

## SYMPOSIUM REPORT

# TRPV4 plays an evolutionary conserved role in the transduction of osmotic and mechanical stimuli in live animals

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The TRPV4 ion channel, previously named vanilloid receptor-related osmotically activated channel (VR-OAC), functions *in vivo* in the transduction of osmotic and mechanical stimuli. In *trpv4* null mice, TRPV4 was found to be necessary for the maintenance of systemic osmotic equilibrium, and for normal thresholds in response to noxious mechanical stimuli. In a *Caenorhabditis elegans* TRPV mutant transgenic for mammalian TRPV4, the mammalian transgene was directing the osmotic and mechanical avoidance response in the context of the ASH 'nociceptive' neurone. Molecular mechanisms of gating of TRPV4 *in vivo* are not known at this point and have to be determined.

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## Background

What are the molecular underpinnings of signal transduction in response to osmotic and mechanical stimulation in mammals? What role does the recently discovered osmotically activated TRPV4 nonselective cation channel (Liedtke *et al.* 2000; Strotmann *et al.* 2000; Wissenbach *et al.* 2000; reviewed in (Mutai & Heller, 2003; Nilius *et al.* 2003) play in this signal transduction pathway?

## Nervous system regulation of osmotic equilibrium

To deliberate answers to the former more fundamental and to the latter more timely question, a brief discussion of the nature of the stimulus is warranted. Osmotic stimulation is a deviation from an osmotic set-point, which in many mammals is  $295 \text{ mosmol l}^{-1}$  (Denton *et al.* 1996; Bourque & Oliet, 1997). Live cells counter-regulate their volume in response to hypotonicity, which leads to fluid influx initially, as well as to hypertonicity, which leads to fluid efflux initially (Nilius *et al.* 2001; Albers & Bradley, 2004; Huang & Tunnacliffe, 2004; Okada, 2004; Strange, 2004). For a multicellular organism with an internal milieu, hypertonicity can be considered the more

