REVIEW

Transient receptor potential vanilloid channels functioning in transduction of osmotic stimuli

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Abstract

In signal transduction of metazoan cells, ion channels of the family of transient receptor potential (TRP) have been identified to respond to diverse external and internal stimuli, amongst them osmotic stimuli. This review will highlight findings on the TRPV subfamily, both vertebrate and invertebrate members. Out of the six mammalian TRP vanilloid (TRPV) channels, TRPV1, TRPV2, and TRPV4 were demonstrated to function in transduction of osmotic stimuli. TRPV channels have been found to function in cellular as well as systemic osmotic homeostasis in vertebrates. 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 stimuli in *C. elegans*. In a striking example of evolutionary conservation of function, mammalian TRPV4 has been found to rescue osmo- and mechanosensory deficits of the TRPV mutant strain osm-9 in *C. elegans*, despite not more than 26% orthology of the respective proteins.

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Introduction: response to osmotic stimuli – a function of TRPV ion channels, apparent since 'birth' of this subfamily

Within the transient receptor potential (TRP) superfamily of ion channels (Cosens & Manning 1969, Montell & Rubin 1989, Wong *et al.* 1989, Hardie & Minke 1992, Zhu *et al.* 1995), the TRP vanilloid (TRPV) subfamily stepped into the spotlight in 1997 (Caterina *et al.* 1997, Colbert *et al.* 1997). The spectacular finding of the capsaicin receptor TRPV1 led to subsequent research in the direction of study of responses to ligand (capsaicin), acidity, and thermal stimuli. Slightly less attention was perhaps dedicated to the other founding member, the *Caenorhabditis elegans osm-9* gene. The discovery of *osm-9* carried the suggestion with it that the TRP channels might subserve critical roles in transduction of osmotic and mechanical stimuli. Subsequently, TRPV2, TRPV3, and TRPV4 were identified by a candidate gene approach (Caterina *et al.* 1999, Kanzaki *et al.* 1999, Liedtke *et al.* 2000, Strotmann *et al.* 2000, Wissenbach *et al.* 2000, Peier *et al.* 2002, Smith *et al.* 2002, Xu *et al.* 2002). The latter strategy also led to the identification of four additional *C. elegans ocr* genes (Tobin *et al.* 2002) and two *Drosophila trpv* genes, Nanchung (NAN) and Inactive (IAV; Kim *et al.* 2003, Gong *et al.* 2004). The TRPV channels can be sub-divided into four branches by sequence comparison (see dendrogram in Fig. 1). Alluding to their function, TRPV1, TRPV2, TRPV3, and TRPV4 have been named 'thermo-TRPs'; review articles on 'thermo-TRPs' are available for interested readers (Caterina & Julius 1999, Clapham 2003, Tominaga & Caterina 2004, Caterina & Montell 2005, Patapoutian 2005). TRPV5 and TRPV6, possibly function in Ca^{2+} uptake in the kidney and intestine (Hoenderop *et al.* 1999, 2003, Peng *et al.* 1999, 2003, den Dekker *et al.* 2003). Regarding the invertebrate TRPV channel genes, one invertebrate branch includes *C. elegans* OSM-9 and *Drosophila* IAV and the other branch includes OCR-1 to OCR-4 of *C. elegans* and *Drosophila* NAN. In case heterologous expression system data were available for TRPV channels, their non-selective conductance of cations with a (slight) preference for Ca^{2+} was apparent. This means that Ca^{2+} influx through the respective TPRPV channel is the critical signaling mechanism.

This review will provide some discussion on the role of mammalian and also invertebrate TRPV channels (focus on *C. elegans*) in signal transduction in response to osmotic, and also mechanical stimuli, because these submodalities are

