be gated by vanilloid ligands, noxious heat > 42 °C and acidity [13]. The identification of TRPV1 as a thermal transducer molecule subsequently led to the discovery of additional 'thermo-TRPs', namely TRPV2 [14], -V3 [15–17], -V4 [18–20], -M8 [21, 22] and -A1 [23], which appear to respond to different temperatures from cold to hot. To date, some 70 TRP channels have been found in genomes of humans, *C. elegans*, and *Drosophila* [24] and have been categorized into 7 subfamilies by sequence homology: TRPC, TRPV, TRPM, TRPN, TRPA, TRPP and TRPML [25–27].
Emerging features of the TRP family include their physiological role in sensory transduction with different sub-

* Corresponding author.

modalities [28–33]. TRP channels are gated by diverse external stimuli such as heat, cold, mechanical forces and endogenous stimuli including intracellular signaling components downstream of phospholipase-C [29, 34–36] as well as lipid signaling molecules [35, 37–44]. Recently, it was reported that in some TRP channels gating was tightly modulated by membrane potential [45–48]. Most TRP channels non-selectively mediate influx of cations, but some members are very selective, for example, TRPV5 and -V6 exhibit Ca^{2+} selectivity [49–52], and TRPM4b and -M5 are permeant only to monovalent cations [46, 53–55]. With respect to their predicted structure, TRP channels also show diversity; the TRPC and TRPV subfamilies contain 3~5 multiple ankyrin-binding repeats N-terminal to the transmembrane segments [26]. The TRPA and TRPN subfamilies encompass 8~29 ankyrin repeat superdomains [5, 7, 23], but the TRPM and TRPP subfamilies lack ankyrin repeats [26]. Some members in the TRPM subfamily have a long C-terminus with enzymatic domains such as an ADP-ribose phosphatase-related NUDIX domain (TRPM2) [56] and an atypical kinase domain (PLIK: phospholipase C-interacting kinase) (TRPM6, TRPM7) [57–59]. Despite their generally low sequence homology, all TRP channels share a basic building plan, namely six predicted transmembrane domains with cytoplasmic N-terminus and C-terminus and a pore region between TM5 and TM6. This topology bears a principal resemblance to voltage-gated potassium and also cyclic nucleotide-gated channels [25], suggesting that they originate from a single proto-ancestor.

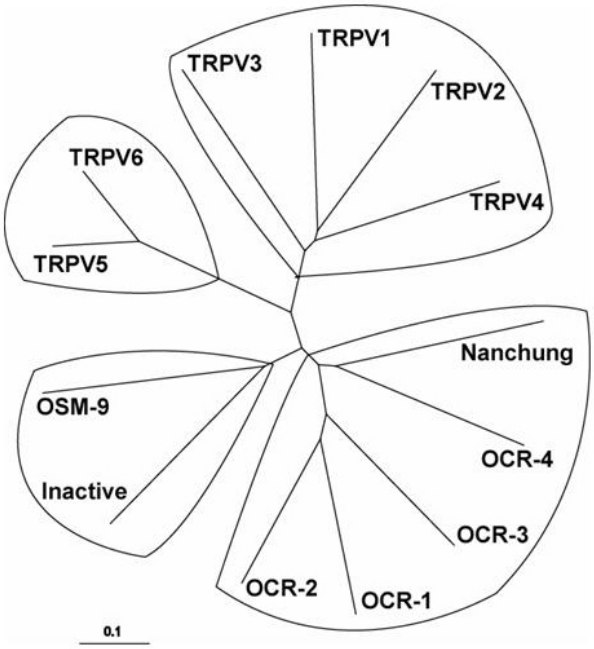

Figure 1. A phylogenetic tree shows four branches of TRPV family. TRPV1, -V2, -V3, -V4, -V5, -V6, mammalian TRPV channels. Nanchung and Inactive, *Drosophila* TRPV channels. OSM-9, OCR-1,-2,-3, *C. elegans* TRPV channels. The distance scale is expressed as the number of substitutions per amino acid.

The TRPV subfamily

The founding members of the TRPV subfamily are OSM-9 in *C .elegans* and TRPV1 in mammals, both reported in 1997. OSM-9 was identified through genetic screening for defects in osmotic avoidance [9]. TRPV1, -V5 and -V6 were identified by an elegant expression cloning strategy [12, 60, 61]. The remaining mammalian TRPV channels, TRPV2, -V3 and -V4 were identified by a candidate gene approach, respectively [14–20]. The latter strategy also led to the identification of four additional *ocr* genes in *C. elegans* [10] and two *Drosophila* TRPV channels, Nanchung (NAN) and Inactive (IAV) [6, 62]. The TRPV channels can be subgrouped into four branches by sequence homology (fig. 1). One branch includes four members of mammalian TRPVs, TRPV1, -V2, -V3 and -V4; in vitro whole cell recording showed that they respond to temperatures higher than 42, 52, 31 and 27 C, respectively, suggesting that they are involved in thermosensation, hence the term 'thermo-TRPs'. Insightful review articles on 'thermo-TRPs' are available [31,63-66], and we refer the interested reader to these papers. The second mammalian branch includes the Ca^{2+}-selective channels TRPV5 and TRPV6. Their physiological function is likely to be in Ca^{2+} uptake in the kidney and intestine [67–9]. One invertebrate branch includes *C. elegans* OSM-9 [9] and *Drosophila* IAV [62]; the other branch contains four *C. elegans* OCRs [10] and *Drosophila* NAN [6].

This review will elaborate on the role that mammalian and invertebrate TRPV channels play in signal transduction in response to osmotic and mechanical stimuli. We coin the terms'osmo-TRP'and 'mechano-TRP'which denotes the involvement of such a TRP channel in transduction in response to an osmotic and/or mechanical stimulus. Certainly, osmo- and mechano-TRPs comprise family members that do not belong to the TPRV subfamily, e. g. one would refer to TRPA1, NOMPC (and others) as a mechano-TRP, but the scope of our review will remain focused on the TRPV subfamily and the following pertinent questions. Do TRPV ion channels function in sensing and transduction of mechanical stimuli? What about their role in response to osmotic stimuli? If the answer to these questions is affirmative, how exactly does it go? Which molecular mechanisms are involved? Are the responses to either stimulus linked, possibly via the transduction of membrane tension? Two main reasons for the preliminary nature of arising answers is (i) that the field of TRPV ion channels is 'on the market' for

less than a decade [9, 12], and (ii) that the methodology to answer the above questions with respect to TRPV ion channels is challenging and complex.