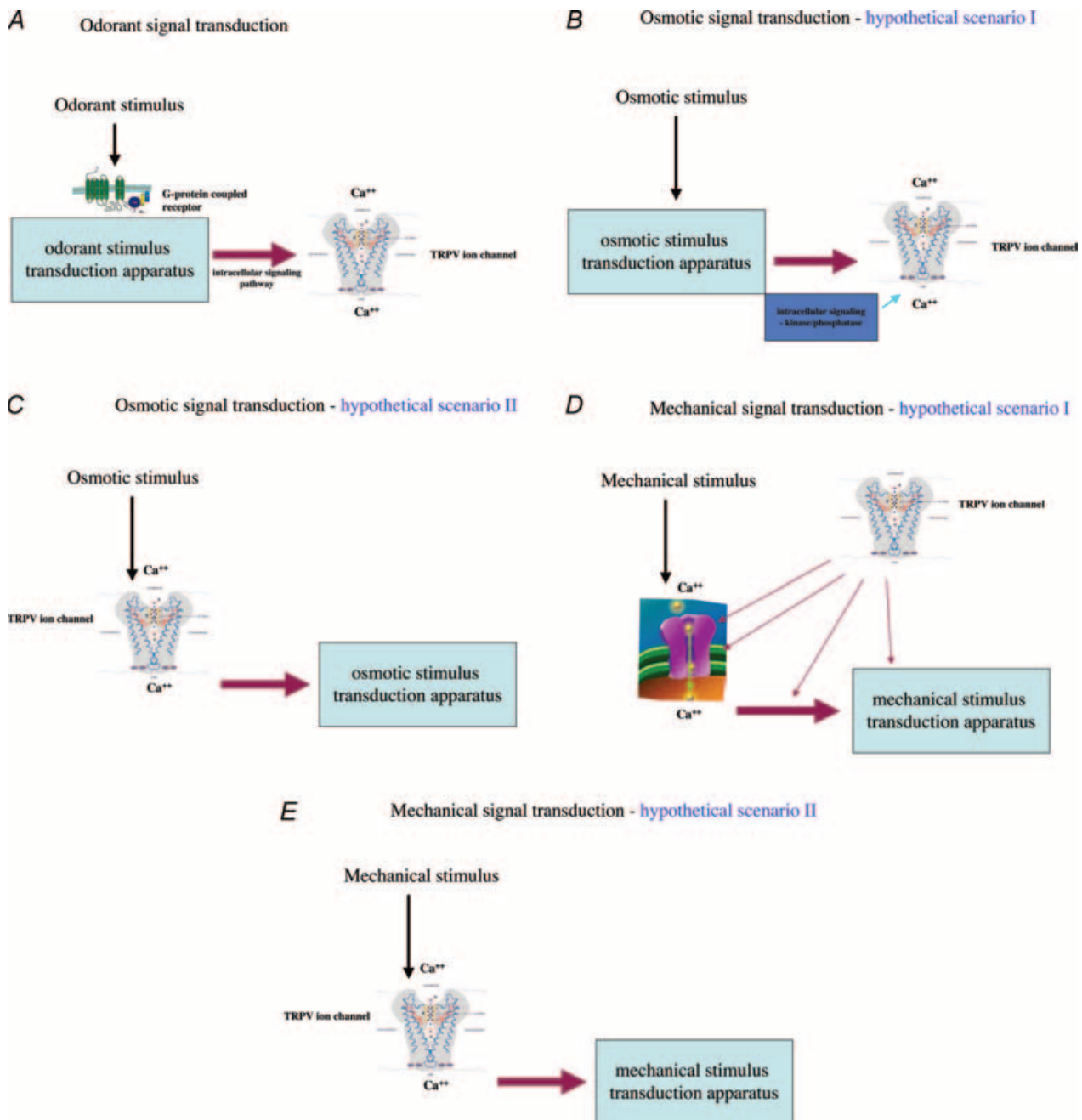
relevant stimulus because accumulation of osmolytes is an unavoidable consequence of metabolism. When confronted with a deviation from the iso-osmotic setpoint, cells respond with initial swelling or wrinkling, and subsequently with volume regulation. Mammals respond to deviations from the osmotic set-point by a complex set of measures that consist of changes in secretion of a water-saving hormone, antidiuretic hormone (ADH), also called vasopressin, and alterations in water intake (McKinley *et al.* 1992; Johnson & Thunhorst, 1995; Denton *et al.* 1996; Bourque & Oliet, 1997). 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 (McKinley *et al.* 1999). In the lamina terminalis, the sensory circumventricular organs, organum vasculosum laminae terminalis (OVLT) and subfornical organ (SFO), do not possess a blood–brain barrier (McKinley *et al.* 1989; Johnson & Loewy, 1990; McKinley *et al.* 2001). Sensory neurones relay synaptically to the supraoptic and paraventricular nucleus where antidiuretic hormone is being synthesized in magnocellular neurones (Bourque & Renaud, 1983).

## Signal transduction in response to osmotic stimulation

At the cellular level, an osmotic stimulus can be understood as a mechanical stimulus because a change in volume

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**Figure 1. Schematic drawings showing the specifics of signal transduction in sensory (nerve) cells in response to odorant (A), osmotic (B–C) and mechanical (D–E) stimuli**