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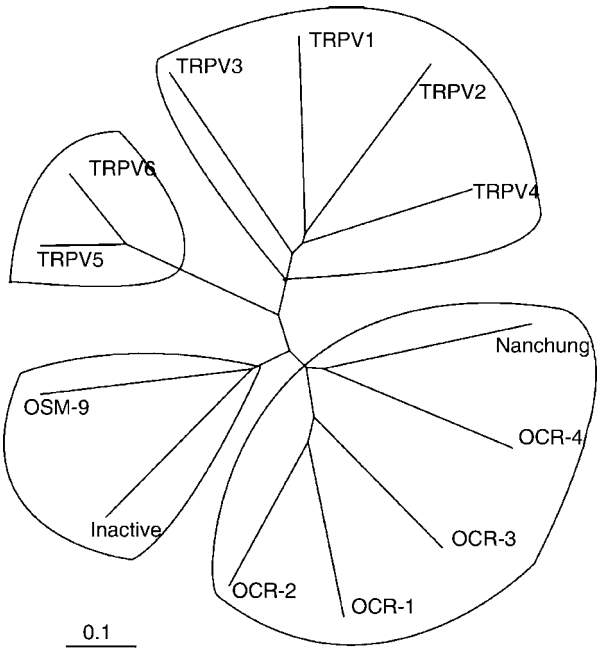


Figure 1 Dendrogram of mammalian (TRPV1–6), *Caenorhabditis elegans* (OSM-9 and OCR-1 to OCR-4), and *Drosophila melanogaster* (NAN and IAV) TRPV ion channels. From Liedtke W & Kim C (2005) Functionality of the TRPV subfamily of TRP ion channels: add mechano-TRP and osmo-TRP to the lexicon! Cellular and Molecular Life Sciences 62 2985–3001 ©Springer Reprinted with permission from Birkhäuser Basel.

related via membrane tension. These 'osmo- and mechano-TRPs' (Liedtke & Kim 2005) are TRPV1, TRPV2, TRPV4, OSM-9, OCR-2, NAN, and IAV. Other TRPV channels might join this functional group within the TRP superfamily which certainly also comprises non-TRPV channels, e.g., transient receptor potential ankyrin 1 (TRPA1; Corey 2003, Nagata *et al.* 2005) or no mechano-receptor potential mutant C (Walker *et al.* 2000). The available evidence will be summarized, gene by gene, in Table 1, guided by the question: do TRPV ion channels function in transduction of osmotic (and mechanical) stimuli, and by which molecular mechanism?

In vivo findings implicate products of the *trpv1* gene in transduction of osmotic and mechanical stimuli

In heterologous cellular expression systems, there have not been reports on transduction of osmotic and mechanical stimuli involving TRPV1. Genetically engineered *trpv1*$^{-/-}$ mice, which have previously been shown to lack thermal hyperalgesia following inflammation (Caterina *et al.* 2000, Davis *et al.* 2000), also showed an altered response of their magnocellular hypothalamic neurons to tonicity stimuli. Very recently, Reza Sharif Naeini from Charles Bourque's group reported that *trpv1*$^{-/-}$ mice failed to express an N-terminal variant of the *trpv1* gene in magnocellular neurons of the supraoptic and paraventricular nucleus of the hypothalamus (Naeini *et al.* 2006). As these neurons are known to secrete vasopressin, the *trpv1*$^{-/-}$ mice were found to have a profound impairment of antidiuretic hormone (ADH) secretion in response to systemic hypertonicity, and their magnocellular neurons did not show an appropriate bioelectrical response to hypertonicity. These findings led Bourque and colleagues to conclude that this *trpv1* N-terminal variant, which could not be identified at the molecular level, is likely involved as (part of) a tonicity sensor of intrinsically osmo-sensitive magnocellular neurons.

Table 1 Response of transient receptor potential vanilloid ion channels to osmotic (and mechanical) stimuli, and the molecular mechanism involved. A synopsis of the respective *trpv* genes covered in this review, following the ductus of the narrative