Mammalian TRPV channels and mechano- and osmosensation

TRPV channels in mechanotransduction in mammals: TRPV1 and TRPV4

With respect to mammalian TRPV channels, *trpv1* null mice, which have previously been shown to be devoid of thermal hyperalgesia following inflammation [70,71], also displayed an altered response of their bladder to distension [72]. The TRPV1 protein could be localized to sensory and autonomous ganglia nerve cells, and also to urethelial cells lining the pyelon, ureter and bladder. When bladder and urethral-epithelial cells were cultured, their response to mechanical stress was significantly different from wild-type. More specifically, the TRPV1$^+$-excised bladders secreted ATP upon mechanical stretch which, in turn, is known to excite nerve fibres in the submucosa of the bladder. This response to a mechanical stimulus was greatly diminished in bladders excised from *trpv1* null mice. It appears likely that this mechanism, which is operative in the mouse, also plays a role in human bladder epithelium. Intravesical instillation of TRPV1-activating ligands is being used to treat hyperactive bladder syndromes, as in spinal cord disease, although the exact pathophysiology and its modulation by TRPV1 agonists is not clear at this point [73–76]. Another instance of an altered response to mechanical stimulation in *trpv1* null mice pertains to the response of the jejunum to mechanical distension [77]. Afferent jejunal nerve fibres were found to respond with lowered frequency of discharge in *trpv1* null mice than in wild-type (w.t.) littermates. In humans, in the rectum, TRPV1-positive fibres were found significantly increased in patients suffering from fecal urgency, a pathologic hypersensitivity of the rectum to mechanical distension [78]. Expression of TRPV1$^+$ fibres in rectal specimens from these patients was correlated with a lowered threshold to mechanical distension (the TRPV1$^+$ fibres were also correlated with a subjective feeling of heat). Another recent study focused on possible mechanisms of signal transduction in response to mechanical stimuli in vessels [79]. Elevation of intraluminal pressure in mesenterial arteries was found to be associated with generation of 20-hydroxyeicosatetraenoic acid, which in turn activated TRPV1 on C-fibre nerve endings, which resulted in depolarization of nerves and consequent vasoactive neuropeptide release. With respect to nociception, TRPV1 was shown to be involved in inflammatory thermal hyperalgesia, but not mechanical hyperalgesia in mice [80, 81]. However, a specific blocker of TRPV1 reduces mechanical hyperalgesia in the rat [82]. This finding in rats is not in keeping with a lack of a difference between *trpv1* null mice and w.t. littermates. Either this may be due to a species difference between rat and mouse with respect to signal transduction in inflammation-induced mechanical hyperalgesia and the role of TRPV1, or it may be due to the different mechanisms that affect the signaling in a general gene knockout (i. e. of the *trpv1* gene) vs. a specific pharmacological blocking (of the TRPV1 ion channel protein, which most likely participates in a multiple-protein signaling complex).

In *trpv4* null mice, the response to mechanical stimulation is altered [83, 84]. In the absence of TRPV4, which in w.t. could be demonstrated to be expressed in sensory ganglia [83] and, in skin, in subcutaneous nerve fibres and keratinocytes (epithelial cells) [85, 86], the threshold for noxious mechanical stimulation was significantly elevated. This result was obtained using two standard tests, the Randall-Sellitto test, which applies mechanical pressure by squeezing the paw (or the tail), and an automatized von-Frey test, which applies mechanical pressure by poking the hindpaw from underneath, leading to withdrawal [83]. In rats, however, an antisense-mediated knock-down of TRPV4 protein in dorsal root ganglion neurons did not increase the threshold for mechanical noxious stimuli [87]. In mice, paw withdrawal in response to a noxious thermal stimulus, an infra-red beam applied to the paw (Hargreave's test), was no different between *trpv4* null and w.t., neither was the escape response of the mice in response to the hot-plate test [83, 84]. However, a more sophisticated evaluation of abnormalities toward thermal stimuli revealed an abnormal inflammatory hyperalgesia in *trpv4* null mice, and altered behavior in a thermal gradient [88, 89]. When the rats were sensitized with taxol, their threshold for noxious mechanical stimuli was drastically lowered as a result of the taxol-induced neuropathy (taxol is a chemotherapeutic agent used in patients suffering from breast and ovarian cancer, and is known to induce a painful neuropathy in humans when surpassing a limiting dose) [90, 91]. When these rats were treated intrathecally with the TRPV4-specific antisense oligonucleotide, the threshold for mechanical painful stimuli went up [92]. This effect unambiguously suggests a role for TRPV4 in mediating hyperalgesia in response to mechanical stimuli in a neuropathic pain model. Last, but not least, with respect to mechanoreception in *trpv4* null animals, *trpv4* null mice do not show any sign of inner ear dysfunction, including deafness [83], which has to be viewed against the expression pattern of the *trpv4* gene in the inner ear [18, 93]. *trpv4* messenger RNA (mRNA) could be detected in the secretory epithelia of the stria vascularis/tegmentum vasculare and in neurosensory inner ear hair cells of both rodents and birds. This negative finding in vivo does, however, not exclude a role for *trpv4* in inner ear function.

With respect to the functioning of TRPV2 in vivo, we cannot tell because the *trpv2* null mouse has not yet been

published. Findings in a *trpv3* null mouse were reported very recently [94]. Deletion of the *trpv3* gene led to abnormalities of thermosensation and thermal preference. Inflammatory hyperalgesia in response to mechanical stimulation was not found to be different between *trpv3* null mice and w.t. littermate controls. Testing of the threshold for noxious mechanical stimuli was not reported.

TRPV channels in transduction of osmotic stimuli in mammals: TRPV1 and TRPV4

In regard to the response of TRPV channels to osmotic stimuli in vivo, we comment on what we know about the role of TRPV1 and -4 in response to osmotic stimuli in live animals. Before we tackle this subject, it is worthwhile considering the nature of the osmotic stimulus on a cell and on a mammalian organism [95, 96]. Osmotic stimulation is any deviation from an osmotic set point, which in most mammals is 295 mosmol/l [97, 98]. For a multicellular organism with an internal milieu, hypertonicity can be considered the more relevant stimulus because accumulation of osmolytes is an unavoidable consequence of metabolism, i.e. if metabolism is necessary for life itself, then life can be considered a race against hypertonicity. When confronted with a deviation from the iso-osmotic set point, cells respond with initial swelling or shrinkage, and then with counter-regulatory volume regulation. Mammalian organisms respond to deviations from the osmotic set point by a complex set of measures that consist mainly of alterations of secretion of a water-saving hormone, anti-diuretic hormone (ADH), also called vasopressin, and alterations in water intake [97–100]. Deviations from systemic tonicity are being sensed in the lamina terminalis, the anterior wall of the third ventricle adjacent to the anterior hypothalamus in the central nervous system (CNS) [101]. In the lamina terminalis, the sensory circumventricular organs, organum vasculosum laminae terminalis (OVLT) and subfornical organ (SFO), do not possess a blood-brain barrier [102–104]. ADH is being synthesized in magnocellular neurons in the supraoptic and paraventricular nucleus of the hypothalamus [105]. These neurons have been demonstrated to be directly sensitive to osmotic stimulation by activation of stretch-inactivated cation channels of a yet unknown molecular identity [98]. However, these neurons are not located outside the blood-brain barrier, and their known projections to the circumventricular organs involve synaptic relay [98, 99, 101]. At the cellular level, an osmotic stimulus can be regarded as a mechanical stimulus, because a change in volume alters cell membrane tension, a mechanical force parallel to this membrane. However, a change in cell volume could conceivably lead to only a small or even no change in membrane tension provided the membrane is very 'loose', possibly being involved in the formation of multiple processes. From yeast cells we also know that another response to osmotic stimulation can be the specific activation of intracellular phosphorylation/dephosphorylation signaling cascades [106–108]. This appears to be true also in mammalian cells. These two basic response patterns – osmotic stimulus leading to mechanical stimulation of the cell membrane vs. osmotic stimulus leading to activation of intracellular phosphatase/kinase signaling cascades – need not be mutually exclusive (fig. 2).