A, the odorant activates the TRPV ion channel via a G protein-coupled receptor mechanism. This happens, e.g. in the ASH sensory neurone of *C. elegans* in response to 8-octanone, an aversive odorant. The TRPV channel, OSM-9 or OCR-2, is down-stream of the G protein-coupled receptor. Calcium influx through the TRPV channel is an amplification mechanism which is necessary for this signalling pathway. B, one hypothetical scenario where, analogous to A, the TRPV channel functions down-stream of an – as yet unknown – osmotic stimulus transduction apparatus. Intracellular signalling via phosphorylation (dephosphorylation)-dependent pathways activates the channel. For heterologous cellular expression systems, two groups have obtained – contradictory – data that suggest phosphorylation of TRPV4 to be of relevance (Vriens *et al.* 2003; Xu *et al.* 2003). C, another hypothetical scenario where the TRPV channel is on top of the signalling cascade. Scenario I and II 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

alters cytoplasmic membrane tension, a mechanical force parallel to this membrane. However, a change in cell volume could conceivably lead to little change in membrane tension provided the membrane is loose, possibly being involved in process-formation. From yeast cells we also know that another response to osmotic stimulation can be the specific activation of intracellular phosphorylation/dephosphorylation signalling cascades (Brewster *et al.* 1993; Brewster & Gustin, 1994; Hohmann, 2002). This also appears to be true in mammalian cells. These two basic response patterns – osmotic stimulus leading to mechanical stimulation of the cytoplasmic membrane *versus* osmotic stimulus leading to activation of intracellular phosphatase/kinase signalling cascades – need not be mutually exclusive. Whereas an osmotic stimulus can be a mechanical stimulus, a mechanical stimulus can certainly be exerted on a cell without a change in tonicity. A cell bearing processes is conceivably more readily equipped for mechanotransduction than a simply shaped cell. This prerequisite is not necessarily true for cells responding to osmotic stimuli.

#### Function of *trpv4* in vivo – gene-targeted mice

Returning to the question which roles TRPV4 plays in the response to osmotic and mechanical stimulation in live animals, let us begin with *trpv4* gene-targeted mice. *trpv4* null mice, when challenged with systemic hypertonicity, did not regulate their systemic tonicity as proficiently as wild-type littermates (Liedtke & Friedman, 2003). Their drinking was diminished, and systemic osmotic pressure was significantly elevated. Antidiuretic hormone synthesis in response to osmotic stimulation was impaired in *trpv4*<sup>-/-</sup> mice. Hypertonic stress led to diminished expression of c-FOS<sup>+</sup> cells in the circumventricular organ, OVLT, indicative of impaired osmotic activation. These findings in *trpv4*<sup>-/-</sup> mice point towards a defect in osmotic sensing in the central nervous system. Thus, TRPV4 is necessary for the maintenance of osmotic equilibrium in mammals. It is conceivable that TRPV4 acts as an osmotic sensor in the CNS. The impaired osmotic regulation in *trpv4* null mice reported in our paper 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 by Mizuno *et al.* (2003) suggest that there is an accentuated ADH response to water deprivation and subsequent systemic administration of propylene glycol

to mice (see Table). The reasons for this discrepancy are perhaps methodological differences. A blunted ADH response and diminished c-FOS response in the OVLT in *trpv4* null mice upon systemic hypertonicity suggests, as one possibility, an activation of TRPV4<sup>+</sup> cells in the OVLT by hypertonicity, which is contrary to what we observe in heterologous cellular expression systems. With respect to mechanical stimulation, thresholds for noxious mechanical somatosensory stimuli were significantly elevated in the absence of TRPV4 whereas thresholds for noxious thermal stimuli were not affected.

#### Function of *trpv4* in heterologous expression systems

When exploring the physiology of ion channels, heterologous cellular expression systems permitted fruitful investigations, e.g. for voltage- and ligand-gated channels. It is perhaps underappreciated that this concept cannot be translated seamlessly into the investigation of channels that respond to osmotic and mechanical stimuli. When applying the osmotic solution by means of streaming bath solution, one has to realize that a mechanical stimulus is coapplied to the cell, namely flow, notably so where the cell bears a process/processes. Also, it is important to consider that osmotic stimuli gating ion channels are distinctly different from specific ligands/activators such as GABA or NMDA. It can be safely assumed that most given cells harbour an innate response to osmotic stimulation and perhaps also to mechanical stimuli. With respect to the latter, methods to apply them with precision in heterologous tissue culture cells are clearly missing. Thus, 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 and mechanical, osmotic and ionic stimuli, will have to be interpreted with caution. Findings from live animals are assuming an increased significance in this respect.

