

Molecular Mechanisms of TRPV4-Mediated Neural Signaling

Wolfgang Liedtke

Duke University, Center for Translational Neuroscience, Durham North Carolina, USA

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, among them osmotic stimuli. This review highlights a specific member of the TRPV subfamily, the TRPV4 channel, initially named vanilloid-receptor related osmotically activated channel (VR-OAC) or OTRPC4. 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 *Caenorhabditis elegans*. This is an astounding finding given the <26% orthology between OSM-9 and TRPV4 proteins. Here, recent findings pertaining to TRPV4's mechano- and osmosensory function in endothelia, in the alveolar unit of the lung, and in intestinal sensory innervation are reviewed, namely, transduction of mechanical shear stress in endothelia, maintenance of alveolar integrity on the endothelial side, and intestinal mechanosensation of noxious stimuli by dorsal root ganglion sensory neurons, which can be potently sensitized to mechanical stimuli by activation of the proteinase-activated receptor 2 (PAR-2), in a strictly TRPV4-dependent manner.

Key words: TRP; TRPV; osmotic stimuli; osmotransduction; mechanical stimuli; mechanotransduction; sensory neuron; intestinal innervation; endothelia; shear stress; lung alveolar edema; proteinase-activated receptor 2 (PAR2)

Introduction: Response to Osmotic and Mechanical Stimuli by TRPV Ion Channels

Within the transient receptor potential (TRP) superfamily of ion channels,^{1,2-5} the TRPV subfamily stepped into the spotlight in 1997.^{6,7} The spectacular find of the capsaicin-receptor-TRPV1 led to burgeoning research focusing on responses to ligand (capsaicin), acidity, and thermal cues. Slightly less attention was perhaps dedicated to the other founding member, the *Caenorhabditis elegans osm-9* gene. The discovery of *osm-9* implied that TRP channels might subservise critical roles in transduction of osmotic and mechanical stimuli. Subsequently, TRPV2, -V4, and -V3 were cloned

by a candidate gene approach.⁸⁻¹⁵ The latter strategy also led to the identification of four additional *C. elegans ocr* genes¹⁶ and two *Drosophila trpv* genes, Nanchung (NAN) and Inactive (IAV).^{17,18} TRPV channels have been classified by their evolutionary relationships, using dendrograms (Fig. 1). Alluding to their function, TRPV1, -V2, -V3, and -V4 have been referred to as “thermo-TRPs” (insightful review articles provided by Refs. 19–23). TRPV5 and TRPV6 function in Ca²⁺ uptake in the kidney and intestine.²⁴⁻²⁸ In case heterologous-expression-system data were available for TRPV channels, their nonselective conductance of cations with a (slight) preference for Ca²⁺ was apparent. This indicates that Ca²⁺ influx through the respective TRPV channel is a critical signaling mechanism.

Here we will review the role of TRPV4 in signal transduction in response to osmotic

Address for correspondence: Dr. Wolfgang Liedtke, DUMC Neurology, Box 2900, Durham, NC 27710. wolfgang@neuro.duke.edu

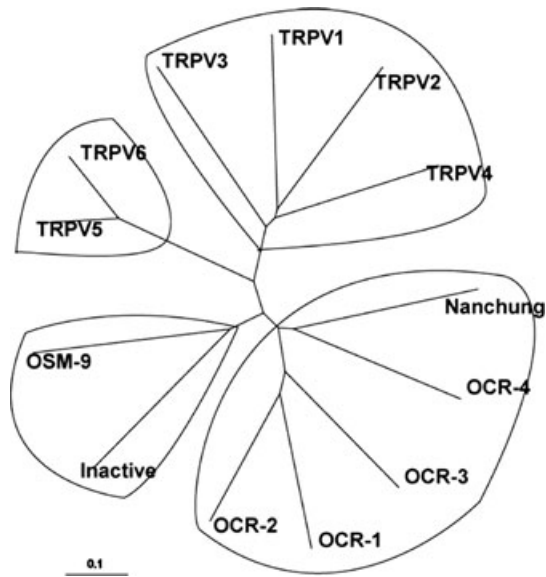


Figure 1. Dendrogram of mammalian (TRPV1–6), *Caenorhabditis elegans* (OSM-9 and OCR-1 to -4), and *Drosophila melanogaster* [Nanchung (NAN) and Inactive (IAV)] TRPV ion channels. (From Liedtke and Kim.²⁹ Reproduced with permission.)