Returning to the question which roles TRPV1 and TRPV4 play in the response to osmotic stimulation in live animals, the focus will first be on the latter channel. *trpv4* null mice, when challenged with systemic hypertonicity, did not counter-regulate their systemic tonicity as efficiently as w.t. littermates [83]. Their drinking was reduced, and systemic osmotic pressure was significantly elevated. Continuous infusion of the ADH analogue dDAVP led to systemic hypotonicity, whereas renal water readsorbtion capacity was not impaired in either genotype. ADH synthesis in response to osmotic stimulation was impaired in *trpv4*$^{-/-}$ mice. Hypertonic stress led to diminished expression of c-FOS^{+} cells in the circumventricular organ, OVLT, indicative of impaired osmotic activation. These findings in *trpv4*$^{-/-}$ mice point toward a defect in osmotic sensing in the CNS. Thus, TRPV4 is necessary for the maintenance of osmotic equilibrium in mammals. It is conceivable that TRPV4 acts as part of an osmotic sensor in the CNS. The impaired osmotic regulation in *trpv4* null mice reported in the paper referenced above differs from that published in another report. While our experiments showed that *trpv4* null mice secrete reduced amounts of ADH in response to hypertonic stimulation, the results from Mizuno et al. [109] suggest that there is an accentuated ADH response to water deprivation and subsequent systemic administration of propylene glycol to mice. The reasons for this discrepancy are not clear; perhaps they are due to methodological differences. A blunted ADH response and diminished cFOS response in the OVLT in *trpv4* null mice upon systemic hypertonicity suggests, as one possibility, an activation of TRPV4^{+} sensory cells in the OVLT by hypertonicity. This consideration, in contradiction to what can be learned from heterologous cellular expression systems, is important and will be followed up further below. When using osmotic stimuli as noxious stimuli, hypotonicity-induced paw withdrawal in mice and rats did not differ in *trpv4* knock-out or knock-down animals when compared with w.t. [87, 92]. However, when paws were pre-sensitized with prostaglandin E2, w.t. controls were significantly more sensitive to a hypotonic aversive stimulus than *trpv4* knock-out or knock-down animals. With respect to the *trpv1* gene, the bladder epithelial cells of *trpv1* null mice did not respond to hypotonic osmotic stimulation in explant culture when compared with w.t. littermates [72]. Recently, Bourque and colleagues communicated that the *trpv1* gene is

necessary for osmotic sensing and secretion of ADH in response to osmotic stimuli in the CNS [158–159].

Considerations regarding heterologous cellular expression systems

When exploring the function and physiology of ion channels, heterologous cellular expression systems permitted the most rewarding investigations, e. g. for voltage-gated channels, but also for ligand-gated channels such as the nicotinic acetylcholine receptor, GABA-ergic channels and NMDA receptors. It is perhaps slightly underappreciated that this concept cannot be translated seamlessly to the investigation of channels that respond to osmotic and mechanical stimuli. Non-specific effects may result from perturbation of the cells through purely physical effects of these stimuli. With respect to mechanical stimulation, a substantial problem lies in the application of the stimulus, e. g. stretching the cell, and the measurement of the subsequent cellular response. First, a latency has to be determined in order to be able to differentiate direct activation of the channel, i. e. mechanotransduction happens exclusively by activation of the channel without other signaling molecules directly involved in this signaling, vs. an indirect response, i. e. mechanotransductory channel activation occurs downstream. For a direct response, a latency shorter than 1 ms is required [110, 111]. With currently available technology, this means that patch-clamp recordings have to be performed. In this setting, a substantial challenge is the application of the mechanical stimulus without disturbing recording. With respect to tonicity, the precise beginning of the osmotic stimulus cannot be defined. When applying the osmotic solution by means of streaming bath solution, one has to realize that a mechanical stimulus is co-applied, namely flow, notably so in case the cell bears any mechanosensitive processes. Also, it is important to consider that osmotic and mechanical stimuli as activators of ion channels are distinctly different from specific ligands/activators (e. g. GABA, NMDA). It can reasonably be assumed that most given cells harbor an innate response to primal biophysical stimuli such as tonicity and touch; i. e. heterologously expressing a given channel in this context is supplementing a pre-existing signaling apparatus by one more molecule. It is quite clear, on the other hand, that the situation is different for e. g. the response of an epithelial or fibroblast cell to a nervous system-specific ligand/activator such as GABA or NMDA.

Heterologous expression data for TRPV4

Bearing in mind these qualifiers, a response to osmotic and mechanical stimuli in heterologous cellular expression systems could be demonstrated for TRPV4 and TRPV2. Chinese hamster ovary (CHO) tissue culture cells responded to application of hypotonic solution when they were (stably) transfected with TRPV4 [18]. HEK-293T cells were found to harbor *trpv4* complementary DNA (cDNA), which was cloned from these cells. However, *trpv4* cDNA was absent in other batches of HEK 293T cells, so that this cell line was used as heterologous expression vehicle by other investigators [19, 20]. Notably, when comparing the two settings it was obvious that the single-channel conductance was very different [18, 19]. This clearly underscores the relevance of the comprehensive gene expression pattern in a heterologous cell system for the functioning of TRPV4 in response to a very basic biophysical stimulus. It is conceivable that this also applies to other TRP channels and to other such stimuli. It will be interesting to learn about the specific TRP(V) channel protein 'interactomes' in a given type of cell. All interaction partners, particularly other ion channel subunits, can be regarded as candidate regulators of channel activity. Also, it was found that the sensitivity of TRPV4 could be tuned by warming of the media. Peak sensitivity of gating in response to hypotonicity was recorded at core body temperature of the respective organism, and TRPV4 channels from both birds (chick, core body temperature 40 °C) and mammals (rat, 37 °C) were compared in CHO cells [18]. This experiment was later recapitulated with mammalian TRPV4 in HEK-293T cells [112]. In addition, in this investigation, the cells were mechanically stretched, without a change of osmotic pressure. At room temperature, there was no response upon mechanical stimulation; however, at 37 °C the isotonic response to stretch resulted in the maximum calcium influx of all conditions investigated. In two other investigations, TRPV4 was found to be responsive to changes in temperature [86, 113]. Change in temperature was accomplished by heating the streaming bath solution (see above considerations regarding flow). Gating was increased when hypotonic solution was used as bath solution. In one investigation, temperature stimulation could not activate the TRPV4 channel in cell-detached inside-out patches [113]. With respect to the gating mechanism of TRPV4 by hypotonicity (as discussed above for the dualism [change in tonicity leads to change in membrane tensile strength] vs. [change in tonicity leads to altered phosphorylation states in intracellular signaling cascades]), other investigations report conflicting results on phosphorylation sites of TRPV4 that are necessary for the response to hypotonicity.

One paper reported that TRPV4 was tyrosine-phosphorylated in HEK-293T cells and in murine distal convoluted tubule cells from kidney [114, 115]. Tyrosine phosphorylation was sensitive to a specific inhibitor of the Src family tyrosine kinases. The Lyn tyrosine kinase was found to coimmunoprecipitate TRPV4 protein and to bear a prominent role in phosphorylation of the TRPV4 protein, in which the amino acid residue Y253 was found to be pivotal. A point mutation of Y253 greatly reduced

hypotonicity-induced channel activity. On the other hand, in another investigation, in HEK-293T cells, hypotonicity activated TRPV4 by means of phospholipase-A2 formation of arachidonic acid via a cytochrome P450 epoxygenase pathway [116]. In HEK cells, this signaling mechanism did not apply for activation of TRPV4 by heat or by the phorbol-ester 4-alpha PDD. This latter activation mechanism was found to be dependent on phosphorylation of Y555 of TRPV4. However, the authors of this study could not replicate the aforementioned finding, namely that tyrosine kinase phosphorylation of residue Y253 of the TRPV4 channel was pivotal for hypotonicity-induced gating. In yet another investigation, mentioned above in respect to response of live animals to painful mechanical stimuli, the alteration of mechanical hyperalgesia in a taxol-induced neuropathy pain model in the rat was found to be sensitive to blocking of Src-type of tyrosine kinase inhibitors [92]. This was also true for the Ca^{2+}-influx response of explanted DRG ganglion neurons to hypotonicity.