Gene	Evidence
trpv1	Loss-of-function studies *in vivo*/dissociated cells
	trpv1$^{-/-}$ mice show abnormalities in tonicity homeostasis, response to mechanical stretch and tonicity response of bladder, bowel and vessels
	Pharmacological inhibition of TRPV1 diminishes mechanical hyperalgesia
trpv2	Heterologous expression and loss-of-function studies in dissociated cells
	De novo/diminished reaction to hypotonicity and mechanical stretch
trpv4	Heterologous expression
	De novo reaction to hypotonicity and mechanical stretch
	Loss-of-function studies *in vivo*
	trpv4$^{-/-}$ mice show abnormalities in tonicity homeostasis, elevated thresholds for mechanically and osmotically induced pain
	Possible regulation of channel function by *N*-glycosylation
	Involved in volume regulation in response to hypotonic swelling
osm-9	*C. elegans* mutation with defects in avoidance of osmotic, mechanical and odorant avoidance
	Related *C. elegans* TRPV gene, *ocr-2* with identical phenotype
	Transgenic rescue by TRPV4, expression directed to one sensory neuron, of osmotic and mechanical, not odorant defects of *osm-9* mutant worms

trpv1$^{-/-}$ mice also showed an abnormal response of their bladder to stretch (Birder *et al.* 2002). TRPV1 could be localized to sensory and autonomous ganglia neurons innervating the bladder, and also to urethelial cells. When bladder and urothel-epithelial cells were cultured, their response to mechanical stretch and hypotonicity was different from wild-type controls. Specifically, the TRPV1^{+} bladders secreted ATP upon stretch and hypotonicity, which, in turn, is known to activate nerve fibers in the urinary bladder. This response to mechanical stimulation was greatly reduced in bladders excised from *trpv1*$^{-/-}$ mice. It appears likely that this mechanism, functional in mice, also plays a role in human bladder epithelium. Intravesical instillation of TRPV1 activators is used to treat hyperactive bladder in spinal cord disease (Dinis *et al.* 2004, Lazzeri *et al.* 2004, Stein *et al.* 2004, Apostolidis *et al.* 2005). Another instance of an altered response to mechanical stimuli in *trpv1*$^{-/-}$ mice relates to the response of the jejunum to stretch (Rong *et al.* 2004). Afferent jejunal nerve fibers were found to respond with decreased frequency of discharge in *trpv1*$^{-/-}$ mice when compared with wild type. In humans, in the rectum, TRPV1-positive fibers were found significantly increased in patients suffering from fecal urgency, a condition with rectal hypersensitivity in response to mechanical distension (Chan *et al.* 2003). Expression of TRPV1^{+} fibers in rectal biopsy samples from these patients was positively correlated with a decreased threshold to stretch. In addition, the occurrence of TRPV1^{+} fibers was also correlated with a dysaesthesia, described as a burning sensation by the patients. Another recent study focused on possible mechanisms of signal transduction in response to mechanical stimuli in blood vessels (Scotland *et al.* 2004). Elevation of luminal pressure in mesenterial arteries was shown to be associated with generation of 20-hydroxyeicosatetraenoic acid, which, in turn, activated TRPV1 expressed on C-fibers leading to nerve depolarization and vasoactive neuropeptide release. With respect to nociception, using *trpv1*$^{-/-}$ mice, *trpv1* was shown to be involved in inflammatory thermal hyperalgesia, but not inflammatory mechanical hyperalgesia (Caterina & Julius 1999, Gunthorpe *et al.* 2002). However, a specific blocker of TRPV1 was found to reduce mechanical hyperalgesia in rats (Pomonis *et al.* 2003). This latter result appears contradictory in view of the obvious lack of difference between *trpv1*$^{-/-}$ and wild-type control mice. This discrepancy is either due to a species difference between mouse and rat or may be due to the different mechanisms that affect signaling in a *trpv1* general knockout versus a specific temporal pharmacological blocking of TRPV1 ion channel proteins, which very likely participate in signaling multiplex protein complexes.

Taken together, loss-of-function studies using *trpv1*$^{-/-}$ mice clearly imply the *trpv1* gene as playing a significant role in transduction of osmotic and mechanical stimuli. Despite this phenotypical clarity, the details and molecular mechanisms await further investigation.