and mechanical stimuli, because these submodalities are related via membrane tension. TRPV4 is part of a functional group of “osmomechano-TRPs,”²⁹ which comprise TRPV1, -2, -4, TRPA1, TRPM3, TRPM7, TRPC1, TRPP2, TRPML3, and the invertebrate channels OSM-9, OCR-2, NAN, NompC, and IAV.^{30–32}

Role of *trpv4* in Osmo-mechanotransduction, Including Hydromineral Homeostasis and Pain

CHO immortalized tissue culture cells responded to hypotonicity when they were (stably) transfected with TRPV4.¹³ HEK-293T cells, when maintained by the same group, were found to express *trpv4* cDNA, which could be cloned from these cells. However, *trpv4* cDNA was not found in other batches of human embryonic kidney (HEK) 293T cells, so that this cell line was used for heterologous expression

by other groups.^{14,15} Notably, when comparing the settings it became obvious that the single-channel conductance of TRPV4 was different.^{13,14} This underscores the relevance of complementary gene expression in heterologous cellular systems for the functioning of TRPV4 in response to basic biophysical cues. Also, it was found that the sensitivity of TRPV4 could be modulated by warming of the media. Similar results were found by another group when expressing TRPV4 in HEK-293T cells,³³ reviewed in Refs. 34 and 35. In addition, in the latter study, 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 Ca²⁺ influx compared to all other conditions. This finding is in keeping with the results from the original cloning study,¹³ in that mammalian TRPV4 showed its peak sensitivity in response to hypotonicity at 37°C, with strikingly declining activity above that temperature, whereas avian TRPV4 peaked at 40°C and declined in activity above 40°C. In two subsequent investigations, heterologously expressed TRPV4 was found to be responsive to changes in temperature.^{36,37} Temperature cues were applied by heating of the streaming bath solution. This method of applying a temperature stimulus represents a mechanical stimulus per se via shear stress of streaming flow. Gating of TRPV4 was found to be augmented when hypotonic solution was used as streaming bath. In one of the studies, temperature stimuli could not activate the TRPV4 channel in cell-detached inside-out patches.³⁷

In regard to maintenance of systemic osmotic pressure in live animals, *trpv4*^{-/-} mice, when exposed to systemic anisotonicity, did not regulate their osmotic equilibrium as efficiently as did wild-type (w.t.) controls.³⁸ Their drinking was reduced, and systemic tonicity was significantly elevated. At the neuroendocrine level, ADH-AVP synthesis in response to hypertonicity was diminished in *trpv4*^{-/-} mice. Hypertonic stress led to reduced expression of

nuclear c-FOS in the sensory circumventricular organ, OVLT, indicating an impaired osmotic activation in this brain area known to lack a functional blood–brain barrier. These findings in *trpv4*^{-/-} mice point toward a deficit in central osmotic sensing. Thus, TRPV4 is necessary for maintenance of tonicity equilibrium in mammals. It is conceivable that TRPV4 acts as an osmotic sensor in the CNS. The impaired osmotic regulation in *trpv4*^{-/-} mice differs from that reported 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.*³⁹ 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 c-FOS 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 the *trpv4* gene as playing a significant role in the maintenance of systemic osmotic homeostasis *in vivo*.