What lessons might be learned from these three interesting investigations, all of which were elegant studies conducted by well-respected groups? The discrepancy between the first two studies reiterates the importance of the role of the host cell in heterologous expression systems and, possibly, their respective transcriptome. With respect to mechanical hyperalgesia in rodents with taxol-induced neuropathy, it would be interesting to see whether a mouse knock-in of a distinct point mutation of TRPV4, such as Y253F, would have a diminished mechanical hyperalgesia and whether the DRG ganglion cells of this line of mice would respond differently with respect to their hypotonicity-induced Ca^{2+} influx. In a very recent paper, the ciliary beating frequency of ciliated cells was found to be influenced by gating of TRPV4 [117]. In explanted ciliated cells, and also in heterologously transfected HeLa cells, TRPV4 could be activated (mechanically) by exposing the cells to hyperviscous, isotonic media.

Heterologous expression data for TRPV2

With respect to the TRPV2 ion channel, it was initially described as a temperature-gated ionotropic receptor for stimuli > 50 °C [14]. Recently, TRPV2 was also demonstrated to respond to hypotonicity and mechanical stretch [118]. Arterial smooth muscle cells from various arterial territories expressed TRPV2 protein. These cells responded to hypotonic stimulation with calcium influx. This activation could be reduced by specific down-regulation of TRPV2 protein by antisense oligonucleotides. Heterologously TRPV2-expressing CHO cells displayed a similar response to hypotonicity. These cells were also stretched by applying negative pressure to the patch pipette and by stretching the cell membrane on a mechanical stimulation device. Both maneuvers led to calcium influx that was dependent on heterologous expression of TRPV2. There are no reports on whether these responses depend on tyrosine-kinase-induced phosphorylation of specific TRPV2 residues.

… in aggregate …

Thus, from these in vitro studies we can conclude that both TRPV4 and TRPV2 are ionotropic candidate mechanoreceptors involved in the transduction of osmotic and mechanical signaling. In addition, up to this point, heterologous expression systems did not reveal any evidence that TRPV1, -3, -5 or -6 responded to such stimuli. From the studies in genetically engineered rodents, it appears that both TRPV1 and TRPV4 are involved in the response to mechanical and osmotic stimuli in vivo (with *trpv2* and *trpv6* null mice not yet reported). Clearly, the findings in heterologous expression systems pertaining to TRPV channel activation in response to biophysical stimuli with basic relevance for cellular homeostasis, such as temperature, mechanical, osmotic and ionic stimuli, will have to be interpreted with caution. On the other hand, findings from live animals are of considerable value in this respect. However, with respect to animal data, some important qualifiers have to be kept in mind. First, one needs to ascertain the activity of the respective promoter in a given cell population. Either the cells have to homogeneously express a given TRPV gene, e. g. the TRPV3 or TRPV4 protein in the epidermis of the skin, or the cell harboring an active *trpv* promoter has to be highlighted by a (fluorescent) reporter gene. Rodent transgenic technology will permit the generation of such mouse lines [119, 120], which will have to be crossed into the respective *trpv* null lines. Second, 'knocking out' a given *trpv* gene represents an attractive first-line approach, but one has to be aware of the possibility of developmental compensation leading to 'masking' of a phenotype. This can be avoided by generating 'second generation' knock-out models with cre-lox technology that leads to tissue/cell-specific deletion of a targeted gene, and by 'third generation' knock-out technology that will permit not only tissue/cell-specific targeting, but also feature inducibility, i. e. temporal control.

C. elegans TRPV channels and mechano-, osmosensation

Cloning of the *osm-9* gene, a founding member of the *trpv* gene family

At the end of 1997, about the same time that the landmark TRPV1 paper made the cover of *Nature* [12], a paper was published in the *Journal of Neuroscience* entitled 'OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation and olfactory adaptation in *Caenorhabditis elegans* [9]. The discoveries

reported in these two papers can be regarded as the founding events for the TRPV subfamily of ion channels. The *osm-9* mutant was found in a forward genetics screen in *C. elegans* that applied a confinement assay with a high-molar osmotically active substance. *osm-9* mutants did not respect this barrier, and the mutated gene was found to be a TRP-related ion channel. On closer analysis, *osm-9* mutants did not respond to aversive osmotic stimuli, they did not respond to mechanical stimuli applied to their anterior end ('wormnose') and they displayed sensory deficits in their response to odorant cues. The OSM-9 channel protein was expressed in amphid sensory neurons, the worm's cellular substrate of exteroceptive sensing of chemical, osmotic and mechanical cues. In this respect, it should be reiterated that worms are not capable of perceiving light, and the mechanical sense refers to touch including vibration of the supporting media, but worms cannot hear. The OSM-9 channel protein was expressed in the sensory cilia of the AWC and ASH amphid sensory neurons. Bilateral laser ablation of the ASH neuron is known to lead to a deficit in osmotic, nose touch and olfactory avoidance [121]. ASH has thus been termed the 'nociceptive' neuron [122]. The OSM-9 protein could not, however, be expressed in heterologous cellular expression systems, and explant cultures of amphid sensory neurons were not functional (see below).

Four more *trpv* genes in the *C. elegans* genome; *ocr-2* gene functions in nociception

Next, from the same laboratory, four additional TRPV channels from *C. elegans* were reported, named OCR-1 to -4 (OSM-9 and capsaicin-receptor related channels 1–4) [10]. Of these four channels, only the OCR-2 channel was found to be expressed in ASH. Because of the prominent role of ASH in the response to osmotic and mechanical stimulation, the investigation focused prominently on OCR-2. The *ocr-2* mutant phenotype was virtually identical to the *osm-9* phenotype with respect to 'nociceptive' submodalities, and there was genetic evidence that the two channels were necessary for the proper intracellular trafficking of other TRPV channels in sensory neurons, indicating that these channels interact. When expressing the mammalian capsaicin receptor TRPV1 in the ASH sensory neurons, neither *osm-9* nor *ocr-2* mutants could be rescued for any of their deficits, but *osm-9 ash::trpv1* transgenic worms displayed a brisk avoidance response to TRPV1 ligands such as capsaicin, which normal worms virtually do not respond to.

TRPV4 expression in ASH rescues *osm-9* mechanical and osmotic deficits

Next, TRPV4 was transgenically targeted to the ASH neurons of *osm-9* mutants. Surprisingly, TRPV4 expression in *C. elegans* ASH rescued the *osm-9* animal's defects in avoidance of hyperosmotic stimuli and nose touch [123]. However, mammalian TRPV4 was unable to rescue the odorant avoidance defect of *osm-9*, suggesting that this specific function of TRPV channels differs between vertebrates and invertebrates. This basic finding of the rescue experiments in *osm-9 ash::trpv4* worms has important implications for mechanisms of signal transduction, a schematic representation is shown (fig. 2A–C).

TRPV4 appeared to be integrated into the normal ASH sensory neuron signaling machinery, since the transgene failed to rescue these deficits in *C. elegans* mutants defective in osmosensation and mechanosensation (including OCR-2, bespeaking the specificity of the observed response). A point mutation in the pore loop of TRPV4, M680K, markedly reduced complementation, indicating that TRPV4 very likely functions as an ion channel in the transduction of osmotic and mechanical stimuli in vivo. In an attempt to recapitulate the properties of the mammalian channel in the aversive reaction of the worm, the sensitivity for osmotic stimuli and the effect of temperature on the avoidance responses of *osm-9 ash::trpv4* worms more closely resembled the known functional properties of mammalian TRPV4 than that of normal worms. These data suggest that TRPV4 functions as an osmotically and mechanically gated channel, and that, in this model, TRPV4 directs the osmotic and mechanical avoidance behavior of the worm. Our investigation suggested that TRPV4 functions as (part of) the sensor for osmotic and mechanical stimuli. This is illustrated in figure 2B (tonicity signaling) and -2C (mechanotransduction). The characteristics of the response of *osm-9 ash::trpv4* worms more closely resemble those of the TRPV4 channel than those of w.t. worms in several important respects. *osm-9 ash::trpv4* worms respond to mildly hyper-osmolar solutions that are not sensed by normal worms. In addition, the modulating effect of temperature on the response of *osm-9 ash::trpv4* worms resembles known properties of mammalian TRPV4 in heterologous expression systems. It appears unlikely that these fundamental properties of the response of *osm-9 ash::trpv4* worms would resemble those of mammalian TRPV4 if it were downstream of the sensor. Moreover, TRPV4 does not complement the odorant avoidance deficit of *osm-9* worms, where G-protein-coupled receptors function as the sensors, and TRPV4 did not function down stream of other known mutations that affect nose touch and osmotic avoidance in *C. elegans*. In aggregate, these data and considerations suggest that mammalian TRPV4 was functioning as the osmotic and mechanical sensor or at least a component of it. It should be reiterated that TRPV4 was practically expressed only in ASH, a single sensory neuron, where the mammalian protein, with a similarity to OSM-9 of approximately