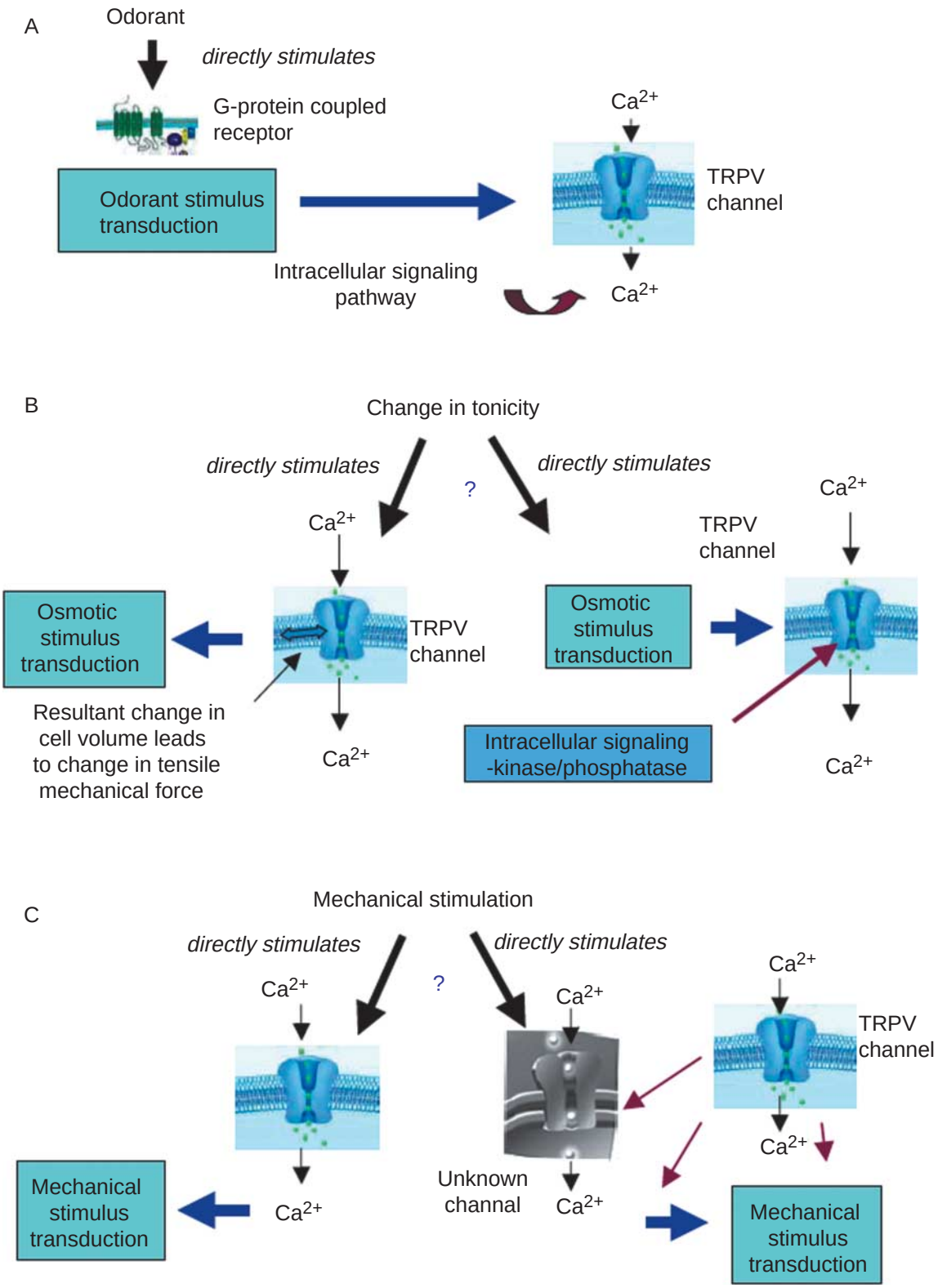
Tissue culture cell data implicate TRPV2 in osmo-mechanotransduction

In heterologous cellular expression systems, TRPV2 was initially described as a temperature-gated channel for stimuli >52 °C (Caterina *et al.* 1999). Recently, TRPV2 was also demonstrated to respond to hypotonicity and mechanical stimuli (Muraki *et al.* 2003). Arterial smooth muscle cells from various arteries expressed TRPV2. These myocytes responded to hypotonicity with Ca^{2+} influx. This activation could be reduced by specific downregulation of TRPV2 by an anti-sense method. Heterologously expressed TRPV2 in Chinese hamster ovary (CHO) cells displayed a similar response to hypotonicity. These cells were also subjected to stretch by suction of the recording pipette and by stretching the cell membrane on a mechanical stimulator. Both maneuvers led to Ca^{2+} influx that was dependent on heterologous TRPV2 expression.