In regard to pain-related behavior in mice, Alessandri-Haber *et al.* described that hypertonic and hypotonic subcutaneous solution leads to pain-related behavior in w.t. mice, which is not present in *trpv4*^{-/-} mice.⁴⁰ 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 ganglia (DRG) neurons upon stimulation with hypertonicity and subsequent Ca²⁺ imaging, which was, on the other hand, feasible for hypotonic stimulation. Taken together, this study documents 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 Ca²⁺, which was dependent on TRPV4. These data imply the *trpv4* gene as playing a significant role in transduction of pain stimuli evoked or amplified by local changes in tonicity, and imply a possible role for the *trpv4* gene in pain transduction. This was reiterated by two studies. First, it was demonstrated that *trpv4* was necessary for noxious mechanical stimulation after treatment of rats with paclitaxel, a rodent pain-model of chemical deafferentiation with close similarity to human disease conditions, namely, development of a painful neuropathy after treatment with paclitaxel and other taxane drugs for breast, ovarian, testicular, and other cancers.⁴¹ Most recently, using *trpv4* null mice generated by the author, it was also revealed that *trpv4* was necessary for mechanical hyperalgesia to develop after application of “inflammatory soup,” consisting of several proalgesic mediators.⁴² These observations were recently extended to be valid for a whole set of painful-neuropathy models. Finally, Bunnett's group, also from the University of California at San Francisco (UCSF), determined that activation of the GPCR proteinase-activated-receptor-2 (PAR-2), led to mechanical hyperalgesia in mice, which was entirely dependent on TRPV4.⁴³ This is an intriguing finding, since activation of PAR-2 has been shown previously to sensitize thermal hyperalgesia via TRPV1.^{44,45} One particular innervated territory, in which this was demonstrated very recently by several groups, using *trpv4* null mice, is the large intestine, where mechanosensitivity is strongly dependent on TRPV4.⁴⁶ This also pertains to noxious mechanical stimuli, and the response can be sensitized strikingly and specifically by activation of PAR-2, for example, by granzyme, which is known to be produced in the large intestine by bacteria.⁴⁷ Moreover, PAR-2 cleaving enzymatic activity was detected in tissue isolated from patients with inflammatory bowel disease.⁴⁸

Taken together with previous studies on PAR-2–TRPV1 interactions,^{44,45} hyperalgesia to temperature is mediated specifically through TRPV1, and hyperalgesia to mechanical

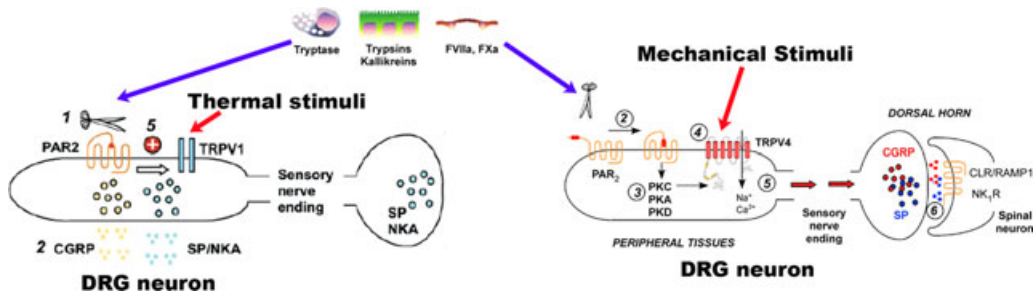


Figure 2. Schematics regarding the “tuning” of transduction of noxious stimuli by activation of PAR-2, for hyperalgesia in response to noxious *thermal* stimuli via TRPV1 (**left-hand side**), for hyperalgesia in response to noxious *mechanical* stimuli via TRPV4 (**right-hand side**). (From Grant *et al.*⁴³ and Amadesi *et al.*^{44,45} Reproduced with permission.)

stimuli is mediated specifically through TRPV4, in the case where hyperalgesia has been primed by PAR-2 signaling. This represents a novel mechanism of TRPV4 activation (Fig. 2).