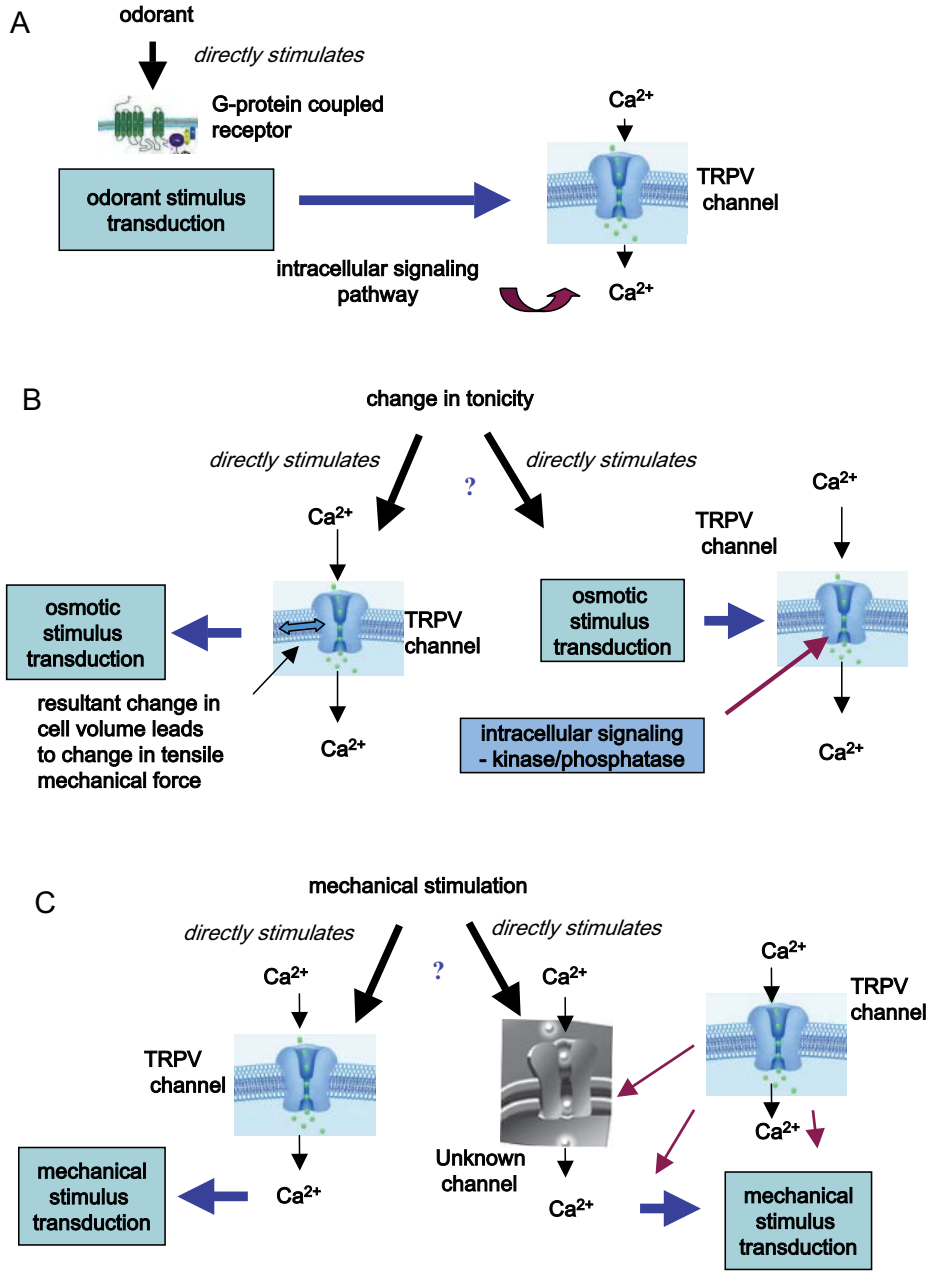

Figure 2. Schematic representations illustrating how signal transduction in sensory (nerve) cells in response to odorant (A), osmotic (B) and mechanical (C) stimuli could possibly function. (A) The odorant activates the TRPV ion channel via a G-protein-coupled receptor mechanism. Such a mechanism is operative in the ASH sensory neuron of *C. elegans* in response to e. g. 8-octanone, a repulsive odorant. Intracellular signaling cascades downstream of the G-protein-coupled receptor activate the TRPV channel, OSM-9 or OCR-2. Calcium influx through the TRPV channel serves as an amplification mechanism, which is required for this signaling pathway to lead to the stereotypical withdrawal response. (B) This drawing represents two possibilities for how tonicity signaling could work. In one alternative scenario, depicted on the right-hand side, the TRPV channel functions downstream of a – yet unknown – osmotic stimulus transduction mechanism, which is directly activated by a change in tonicity. This is conceptually related to what is depicted in (A). Intracellular signaling via phosphorylation (dephosphorylation)-dependent pathways activates the TRPV channel. For heterologous cellular expression systems, two groups have obtained data, contradictory in its detail, that suggest phosphorylation of TRPV4 to be of relevance [114, 116]. On the left-hand side of the representation, note another scenario where the TRPV channel is at the top of the signaling cascade, i. e. it is directly activated by a change in tonicity, which in turn leads to an altered mechanical tension of the cytoplasmic membrane via volume change. Note that the two alternatives need not be mutually exclusive. Apart from phosphorylation of the TRPV channel, which could possibly be of relevance in vivo, a direct physical linkage of the TRPV channel to the cytoskeleton, to the extracellular matrix and to the lipids of the plasma membrane adjacent to the channel has to be entertained. (C) This drawing represents two possibilities for how mechanotransduction could work. Here, depicted on the right-hand side, an unknown mechanotransduction channel responds directly to the mechanical stimulus with calcium influx. This activity and the subsequent signal transduction are modulated more indirectly by the TRPV channel, which acts on the unknown transduction channel, onto the biophysical properties of the membrane, and via other, yet-unknown intracellular signaling mechanisms. The left-hand side depicts another alternative. Here, the TRPV channel is the mechanotransducer itself, i. e. it is activated directly via mechanical stimulation. The data from [123] suggest the scenarios where the TRPV channel (namely TRPV4) is the transduction channel itself, whereas what is depicted in (A) is not functional when the OSM-9 (or OCR-2) TRPV channel is substituted by mammalian TRPV4.