In aggregate, having been discovered as a 'thermo-TRP', TRPV2 appears to be an 'osmo-mechano-TRP' as well. However, in the absence of reports on TRPV2 null mice, this grouping is based on tissue culture data.

In vivo mouse and tissue culture data implicate the *trpv4* gene to function in osmo-mechanotransduction, including hydromineral homeostasis and pain

CHO immortalized tissue culture cells responded to hypotonic solution when they were (stably) transfected with TRPV4 (Liedtke *et al.* 2000). Human embryonic kidney cell line 293, transformed by large-T antigen (HEK-293T) cells, when maintained by the same authors, were found to express *trpv4* cDNA, which was cloned from these cells. However, *trpv4* cDNA was not found in other batches of HEK-293T cells, so that this cell line was used for heterologous expression by other groups (Strotmann *et al.* 2000, Wissenbach *et al.* 2000). Notably, when comparing the two settings, it was obvious that the single-channel conductance of TRPV4 was different (Liedtke *et al.* 2000, Strotmann *et al.* 2000). This underscores the relevance of complimentary gene expression in heterologous cellular systems for the functioning of TRPV4 in response to a basic biophysical stimulation. Also, it was found that the sensitivity of TRPV4 could be modulated by warming of the media. Similar results were found in another investigation when expressing TRPV4 in HEK-293T cells (Gao *et al.* 2003), reviewed in Mutai & Heller (2003), O'Neil & Heller (2005). In addition, in this investigation, the cells were mechanically stretched (at isotonicity). At room temperature, there was no response to mechanical stress; however, at 37 °C, the response to stretch resulted in the maximum Ca^{2+} influx of all conditions. In two other investigations, heterologously expressed TRPV4 was found to be responsive to changes in temperature (Guler *et al.* 2002, Watanabe *et al.* 2002). Temperature change was



accomplished by heating the streaming bath solution. This method of applying a temperature stimulus represents a mechanical stimulus *per se*. Gating of TRPV4 was found to be amplified when hypotonic solution was used as streaming bath. In one of these investigations, temperature stimuli could not activate the TRPV4 channel in cell-detached inside-out patches (Watanabe *et al.* 2002).

In regards to maintenance of systemic osmotic pressure in live animals, *trpv4*$^{-/-}$ mice, when stressed with systemic hypertonicity, did not regulate their systemic tonicity as efficiently as did wild-type controls (Liedtke & Friedman 2003). Their drinking was reduced and systemic tonicity was significantly elevated. Continuous infusion of the ADH analog dDAVP led to systemic hypotonicity, whereas renal water readsorbtion was not changed in both genotypes. ADH synthesis in response to osmotic stimulation was reduced in *trpv4*$^{-/-}$ mice. Hypertonic stress led to reduced expression of c-FOS^{+} cells in the sensory circumventricular organ, organum vasculosum laminae terminalis (OVLT), indicating an impaired osmotic activation in this brain area lacking a functional blood–brain barrier. These findings in *trpv4*$^{-/-}$ mice point towards a deficit in central osmotic sensing. Thus, TRPV4 is necessary for the maintenance of the tonicity equilibrium in mammals. It is conceivable that TRPV4 acts as an osmotic sensor in the central nervous system (CNS). The impaired osmotic regulation in *trpv4*$^{-/-}$ mice reported differs from that published in another paper. While the author's own experiments showed that *trpv4*$^{-/-}$ mice secrete lower amounts of ADH in response to hypertonic stimuli, the results from Mizuno *et al.* (2003) suggest that there is an increased ADH response to water deprivation and subsequent systemic administration of propylene glycol. The reasons for this discrepancy are not obvious. In the author's investigation, a blunted ADH response and diminished cFOS response in the OVLT of *trpv4*$^{-/-}$ mice upon systemic hypertonicity suggests, as one possibility, an activation of TRPV4^{+} sensory cells in the OVLT by hypertonicity. These data imply that the *trpv4* gene plays a significant role in the maintenance of systemic osmotic homeostasis *in vivo*, and a possible role for it in disorders of hydromineral homeostasis.