In aggregate, the *trpv4* gene functions critically in regulation of systemic tonicity and in pain transduction of noxious osmotic and mechanical stimuli in mammals.

Critical Role of *trpv4* in Cellular Volume Regulation in Epithelia and Endothelia with Relevance for the Maintenance of Alveolar Integrity in Lungs

TRPV4 has also been found to play a role in the 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. Bereiter-Hahn's group demonstrated that CHO immortalized tissue culture cells have a poor RVD, which, after transfection with TRPV4, improved strikingly.⁴⁹ This finding was extended by demonstrating that human corneal keratinocytes of the eye are dependent on TRPV4 for hypotonicity-induced RVD.⁵⁰ In yet another study, Valverde's group reported that TRPV4 mediates the cell-swelling induced Ca^{2+} influx into bronchial epithelial cells that triggers RVD via Ca^{2+} -dependent

potassium ion channels.⁵¹ This cell-swelling response did not function in cystic fibrosis (CFTR) bronchial epithelia, where, on the other hand, TRPV4 could be activated by 4α -phorbol 12,13-didecanoate (4α -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. For secretory epithelia, 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.⁵² These findings shed light on molecular mechanisms operative in secretory organs that secrete watery fluids and express TRPV4 such as salivary and sweat glands. 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 actin cytoskeleton (for interaction AQP-5–TRPV4, see also Ref. 53). 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, sicca syndrome of mouth and eye, and in hyperhidrosis.