25%, was trafficked correctly to the ASH sensory cilia. The rescue was specific (not for OCR-2, not by mammalian TRPV1), and it respected genetically defined pathways for osmotic and nose-touch avoidance. Thus, this approach has considerable impact on the understanding of the functioning of TRPV4 and on TRPV channel

functioning in general. However, this investigation also brings up stimulating questions. While TRPV4 restores responsiveness to hyper-osmotic stimuli in *C. elegans osm-9* mutants, it is only gated by hypo-osmotic stimuli in transfected mammalian cells. The basis for this difference is not known. One possibility is suggested by the results of a recent study where a mechanosensitive ion channel, gramicidin A, behaved either as a stretch-inactivated or as a stretch-activated channel depending on the lipid composition of the surrounding lipid bilayer [124]. An alternate possibility is that TRPV4 forms heteromultimeric complexes with other proteins, as was recently shown for the MEC proteins, and that this transduction channel multiplex has different properties than its modular components [125, 126]. TRP ion channels are known to form heteromeric complexes with related family members [127, 128]. OCR-2 and OSM-9 are the only *C. elegans* TRPV family members that are expressed in ASH neurons, and OCR-2 expression is essential for the ability of TRPV4 to rescue the sensory defects of *osm-9* worms [10, 123]. The specific rescue of *osm-9*, but not *ocr-2*, by TRPV4 represents an important instance in which the phenotypes of these two TRPV mutants have been distinguished, and suggests that OCR-2 and OSM-9/TRPV4 channels may well have distinct properties. Lastly, it should not be forgotten that this animal model allows the exploration of the medically relevant TRPV4 channel in a simplistic genetic model organism, which is a multicellular organism with a nervous system that can elicit a set of behavioral responses, amongst them avoidance behavior in response to noxious stimuli. Related to this investigation, it was recently reported that TRPV2 could rescue one particular deficit in the *ocr-2* mutant, namely the dramatic down regulation of serotonin biosynthesis in the sensory ADF neuron, but mammalian TRPV2, unlike TRPV4 directing behavior in *osm-9*, did not rescue the osmotic avoidance reaction present in *ocr-2* [129, 130]. Common to all three investigations is the conservation of TRPV signaling function across phyla that were separated by several hundred million years of molecular evolution, and this despite relatively low sequence homology!

Ca^{2+} imaging in ASH now possible

Until recently, progress regarding the *C. elegans* TRPV channels was impeded by the unavailability of heterologous expression systems, and by a lack of methodology that would permit measurement of intracellular signaling in ASH or other sensory neurons while the worm is being stimulated with sensory stimuli. A recent investigation reports major progress in this area [131]. Calcium sensitive fluorescent proteins were expressed in ASH, and their activation by calcium influx was monitored by fluorescence microscopy. Osmotic stimulation and nose touch did indeed activate ASH, and calcium influx could be readily observed. ASH proved to be a multimodal 'nociceptive' sensory neuron because many other stimuli elicited calcium influx into ASH, e. g. copper ions, SDS, quinine, hypertonic osmotic stimuli, nose touch (needed the presence of serotonin). This multimodal response, which showed adaptability, was greatly diminished in *osm-9* mutants.

Polyunsaturated fatty acids regulate TRPV channel functioning in *C. elegans*

In the *osm-9 ash::trpv4* model we speculated that the lipid composition of the ASH cell membrane might be related to the response to hypertonicity versus hypotonicity in tissue culture cells [123]. In a landmark paper, published very recently, it was demonstrated that specific polyunsaturated fatty acids drove TRPV-dependent avoidance reactions in *C. elegans*, and the molecular identity of some of these lipids was defined for the first time [132]. A subset of 20-carbon polyunsaturated fatty acids (PUFAs) could be implicated in nociceptive avoidance behaviors. PUFA signaling led to rapid TRPV-dependent calcium transients in ASH and other sensory neurons. It was concluded that a subset of PUFAs (with omega-3 and omega-6 acyl groups) could act as endogenous modulators of TRPV signal transduction in *C. elegans* nociception.

Drosophila TRPV channels and hearing

Drosophila affords a useful model system particularly in the field of hearing since one can easily measure sound-evoked extracellular membrane potentials upon acoustic stimulation [133, 134] as well as perform intricate genetic analysis. The *Drosophila* genome encodes two TRPV family ion channels, NAN (CG5842) and IAV (CG4536). Recent molecular analysis revealed that the two channels are exclusively expressed in chordotonal neurons, which function as cellular stretch receptors in transduction of sound and proprioception. Consistent with their expression pattern, mutations in NAN and IAV cause deafness and proprioception defects [6, 62]. As established for other TRP channels, NAN and IAV are likely to form heteromers in vivo. In this section, we will discuss whether the NAN-IAV complex is an auditory transducer.

Drosophila hearing organs and cilia

Auditory communication in *Drosophila* plays a critical role during courtship and mating [135]. During this behavior, a mature male fly approaches a female, then extends one of its wings and generates vibrations with it, thereby producing a species-specific song, colloqui-

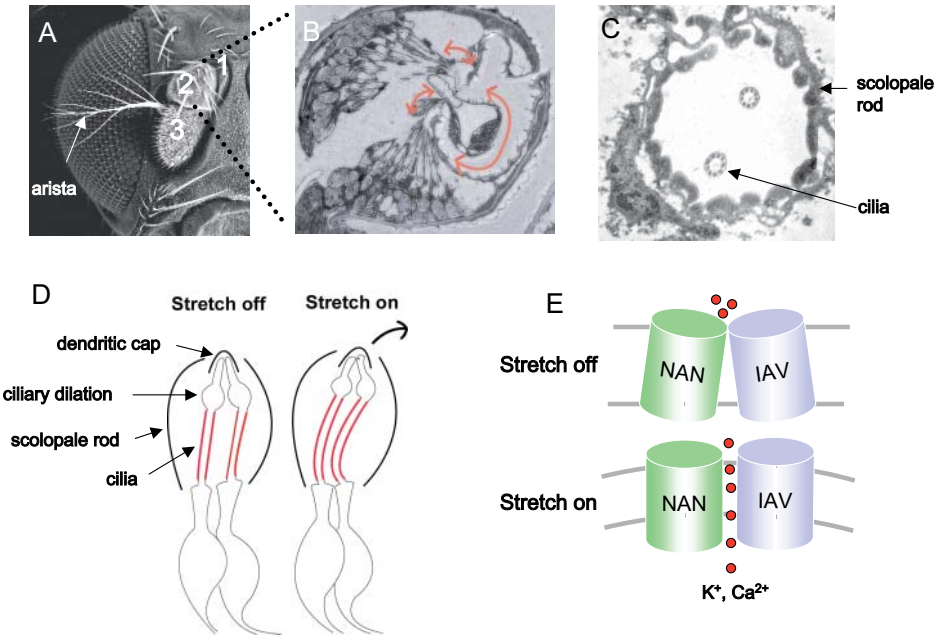

Figure 3. A hypothetical model of the NAN-IAV channel gating. (A) Scanning electron microscopy of a *Drosophila* eye, next to it the *Drosophila* antenna. (B) Higher magnification transmission electron microscopy of ultra-thin sections of antenna; depicted is the Johnston's organ. Arrows indicate the vibration of cuticles caused by sound. (C) Higher magnification transmission electron microscopy shows a transverse section of scolopidia. (D) Schematic drawing of scolopidia. NAN-IAV heteromeric channels are located in the proximal region of the cilia (depicted In red) [62]. Stretch causes bending of cilia at the proximal region [156]. (E) Stretch opens the NAN-IAV heteromeric channel.

ally known as 'love songs' [136–138]. The 'love songs' are composed of pulse and sine components. The sine component of the song consists of an ~160-Hz sinusoidal sound wave [139, 140]. The pulse component of the song is composed of trains of 5- to 10-ms pulses with an ~34 ms interval [135, 137]. When the male is close to female, the near-field amplitude amounts to 95 dB [140]. Upon hearing the male's 'love songs', females halt their movement and become receptive to copulation [135, 141].