In regards to pain-related behavior in mice, Alessandri--Haber *et al.* (2005) described that hypertonic and hypotonic s.c. solution leads to pain-related behavior in wild-type mice, which is not present in *trpv4*$^{-/-}$ mice. When sensitizing nociceptors with prostaglandin E2, the pain-related responses to hypertonic and hypotonic stimulation increased in frequency, and were greatly reduced in *trpv4*$^{-/-}$ mice. The *in vivo* behavioral data for hypertonicity could not be mirrored in acutely dissociated dorsal root ganglion (DRG) neurons upon stimulation with hypertonicity and subsequent Ca^{2+} imaging, which was, on the other hand feasible for hypotonic stimulation. Taken together, this study indicates differences in the response of mice to noxious tonicity depending on the presence/absence of TRPV4. Yet at the level of a critical transducer cell, namely the DRG sensory neuron, only hypotonicity led to a rise of intracellular Ca^{2+}, which was dependent on the presence of TRPV4. These data imply that the *trpv4* gene plays a significant role in transduction of pain stimuli evoked or amplified by local changes in tonicity.

In aggregate, the *trpv4* gene functions critically in regulation of systemic tonicity and in pain transduction of noxious osmotic stimuli in mammals. Heterologous cellular expression studies imply TRPV4 to confer responsiveness to hypotonicity (both aspects also reviewed in Voets *et al.* (2002), Liedtke & Kim (2005)).

Figure 2 Signal transduction in sensory (nerve) cells in response to odorant (A), osmotic (B), and mechanical (C) stimuli. (A) The odorant activates the TRPV ion channel via a G-protein-coupled receptor mechanism. Such a mechanism is functional in the ASH sensory neuron of *C. elegans* in response to, e.g., 8-octanone, a repulsive odorant cue. Intracellular signaling cascades downstream of the G-protein-coupled receptor activate the TRPV channel, OSM-9 or OCR-2. Ca^{2+} influx through the TRPV channel serves as an amplifier mechanism, which is required for this signaling pathway to elicit the stereotypical withdrawal response. (B) This schematic represents two possibilities how tonicity signaling could function. 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, two groups have obtained data, contradictory in detail, that suggest phosphorylation of TRPV4 to be of relevance (Vriens *et al.* 2004*b*, Xu *et al.* 2003). 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, can lead to an altered mechanical tension of the cytoplasmic membrane. 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, extracellular matrix, and the lipids of the plasma membrane in direct vicinity to the channel proteins has to be entertained. (C) This schematic represents two possibilities how mechanotransduction could function. Here, depicted on the right-hand side, an unknown mechanotransduction channel responds directly to the mechanical stimulus with Ca^{2+} 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 possible alternative. Here, the TRPV channel functions as the mechanotransducer itself, i.e., it is activated directly via mechanical stimulation. From Liedtke W & Kim C (2005) Functionality of the TRPV subfamily of TRP ion channels: add mechano-TRP and osmo-TRP to the lexicon! Cellular and Molecular Life Sciences 62 2985–3001 ©Springer Reprinted with permission from Birkhäuser Basel.

Recent developments pertaining to *trpv4* function in osmo-transduction at the cellular level: regulation of TRPV4 channels by *N*-glycosylation and their critical role in cellular volume regulation

Another recent focus in the field of TRP ion channels is intracellular trafficking, post-translational modification and subsequent functional modulation. For TRPV4, it was reported in heterologous cells (HEK-293T) that *N*-glycosylation between transmembrane-domain 5 and pore-loop homeostasis (position 651) decreases osmotic activation via decreased plasma membrane insertion (Xu *et al.* 2006). Interestingly, *N*-glycosylation between transmembrane domains 1 and 2 had a homeostasis similar effect on TRPV5, and the anti-ageing hormone KLOTHO could function as β-glucuronidase and subsequently activate TRPV5 (Chang *et al.* 2005). Thus, it appears feasible that KLOTHO or related, KLOTHO-like hormones function as β-glucuronidases regulating plasma membrane insertion of TRPV4. How critical this mechanism is *in vivo*, remains to be determined.