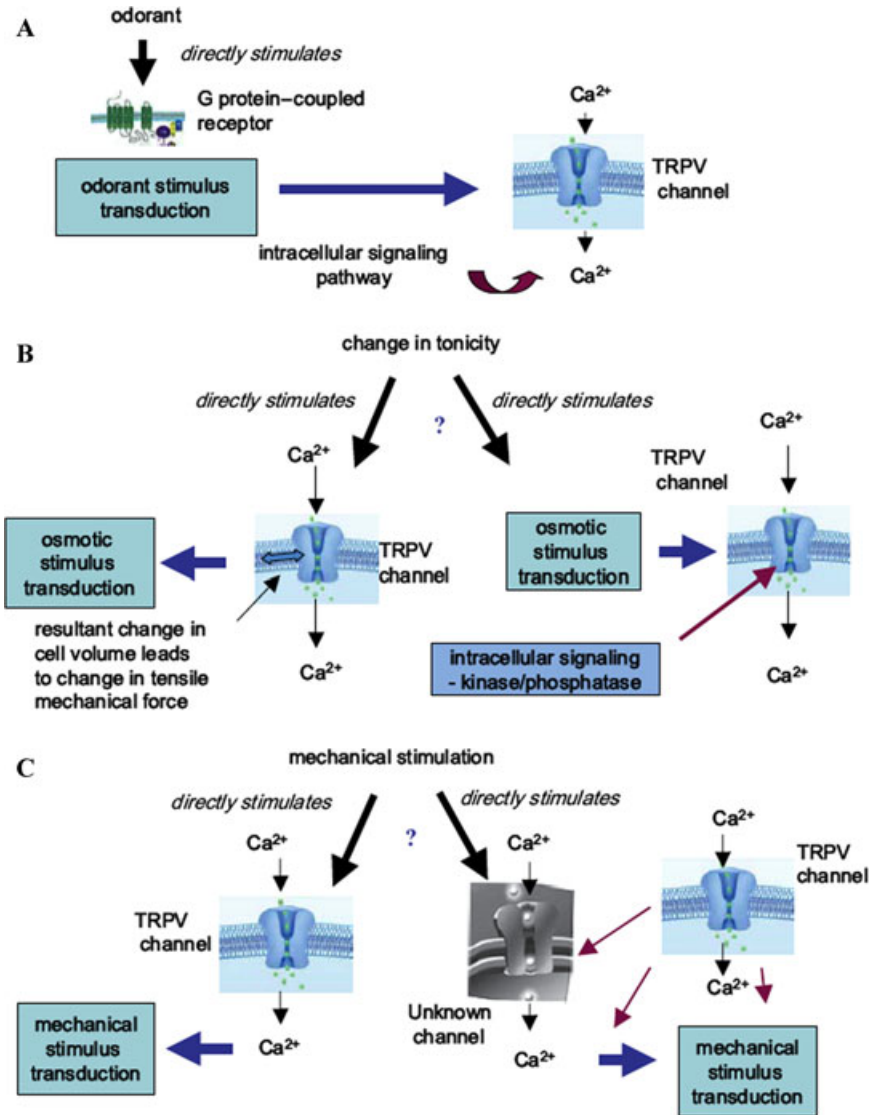


Figure 3. Signal transduction in sensory (nerve) cells in response to odorant (A), osmotic (B), and mechanical (C) stimuli. **(A)** The odorant activates the TRPV ion channel via a G protein-coupled receptor (GPCR) mechanism. Such a mechanism is functional in the (ASH) sensory neuron of *Caenorhabditis elegans* in response to, for example, 8-octanone, a repulsive odorant cue. Intracellular signaling cascades downstream of the GPCR activate the TRPV channel, OSM-9, or OCR-2. Ca²⁺ 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 of 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.^{71,72} On the left-hand side of the representation, note another scenario where the TRPV channel is at the top of the signaling cascade, that is, it is directly activated by a change in tonicity, which in turn can lead to an altered mechanical tension of the cytoplasmic membrane.

More unexpected findings have emerged from the study of airways and lungs. It was found that TRPV4 is involved in the structural integrity of the alveolar unit in a sense that activation of TRPV4 in an *ex vivo* lung model leads to alveolar leakiness, which is fully eliminated in the absence of *trpv4*.⁵⁴ In a recent follow-up paper, Townsley's group pointed out that TRPV4 can be activated in alveolar capillary endothelial cells by increased perfusion pressure, again leading to alveolar leaking, absent in *trpv4* null mice.⁵⁵ Increasing pressure from the other side of the alveolar unit, by application of increased ventilation pressure also compromised the integrity of the alveolar septal barrier, mimicking the clinical context of lung edema associated with high-pressure ventilation.⁵⁶ Again in striking similarity to the clinical situation, where fever worsens this pathophysiology, an increase in temperature increased the magnitude of alveolar leakage. All of these abnormalities were fully eliminated in *trpv4* null mice, pointing to the essential role of TRPV4 in the alveolar septal barrier in the lung. Of note, apart from chemical activation, using the synthetic compound, 4 α -PDD, TRPV4 was activated in these experiments by means of (patho-)physiologically relevant mechanical stimuli in the lung's circulation and respiratory tree. A very recent follow-up study by Kuebler's group from Berlin elaborated that TRPV4, in pulmonary capillary endothelia, is negatively regulated by cyclic guanosine monophosphate (cGMP), which exerted a limiting effect on TRPV4-mediated

Ca²⁺-influx, thus providing a negative feedback loop that functions to protect the lung microvascular barrier.⁵⁷ Alveolar endothelial cGMP could be upregulated by nitric oxide (NO). Therapeutically, this was elegantly translated into indirectly inhibiting TRPV4 by use of sildenafil, better known as "Viagra," which increased NO in pulmonary endothelia. In this study, rats with induced myocardial infarction did not suffer lung edema when treated with sildenafil.