#### Function of *trpv4* in vivo – *Caenorhabditis elegans*

Hence, we will now shift the focus onto another species of animal, the roundworm *C. elegans*, where a relevant discovery with respect to TRPV channels was made in 1997. At the same time that the landmark TRPV1 paper was published in *Nature*, a paper was published in the *Journal of Neuroscience* by Colbert *et al.* (1997). The

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membrane adjacent to the channel has to be considered. *D*, a hypothetical scenario *re* mechanotransduction. Here, an unknown mechanotransduction channel responds to the mechanical stimulus with calcium influx. This activity and the subsequent signal transduction are modulated by the TRPV channel. *E*, another hypothetical scenario *re* mechanotransduction. Here, the TRPV channel is the mechanotransducer itself. The data that we have obtained suggest the scenarios in *C* and *E*, whereas what happens in *A* does not happen when the OSM-9 channel is replaced with TRPV4 (Liedtke *et al.* 2003).

**Table 1. Findings pertaining to osmotic regulation and mechanosensation in *trpv4* gene targeted mice generated by Liedtke & Friedman (2003) and Mizuno *et al.* (2003)/Suzuki *et al.* (2003)**

	Liedtke & Friedman	Suzuki <i>et al.</i> /Mizuno <i>et al.</i>
k.o. strategy	<i>lox-cre</i> mediated excision of exon 12 (encodes pore)	<i>neo</i> -replacement of exon 4
Randall-Selitto	threshold elevated	nd
Automated von-Frey	threshold elevated	nd
Manual von-Frey	nd	no difference
Tail pressure (not specified)	nd	threshold elevated
Inner ear function	no pathological findings	nd
Location in trigeminal ganglion	yes, neurone +	nd
Location in DRG	yes, neurone +	yes (?); cell type not clear
Blood pressure	nd	unchanged (tail cuff)
Serum electrolytes	nd	unchanged
Urine concentration after salt load	nd	complex regulation
Plasma ADH after intraperitoneal 0.5 M NaCl challenge	decreased ADH <i>versus</i> w.t. (sampled after 30 min; systemic tonicities of 325–335 mosmol/l)	nd
Serum ADH after intraperitoneal propylene glycol challenge +48 h dehydration	nd	increased ADH <i>versus</i> w.t. (sampled after 10 s; systemic tonicities > 400 mosmol/l)
c-FOS IR lamina terminalis	decreased in <i>trpv4</i> null in the OVLT after stimulation	nd
dDAVP chronic infusion	decreased reduction of drinking and systemic hyptonicity in <i>trpv4</i> null	nd
Urine osmolality with dDAVP	> 3500 mosmol l <sup>-1</sup> in both genotypes	nd

nd – not determined; w.t. – wild type; ADH – antidiuretic hormone, vasopressin; IR – immunoreactivity.

*osm-9* mutant was found in a forwards genetics screen in *Caenorhabditis elegans* that applied a confinement assay with an 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 had sensory deficits in response to odors. The OSM-9 channel protein was expressed in amphid sensory neurones, the worm's cellular substrate for communication with the outer world. In this respect, it should be reiterated that worms are blind, and the mechanical sense refers to touch including vibration of the support, but worms also are deaf. The OSM-9 channel protein was expressed in the sensory cilia of the AWC and ASH amphid sensory neurones. Bilateral laser ablation of the ASH neurone is known to lead to a deficit in osmotic, nose touch and olfactory avoidance (Kaplan & Horvitz, 1993). ASH has thus been appropriately named the 'nociceptive' neurone (Bargmann & Kaplan, 1998). The OSM-9 protein could not be expressed in heterologous cellular expression systems, and explant cultures of ASH were not functional.