TRPV4 also has been found to play a role in maintenance of cellular osmotic homeostasis. One particular cellular defense mechanism of tonicity homeostasis is regulatory volume change, namely regulatory volume decrease (RVD) in response to hypotonicity. In a recent paper, Bereiter-Hahn's group demonstrated that CHO immortalized tissue culture cells have a poor RVD which, after transfection with TRPV4, improved strikingly (Becker *et al.* 2005). In yet another study, Miguel Valverde's group published that TRPV4 mediates the cell-swelling induced Ca^{2+} influx into bronchial epithelial cells that triggers RVD via Ca^{2+}-dependent potassium ion channels (Arniges *et al.* 2004). This cell swelling response did not function in cystic fibrosis transmembrane resistance (CFTR) bronchial epithelia, where, on the other hand, TRPV4 could be activated by 4-β-PDD, leading to Ca^{2+} influx. This indicates that TRPV4 is downstream of the signaling step that is genetically defective in cystic fibrosis, the CFTR chloride conductance. These findings raise the intriguing possibility that activation of TRPV4 could be used therapeutically in cystic fibrosis. Yet in another recent investigation, Ambudkar and colleagues found the concerted interaction of the water channel aquaporin-5 (AQP-5) with TRPV4 in hypotonic swelling-induced RVD of salivary gland epithelia (Liu *et al.* 2006). These findings shed light on molecular mechanisms operative in secretory organs that secrete watery fluids. This basic physiological mechanism appears to be maintained by a concerted interaction of TRPV4 and AQP-5, which was found to be dependent on the cytoskeleton (for interaction AQP-5–TRPV4, see also Sidhaye *et al.* (2006)). In regards to volume regulation of cells in the CNS, Andrew *et al.* (2006) reported very recently on neuronal RVD in response to hypotonic stimulation in brain slice culture. Perplexingly, the neurons were resistant to changes in tonicity, yet swelled readily when deprived of oxygen–glucose or when depolarized by potassium. This investigation raises once again the unresolved question of the molecular nature of the neuronal water conductance. The behavior of the neurons appears in sharp contrast to the above AQP-5–TRPV4 interaction described for hypotonic swelling and subsequent RVD by secretory epithelial cells. Taken together, TRPV4 also plays a role in regulatory volume decrease in response to tonicity-induced cell swelling, suggested for epithelial cells in airways and exocrine glands but not in nerve cells. An exciting possibility opens up in which TRPV4 could become a translational target in cystic fibrosis.

Mammalian TRPV4 directs osmotic avoidance behavior in *C. elegans*

Cloning of the C. elegans *gene* osm-9*, the other founding member of the* trpv *gene family*

As referenced in the introduction, the *osm-9* mutant line was first reported in 1997 (Colbert *et al.* 1997). The forward genetics screen in *C. elegans* applied a confinement assay with a high-molar osmotically active substance. *osm-9* mutants did not respect this osmotic barrier, and the mutated gene was found to be a TRP channel. On closer analysis, *osm-9* mutants did not respond to aversive tonicity stimuli, they did not respond to aversive mechanical stimuli to their 'nose', and they did not respond to (aversive) odorants. The OSM-9 channel protein was found to be expressed in amphid sensory neurons, the worm's cellular substrate of exteroceptive sensing of chemical, osmotic, and mechanical stimuli. At the subcellular level, the OSM-9 channel was also expressed in the sensory cilia of the AWC and ASH sensory neurons. Bilateral laser ablation of the ASH neuron, referred by some researchers as the worms' equivalent of the trigeminal ganglion or the 'nociceptive' neuron (Bargmann & Kaplan 1998), has been shown to lead to a deficit in osmotic, nose touch, and olfactory avoidance (Kaplan & Horvitz 1993). Next, four more TRPV channels from *C. elegans* were isolated, named OCR-1 to OCR-4 (Tobin *et al.* 2002). Out of these four channels, only OCR-2 was expressed in ASH. The *ocr-2* mutant phenotype was virtually identical to the *osm-9* phenotype with respect to worm 'nociception', and there was genetic evidence that the two channels were necessary for proper intracellular trafficking of each other in sensory neurons, indicating an interaction between OSM-9 and OCR-2. When expressing the mammalian capsaicin receptor TRPV1 in the ASH sensory neurons, neither *osm-9* nor *ocr-2* mutants could be rescued, but *osm-9 ash*::*trpv1* transgenic worms displayed a strong avoidance to capsaicin, which normal worms do not respond to.