Another study is noteworthy in the context of endothelial TRPV4 expression. TRPV4 was found to function in transduction of mechanical shear stress elicited by high-viscosity perfusate and reperfusion in an *ex vivo* carotid artery preparation, where explanted arteries from *trpv4* null mice were compared to controls.^{58,59} Absence of a response to shear stress in *trpv4* nulls was detected in a strictly endothelia-dependent fashion. In another study, TRPV4's function in endothelia was found to be dependent on caveolin-1, indicative of a functional association of TRPV4 with lipid rafts.⁶⁰

Mammalian TRPV4 Directs Osmotic Avoidance Behavior in *C. elegans*

As referenced in the introduction, the *osm-9* mutant was first reported in 1997.⁶ The forwards genetics screen in *C. elegans* applied a confinement assay with a high-molar osmotically

← 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 in the direct vicinity to the channel proteins has to be entertained. (C) This schematic represents two possibilities of how mechanotransduction could function. Here, depicted on the right-hand side, an unknown mechanotransduction channel responds directly to the mechanical stimulus with Ca²⁺ 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, that is, it is activated directly by mechanical stimulation. (From Liedtke and Kim.²⁹ Reproduced with permission.)

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 to by some researchers as the worms’ equivalent of the trigeminal ganglion or the “nociceptive” neuron,⁶¹ has been shown to lead to a deficit in osmotic, nose touch, and olfactory avoidance.⁶² Next, four more TRPV channels from *C. elegans* were isolated, named OCR-1 to -4.¹⁶ 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, implicating an interaction between OSM-9/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 capsaicin avoidance, not present in nontransgenic worms. Next, TRPV4 was directed to ASH sensory neurons of *osm-9* mutants. Surprisingly, TRPV4 expression in *C. elegans* ASH rescued *osm-9* mutants’ defects in avoidance of hypertonicity and nose touch.⁶³ 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 understand-

ing of mechanisms of signal transduction (see Fig. 3).

Proposed TRPV4 Transduction Mechanism in *osm-9 ash::trpv4* Worms

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 of 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 transducing 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, GPCRs 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. The rescue was specific (not for mutated *ocr-2*, not by mammalian TRPV1-capsaicin-receptor), and it respected genetically defined pathways.