Drosophila's hearing organ resides in the antennae [133, 139], which are composed of three segments including (from proximal to distal) the first (scape), the second (pedicel) and the third segment (funiculus) to which arista, a tree like structure, is bonded (fig. 3A). The second segment contains Johnston's organ, the antennal chordotonal organ that responds to sound. In chordotonal organs, neurons elaborate cellular processes, called sensory cilia, with an axonemal segment of a 9 x 2 + 0 arrangement of microtubules [142, 143]. This structure is encased in actin-rich scolopidia and is thought to be bathed in a high K^+, low Ca^{2+} endolymph in analogy to that of external sensory organs [144]. The distal end of the sensory cilia is attached to the dendritic cap that contains the NOMPA [145]. The arista and the third segment are the fly's sound receiver, which oscillates about the longitudinal axis of the third segment in response to the 'love song' [137, 146, 147]. The articulation resides at the second and third joint. In this configuration, the mechanical oscillation of the third segment causes stretching of the sensory cilia of the chordotonal neurons in the second segment (fig. 3B). A mechanosensitive channel presumed to be located in the cilia is thought to respond to the mechanical tension generated by the oscillation of the third segment and to allow influx of cations into the cytoplasm, which in turn generates membrane depolarization. The identity and localization of this mechanosensitive channel has been elusive.

The previous electrophysiological conclusion that neurons in the second segment are auditory receptors is further confirmed using atonal mutant flies, which lack the Johnston's organ and are defective in sound-evoked potentials [134]. Recently, flies lacking only outer segments of dendrites were generated and shown to lack sound-evoked potentials as well, indicating that the cilia mediate sound-induced mechanical sensory transduction [148–150]. The delicate structure of chordotonal cilia appears to be important for mechanosensation, as revealed by two auditory transduction mutants, *touch-insensitive larva B* (*tilB*) and *beethoven* (*btv*) [134]. *tilB* and *btv* mutants were isolated through genetic screening for uncoordination [151] and defects in an auditory behavior assay, respectively [152]. The two mutants lack sound-evoked potentials but mechanoreceptor potentials in bristles are intact [134]. The fact that *tilB* and *btv* are specific to chordotonal mechanosensation is interesting given that bristle and chordotonal mechanosensation have been considered to be very similar, possibly sharing underlying genetic pathways [134]. Of note, ultrastructural analysis revealed that the *tilB* and *btv* mutants exhibit subtle axonemal defects of the sensory cilia of chordotonal organs [134], implying that the axoneme of chordotonal cilia plays a critical role for chordotonal-specific mechanotransduction.

Table1. Properties of TRPV family ion channels.

Nomenclature	Other names	Expression	Agonists	Physiological roles
Mammalian TRPV channels				
TRPV1	VR1	DRG, CNS, bladder, vessels	capsaicin, protons, heat (>42 °C), anandamide 12-(S)-HPETE(12-(S)-hydroperoxyeicosatetraenoyl acid), 5-(S)-HETE, LTB4 (leukotriene B4)	thermal pain sensation, mechanosensation (see this review), vascular regulation, taste transduction [70, 71]
TRPV2	VRL-1, GRC, OTRPC2	DRG, CNS, widely expressed	noxious heat (>52 °C), cell swelling, mechanical force	thermosensation., mechanosensation* [14, 118]
TRPV3	VRL-3	DRG, skin, widely expressed	temperature (>31 °C) and diphenylboronic anhydride	thermosensation (thermal preference), pain sensation [94]
TRPV4	VR-OAC, OTRPC4, Trp12, VRL-2	DRG, kidney, skin, inner ear hair cells, inner ear stria vascularis, endothelium, brain, hypothalamus/ circumventricular organs, trachea/ lung, fat, heart	cell swelling, mechanical force, 4- PDD (4 -phorbol-12,13-didecanoate), temperature (>27 °C) 5,6'-EET [5',6'-epoxyeicosatrienoic acid]	osmotic regulation by the CNS, mechanically- and osmotically-mediated pain sensation, thermal preference [84, 89, 123]
TRPV5	EcaCl, CaT2,ECaC	intestinal and renal epithelia, CNS	constitutively active in transfected cells	Ca^{2+} uptake in kidney, Ca^{2+} homeostasis and bone structure [157]
TRPV6	EcaC2, CaT1, CaTL	intestinal and renal epithelia, CNS	constitutively active in transfected cells	Ca^{2+} uptake in kidney and intestine* [49]
Drosophila TRPV channels				
Nanchung	CG5842	chordotonal neurons	cell swelling	mechanosensation of hearing and proprioception [6]
Inactive	CG4536	chordotonal nuurons	cell swelling	mechanosensation of hearing and proprioception [62]
*C. elegans TRPV channels***				
Osm-9		OLQ, ADL, ADF, AWA, ASH	PUFAs	chemosensation, mechanosensation, hyperosmolarity sensation [9, 10]
Ocr-1		ADL	not determined	chemosensation [10]
Ocr-2		ADF, AWA, ASH	PUFAs	chemosensation, mechanosensation, hyperosmolarity sensation [10]
Ocr-3			not determined	
Ocr-4		OLQ	not determined	putative: mechanosensation [10]

* No *trpv2*, *trpv6* null mouse reported up to this point.
** No heterologous expression has ever been obtained with *C. elegans* TRPV channels.
• OCR-4 has the highest degree of conservation with NAN, expressed in the mechanosensitive OLQ sensory neuron.

Drosophila TRPV channels

The full-length complementary DNAs from CG5842 and CG4536 predict a protein of 833 and 1123 amino acids, respectively, and both have five cytoplasmic ankyrin repeats in the N-terminus preceding six putative transmembrane domains and a pore region between TM5 and TM6 [6, 62]. CG5842 was named *nanchung* after its mutant phenotype, deaf (*nanchung* means 'deaf' in Korean). CG4536 was identified as Inactive. The previously known IAV mutation featured a mutant phenotype of locomotor inactivity, courtship abnormalities and altered responses to cocaine [153]. NAN is most similar to *C. elegans* OCR-4 (37% amino acid identity) whereas IAV is most similar to *C. elegans* OSM-9 (43% amino acid identity).

In situ hybridization with NAN and IAV antisense probes to *Drosophila* embryos revealed NAN and IAV expression in chordotonal neurons of the embryonic peripheral nervous system, with no detectable expression elsewhere [6, 62]. The NAN-expressing cells were visualized in vivo by fusing a 557-base pair (bp) fragment of the NAN promoter region to DNA encoding the yeast transcrip-

tion activator Gal4, and this construct was used to drive expression of green fluorescent protein (GFP) from UAS-GFP transgenic flies. Transgenic embryos carrying both constructs showed GFP expression exclusively in chordotonal neurons in keeping with in situ staining. Expression was also specific to chordotonal neurons in adults, including those in the legs, antennae and wings [6]. A promoter-driven Gal4/UAS-GFP system using a promoter region of the IAV locus confirmed chordotonal neuronal expression of IAV as well [unpublished observations].