Next, mammalian TRPV4 was transgenically targeted to ASH of *osm-9* mutants. Surprisingly, TRPV4 expression in *C. elegans* ASH sensory neurones rescued *osm-9* animals' osmotic and mechanical avoidance defects (Liedtke *et al.* 2003). However, mammalian TRPV4 was unable to rescue the lack of odorant avoidance of *osm-9* mutants, suggesting

that this specific function of TRPV channels differs between vertebrates and invertebrates. TRPV4 appeared to be integrated into the normal ASH sensory neurone signalling machinery, since the transgene failed to rescue these deficits in other *C. elegans* mutants defective in osmosensation and mechanosensation (including *ocr-2*). A point mutation in the pore loop of TRPV4, M680K (Liedtke *et al.* 2003; for structure–function analysis of the predicted pore see Voets *et al.* 2002), 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, the sensitivity for osmotic and mechanical 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 worms. These data suggest that TRPV4 functions as an osmotically and mechanically gated channel, and that, in this model, TRPV4 directs the osmotic and nose-touch avoidance behaviour of the worm. It appears unlikely that these fundamental properties of the response of *osm-9 ash::trpv4* worms would resemble that 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. TRPV4 was practically expressed only in ASH, a single sensory neurone, where the mammalian protein, with a similarity

to OSM-9 of approximately 25%, was routed correctly to the ASH sensory cilium. The rescue was specific (not for *ocr-2*, not by mammalian TRPV1), and it respected genetically defined pathways for osmotic and mechanical avoidance. Thus, this approach bears a considerable impact on the understanding of the functioning of TRPV4. Figure 1 highlights current concepts for signal transduction involving TRPV channels. Based on this reasoning, stimulating questions have to be addressed. While TRPV4 restores responsiveness to hyper-osmotic aversive 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 elusive. 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 (Martinac & Hamill, 2002). One medically relevant aspect of our results is that the *C. elegans* animal model allows the exploration of TRPV4 in a genetic model organism, which is a multicellular organism with a nervous system that directs a set of behavioural responses, amongst them avoidance behaviour in response to noxious stimuli as an invertebrate model of nociception, relating to pain.

### Recent TRPV-related findings in *Caenorhabditis elegans*

Related to this investigation, it was recently reported that mammalian TRPV2 could rescue a deficit in the *ocr-2* mutant, namely the dramatic down-regulation of serotonin biosynthesis in the sensory ADF neurone (Zhang *et al.* 2004; Sokolchik *et al.* 2005). In the *osm-9 ash::trpv4* model we speculated that the lipid composition of the ASH cytoplasmic membrane might be related to the response to hyper-tonicity versus hypo-tonicity in tissue culture cells (Liedtke *et al.* 2003). In a landmark paper, published very recently, it was demonstrated that specific polyunsaturated fatty acids drove TRPV-dependent avoidance behaviour in *C. elegans*, and the molecular identity of some of these lipids was elucidated (Kahn-Kirby *et al.* 2004).

### Conclusion and outlook

Thus, almost five years after the first reports on TRPV4 (Liedtke *et al.* 2000; Strotmann *et al.* 2000; Wissenbach *et al.* 2000), it has become clear that TRPV4 functions in the transduction of osmotic and mechanical stimuli in live animals. Other physiological and pathophysiological processes appear to involve TRPV4 as well, an exciting area of research that is pursued by many distinguished investigators. However, this short review focused on the role of TRPV4 in transduction of osmotic and mechanical stimuli in live animals because it is these two submodalities where we can observe a conservation of function across

phyla, namely from worm to mammal. Incidentally, observations in worms and mammals led to the founding of the TRPV subfamily of ion channels. TRPV4 is necessary for the maintenance of osmotic equilibrium and mechanosensation thresholds in mammals, and it can drive osmotic and mechanical avoidance responses in a *C. elegans* mutant lacking one particular TRPV channel (OSM-9). The molecular mechanisms of these *in vivo* functions remain to be determined.

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