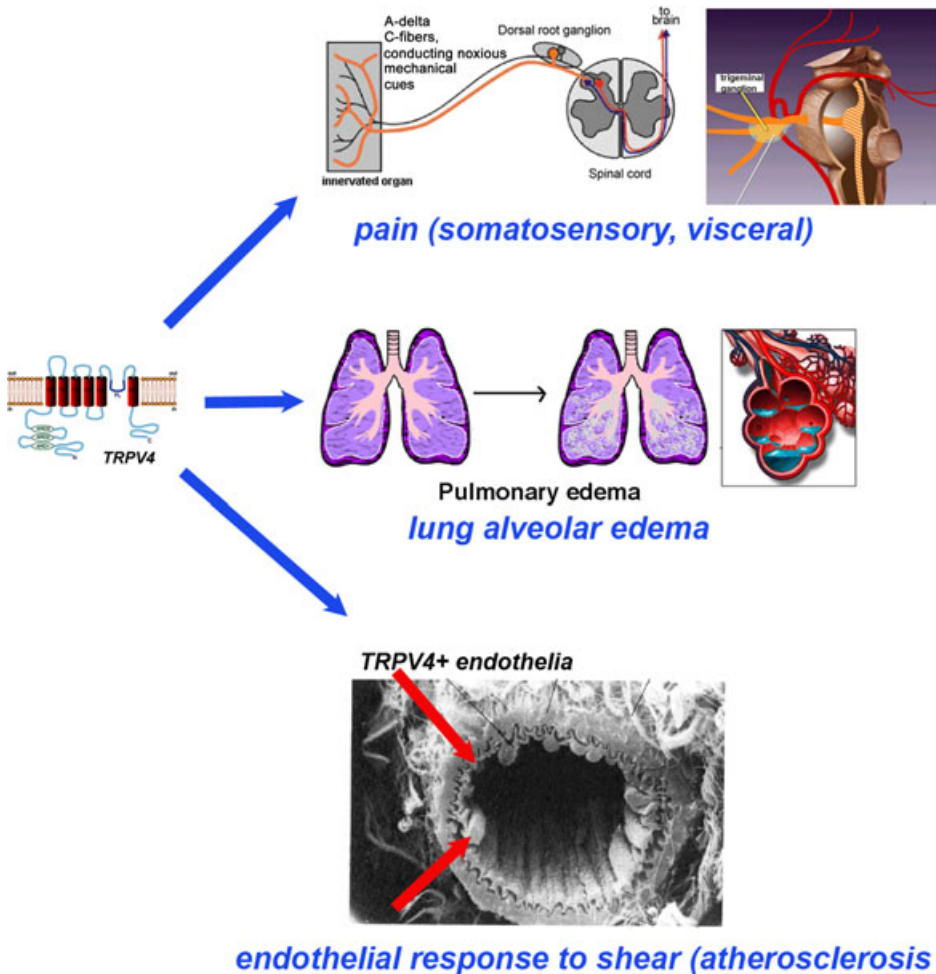


Figure 4. This schematic overview indicates TRPV4 as a possible translational target in somatosensory and visceral pain, mediated by TRPV4-expressing sensory neurons in sensory ganglia, such as dorsal root ganglion and trigeminal ganglion; in lung alveolar edema formation, where functional TRPV4 expression and TRPV4-mediated Ca^{++} influx was shown to facilitate alveolar leakiness and edema; and in endothelia, where TRPV4 was shown to respond to shear stress and re-perfusion, which could lead to pathological Ca^{++} influx as a possible foundation for development of the atherosclerotic lesion.

Outlook for Future Research on TRPV Channels

In regard to TRP channels, one topic for the future is the investigation of the functional significance of protein–protein and protein–lipid interactions of TRP(V) ion channels with to-be-discovered interaction partners (a particularly interesting example of protein–protein interactions of TRPV4 splice variants from airway epithelia was reported recently,⁶⁴ but see also re-

cent insights from Heller's and Nilius', Waltz's, and, again, Valverde's group.^{65–67}

In addition, there is the obvious potential for TRP channels as targets for translational efforts.^{68–70} Secretory disorders could be treated with TRPV4-specific activators, for example, in the case of cystic fibrosis, where such agents, generated by several pharmaceutical and biotech companies, could be used as topical preparations for inhalation. Moreover, sicca-syndrome of the eye and mouth

could be treated with TRPV4 activators. On the other hand, one would want to use (topical application of) TRPV4 blockers for the oftentimes clinically debilitating condition of hyperhidrosis. Just as experimental lung edema was successfully treated with sildenafil, presumably via NO-mediated activation of TRPV4, direct activation of TRPV4 in the pulmonary circulation of intensive care patients appears an attractive option for adjunct treatment of pulmonary alveolar edema (Fig. 4). Age-related declining hydromineral defense mechanisms could possibly be treated by TRPV4 sensitizers, for example, activation of PAR-2, although in this case, a specific topical application appears not practical. Last but not least, pain, both somatosensory and visceral, appears to be a very appealing condition for treatment with TRPV4 blockers, possibly in combination with inhibitors of cyclooxygenase and/or PAR-2 (Fig. 4). For both forms of pain, again, topical application of TRPV4 blockers can be envisioned, maybe in particular for the often debilitating visceral pain of colon, rectum, and bladder. From the vista of the author's own clinical practice, topical applications of TRPV4 blockers via foramen-ovale injections into Meckel's cave (which ensheathes the trigeminal ganglion) represent provocative, yet appealing applications of TRPV4-targeting.

Special Note

Very recent publications strongly suggest an interesting detail of TRPV4 mediated-signaling. In case the TRPV4-expressing cell bears a sensory cilium, TRPV4-expression was observed to be directed to this cellular process. Ciliary TRPV4 expression was found critical for TRPV4 activation.⁷³⁻⁷⁵ These very recent findings reflect earlier observations of TRPV4-expression in a ciliated sensory neuron in *C. elegans*.⁶³

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Conflicts of Interest

The author declares no conflicts of interest.

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