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

Next, TRPV4 was transgenically directed to ASH amphid neurons of *osm-9* mutants. Surprisingly, TRPV4 expression in *C. elegans* ASH rescued *osm-9* mutants' defects in avoidance of hypertonicity and nose touch (Liedtke *et al.* 2003). However, mammalian TRPV4 did not rescue the odorant avoidance defects of *osm-9*, suggesting that this function of TRPV channels differs between vertebrate and invertebrate. This basic finding of the rescue experiments in *osm-9 ash*::*trpv4* worms has important implications for our understanding of mechanisms of signal transduction (Fig. 2).

Proposed mechanism of TRPV4 functioning as transducer of osmotic and mechanical stimuli in C. elegans *ASH sensory neurons*

TRPV4 appeared to be integrated into the normal ASH sensory neuron signaling apparatus, since the transgene failed to rescue the respective deficits in other *C. elegans* mutants lacking in osmosensation and mechanosensation (including OCR-2, bespeaking the specificity of the observed response). A point mutation in the pore-loop of TRPV4, M680K, eliminated the rescue, indicating that TRPV4 likely functions as a transductory ion channel. In an attempt to recapitulate the properties of the mammalian channel in the avoidance behavior of the worm, it was found that 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 properties of mammalian TRPV4 than that of normal *Caenorhabditis*. TRPV4 did not rescue the odorant avoidance deficits of *osm-9* mutants. In odorant transduction, G-protein-coupled receptors function as odorant sensors, and the TRPV channel functions downstream in the signaling cascade. Moreover, TRPV4 did not function downstream of other known mutations that affect touch and osmotic avoidance in *C. elegans*.

When taken together, these findings suggest that mammalian TRPV4 was functioning as the osmotic and mechanical sensor or at least as a component of it. It should be realized that TRPV4 was expressed functionally only in ASH, a single sensory neuron, where the mammalian protein, with a similarity to OSM-9 of approximately 25%, was trafficked correctly to the ASH sensory cilia, a distance of more than 100 μm. The rescue was specific (not for mutated *ocr-2*, not by mammalian TRPV1 capsaicin receptor), and it respected genetically defined pathways.

The above OSM-9–TRPV4 study delivers stimulating points to be addressed in future investigations. Whereas TRPV4 restores responsiveness to hypertonicity in *C. elegans osm-9* mutants, it is only gated by hypo-osmotic stimuli in transfected mammalian cells. The reasons for this discrepancy are not understood. Related to this study, it was recently reported that TRPV2 could rescue one particular deficit of the *ocr-2* mutant, namely the dramatic downregulation of serotonin biosynthesis in the sensory ADF neuron, but mammalian TRPV2, unlike TRPV4 directing behavior in *osm-9*, did not complement the lack of the osmotic avoidance reaction of *ocr-2* (Zhang *et al.* 2004, Sokolchik *et al.* 2005). However, common to these two investigations is the conservation of TRPV signaling across phyla that have separated for several hundred million years of molecular evolution, despite low sequence homology.

In reference to the *Drosophila* TRPV channels, NAN and IAV, the interested reader is directed to original papers (Kim *et al.* 2003, Gong *et al.* 2004) and relevant reviews (Vriens *et al.* 2004*a*, Liedtke & Kim 2005).

Outlook for future research on TRPV channels

In regards to TRP channels, one topic for the future is the investigation of the functional significance of protein–protein interactions of TRPV ion channels with the interaction partners that are to be discovered (a particularly interesting example of protein–protein interactions of TRPV4 splice variants from airway epithelia was reported recently (Arniges *et al.* 2006), but see also Cuajungco *et al.* (2006)). In addition, there is the obvious potential for TRP channels as targets for translational efforts (Nilius *et al.* 2005), such as secretory disorders (e.g., cystic fibrosis), pain, and hydromineral homeostasis.

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