In heterologous expression systems, CHO cells expressing NAN or IAV showed an increase in intracellular calcium concentration after superfusion with hypo-osmotic solution due to an influx of extracellular Ca^{2+} [6, 62]. Both NAN- and IAV-expressing cells were unresponsive to stimuli that can activate other TRP family members, including capsaicin, menthol and temperatures ranging from 10 to 60 °C. Whole-cell recordings showed that the NAN and IAV proteins mediate K^+ and Na^+ currents with similar levels of permeability (P_K/P_{Na} = 0.84 and 0.85 for NAN and IAV, respectively). The permeability of IAV to Ca^{2+} is slightly higher than to Na^+ (P_{Ca}/P_{Na} = 2.79 for IAV).

nan mutant flies (null allele) are viable, but show some proprioceptive defects [6]. When tapped to the bottom of their culture vial, *nan* flies promptly climb up the walls, showing that the flies are intact in geotaxis. However, their climbing ability is impaired when compared with w.t. flies, suggesting a proprioceptive defect. A few *nan* mutant flies fall off the wall when climbing, and many fall off when they reach the top, suggesting that they are not able to properly measure the distance to touch the top surface. After they fall off they are slower to right themselves. These phenotypes are also observed in *iav* mutants and other deaf mutants [134]. Notably, antennal amputation of w.t. flies leads to a locomotor-phenotype similar to that found in *nan* or *iav* mutant flies, implying that the locus of the locomotor defect resides in the antenna. The antennal sound-evoked potentials were completely absent in *nan* and *iav* [6, 62]. Rescue experiments using chordotonal expression of w.t. NAN cDNA or a genomic DNA fragment harboring the complete w.t. *iav* gene restored sound-evoked potentials and full locomotor activity to *nan* and *iav* mutants, respectively. The *nan* mutation does not lead to antennal structural defects that could be related to the mutant phenotype because ultrastructural examination of *nan* mutant antennae did not reveal any abnormality.

NAN-IAV heteromer

NAN and IAV are localized to the same site in the cilia (fig. 3) [6, 62]. As stated, both *nan* and *iav* mutants are completely deaf, indicating that a NAN homomer – provided it exists – in *iav* mutants is not functional in auditory transduction. By similar reasoning, IAV homomers – provided they exist – in *nan* mutants are not functional. As an alternative explanation, their stability or localization may depend on each other. These two possibilities were tested by using anti-NAN and anti-IAV immune sera. Immunostaining of *nan* mutant antennae with anti-IAV sera showed no detectable IAV in the cilia, and conversely, immunostaining of *iav* mutant antennal sections with anti-NAN serum revealed no detectable NAN in the cilia [62]. As for endogenous IAV, the localization of an IAV-GFP fusion protein was also dependent on NAN; no ciliary GFP signal was detected in *nan* mutants containing the same IAV-GFP construct that localized to cilia in w.t. Thus, NAN and IAV are interdependent for their stability and/or localization to the cilium, indicating that NAN forms a complex with IAV. In contrast to the in vivo results, NAN or IAV promotes a hypotonically activated current when individually expressed in heterologous tissue culture systems, meaning that NAN homomers or IAV homomers may be functional in response to osmotic stimuli in transfected cells. As an alternate explanation, it is possible that TRP(V) subunits or yet other unknown proteins that are endogenously expressed in the cultured cells can form heteromeric complexes with the expressed NAN and IAV channels and stabilize it. Based on these results and deliberations, it is reasonable to assume that chordotonal neurons harbor as yet unknown quality control systems, precluding a NAN or IAV homomer from proper trafficking to the cilia. Elucidating the molecular physiology of this quality control system will prove a worthy goal for future studies.

NAN-IAV heteromer: a mechanosensitive channel mediating hearing?

Antisera against the cytoplasmic N- and C-terminal regions of NAN and IAV revealed that NAN and IAV are both localized to the sensory cilia of the chordotonal neurons [6, 62]. Each chordotonal cilium is interrupted at approximately two-thirds of its length by a ciliary dilation composed of a tubular array of unknown composition and function. Notably, a GFP-IAV fusion protein is located to the proximal end of the cilia and thus absent from the region beyond the ciliary dilation [62]. Given the localization of the GFP-IAV fusion protein, the question arises whether the postulated NAN-IAV heteromeric complex is a mechanosensitive transduction channel that mediates hearing in *Drosophila*. If yes, how is NAN-IAV gated? A prevailing model for mechanotranduction is direct gating [154, 155] based on studies of both mammalian inner ear hair cells and *C. elegans mec* body-touch mutants. This model tenders that the mechanosensitive channel is anchored to both the cytoskeleton and to the extracellular matrix.

Relative movement of the intracellular and/or extracellular components exerts a direct mechanical stimulation that opens the channel. If this mechanism were to apply to the chordotonal neurons, then the chordotonal mechanosensitive channel should be located at the tip of the cilia where it is in intimate proximity to a NOMPA-containing dendritic cap. If this is the case, then the NAN-IAV heteromeric complex is rather unlikely to function as auditory mechanotransducer since NAN-IAV appears to be restricted to the proximal part of the cilium so that NAN-IAV cannot interact directly with cap components. However, NAN-IAV below the detection of fluorescence microscopy for the GFP-IAV fusion protein could reside beyond the ciliary dilation**.** On the other hand, the NAN-IAV complex could very well function as an auditory transducer within the confines of an alternate model of mechanotransduction, namely that the cilia would become bent or stretched, which would lead to opening the NAN-IAV complex (fig. 3). This model would reconcile the established localization of the postulated NAN-IAV heteromeric channel by assuming that membrane stretch, such as that resulting from hypotonic stimulation in tissue culture cells transfected with NAN or IAV, would lead to a gating of NAN-IAV similar to the gating in heterologously transfected cells. In support of this model, ciliary bending has actually been observed in stimulated grasshopper femoral chordotonal organs [156]. Future studies will have to address these issues.

Concluding remarks

TRPV channels constitute one of the most exciting arenas of rapid progress in experimental biology today. Clearly, the enthusiasm that has greeted these novel molecular players on the scene has not diminished since it became apparent that temperature and hot peppers are not the only stimuli that can activate these channels. Deciphering molecular mechanisms of TRPV channel functioning is not trivial, because these channels are modulated by stimuli that pertain to cell-autonomous basic homeostasis. We have to bear this qualifier in mind when we interpret data from heterologous cellular expression systems. Also, the specificity of the stimulus needs to be controlled precisely in these systems. Yet heterologous cellular expression systems permit high-throughput screening of chemical compounds/pharmacological agents that can potentially interfere with a specific signaling pathway. The significance of data obtained from genetically altered rodents will assume a pivotal position, although we have to bear in mind that developmental compensation can 'mask' a phenotype. When using explant cultures from these animals, we need to be confident that a given *trpv* promoter is active in a certain cell, and that the investigation is performed in the presence vs. absence of the particular TRPV channel we want to learn about. Invertebrate model organisms will provide another avenue that will carry us in our march toward an increased understanding of molecular mechanisms of TRPV channel regulation. Both *Drosophila trpv* mutants are deaf, and suggestive evidence of mechanosensory involvement of several mammalian TRPV channels, as reviewed above, underscores the biological relevance of these *Drosophila* mutants. *C. elegans* harbors five TRPV channels, two of which are clearly involved in the response to osmotic and mechanical stimuli. The finding that mammalian TRPV4 can direct osmotic and mechanical, not chemical avoidance reactions in the *C. elegans* TRPV mutant *osm-9* is truly inspirational, because it demonstrates an evolutionary conservation of osmotic and mechanical sensing capability in TRPV channels across phyla that cover several hundred million years of molecular evolution. On the other hand, it underscores the general relevance of *C. elegans* and *Drosophila* TRPV channels for understanding the molecular biology of this class of channels. Moreover, a mammalian ion channel that appears relevant for the molecular understanding of pain, TRPV4, can be tested more extensively in an uncomplicated and highly versatile genetic model organism, *C. elegans*. After the widespread acceptance of the handy term 'thermo-TRPs', we believe it is time to add 'mechano-TRP' and 'osmo- TRP' to the lexicon. Last, but not least, the field of TRP channels, TRPV channels in particular, appears to exert an irresistible draw on the biomedical community in the widest sense. The reason for this appears to be the electrifying combination of yet-unraveled molecular mechanisms of ion channel functioning together with undeniable clinical-medical relevance that stretch-activates our scientific imagination and will surely drive us to exciting new pastures.

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