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TRPV4 as osmosensor: a transgenic approach

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Abstract The transient receptor potential vanilloid 4 (TRPV4) ion channel was named initially vanilloid-receptor-related osmotically activated channel (VR-OAC). Preliminary answers to the question, “What is the function of the *trpv4* gene in live animals?” are highlighted briefly in this review. In *trpv4* null mice, TRPV4 is necessary for the maintenance of osmotic equilibrium, and in *Caenorhabditis elegans* transgenic for mammalian TRPV4, TRPV4 directs the osmotic avoidance response in the context of the ASH “nociceptive” neuron. The molecular mechanisms of gating of TRPV4 in vivo need to be determined; in particular, whether TRPV4 in live animals is gated via phosphorylation of defined amino-acid residues or more directly through the osmotic stimulus itself.

lytes is an unavoidable consequence of metabolism. When confronted with a deviation from the iso-osmotic set-point, cells respond with initial swelling or shrinkage and, subsequently, with volume regulation. Mammalian organisms respond to deviations from the systemic 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 [3, 8, 12, 21]. Deviations from systemic tonicity are sensed in the lamina terminalis, the anterior wall of the third ventricle adjacent to the anterior hypothalamus in the central nervous system [22]. In the lamina terminalis, the sensory circumventricular organs, organum vasculosum laminae terminalis (OVLT) and subfornical organ (SFO) do not possess a blood brain barrier [11, 19, 20]. ADH is synthesized in magnocellular neurons in the supraoptic and paraventricular nucleus of the hypothalamus [4].

What are the molecular underpinnings of signal transduction in response to osmotic stimulation in vertebrate organisms? What role does the recently discovered osmotically activated TRPV4 ion channel [15, 29, 32] (reviewed in [24, 25]) play in this signal transduction?

To deliberate on answers to the former, more fundamental, and the latter, more timely question, a brief discussion of the nature of the stimulus is warranted. Osmotic stimulation is any deviation from an osmotic set-point, which in many mammals is 295 mosmol/l [3, 8]. Live cells counter-regulate their volume in response to hypotonicity, which leads initially to fluid influx, as well as to hypertonicity, which leads initially to fluid efflux [1, 10, 26, 28]. For a multicellular organism with an internal milieu, hypertonicity can be considered the more relevant stimulus because accumulation of osmo-

At the cellular level, an osmotic stimulus can be understood as a mechanical stimulus, because a change in volume alters cell membrane tension, a mechanical force parallel to this membrane. However, under circumstances in which the membrane is not stretched, e.g. during involvement in process-formation, a change in cell volume need not necessarily lead to a major change in membrane tension. From yeast cells we also know that another response to osmotic stimulation can be the specific activation of intracellular phosphorylation/dephosphorylation signalling cascades [5, 6, 9]. This also appears to be true in mammalian cells. These two basic response patterns—osmotic stimulus leading to mechanical stimulation of the cell membrane versus osmotic stimulus leading to activation of intracellular phosphatase/kinase signalling cascades—do not need to be mutually exclusive.

Returning to the question of the role played by TRPV4 in the response to osmotic stimulation in live animals, I will first focus on *trpv4* gene-targeted mice. *trpv4* null mice, when challenged with systemic hypertonicity, do not counter-regulate their systemic tonicity as efficiently as wild-type littermates [16, 17]. Their

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drinking is reduced and systemic osmotic pressure rises significantly. Continuous infusion of the ADH analogue desamino-Cys¹-D-Arg⁸-vasopressin (dDAVP) leads to systemic hypotonicity, whereas renal water reabsorption capacity is not impaired in either genotype. ADH synthesis in response to osmotic stimulation is impaired in *trpv4*^{-/-} mice. Hypertonic stress leads to diminished expression of c-FOS⁺ cells in the circumventricular organ, OVLT, indicative of impaired osmotic activation. These findings in *trpv4*^{-/-} 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 part of an osmotic sensor in the CNS. The impaired osmotic regulation in *trpv4* null mice reported in our study [16] differs from that in another study. While our experiments have shown that *trpv4* null mice secrete reduced amounts of ADH in response to hypertonic stimulation, the results of Mizuno et al. [23] suggest an accentuated ADH response to water deprivation and subsequent systemic administration of propylene glycol in mice. The reasons for this discrepancy are not clear, perhaps it reflects 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⁺ cells in the OVLT by hypertonicity, which is contrary to what we observe in heterologous cellular expression systems.

With regard to the latter, when exploring the physiology of ion channels, heterologous cellular expression systems have permitted the most rewarding investigations, e.g. for voltage-gated and ligand-gated channels. It is, perhaps, less well appreciated that this concept cannot be translated seamlessly into the investigation of channels that respond to osmotic stimuli. When applying the osmotic solution by means of streaming bath solution, a mechanical stimulus, i.e. flow, is co-applied to the cell, and may be all the more effective should the cell bear a process. Also, it is important to consider that osmotic stimuli as activators of 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. Thus—and the following generalization beyond osmotic stimulation is legitimate—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, must be interpreted with caution. Findings from live animals are assuming increasing significance in this respect.

We will now focus on one particular species of animal, the roundworm *Caenorhabditis elegans*, in which a relevant discovery with respect to TRPV channels was made almost a decade ago. At the end of 1997, about at the same time that the landmark report on TRPV1 appeared on the cover of *Nature*, another paper appeared in the *Journal of Neuroscience* “OSM-9, a novel protein

with structural similarity to channels, is required for olfaction, mechanosensation and olfactory adaptation in *C. elegans*” [7]. The *osm-9* mutant was found in a forwards genetics screen in *C. elegans* that applied a confinement assay with an osmotically active substance. *osm-9* mutants do not respect this barrier, and the mutated gene is a TRP-related ion channel. On closer analysis, *osm-9* mutants do not respond to aversive osmotic stimuli nor to mechanical stimuli applied to their anterior end (“wormnose”), and they display sensory deficits in their response to odorant cues. The OSM-9 channel protein is 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 blind, and the mechanical sense refers to touch including vibration of the supporting media, but worms also cannot hear. The OSM-9 channel protein is 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 [14]. ASH has thus been named appropriately the “nociceptive” neuron [2]. The OSM-9 protein can not be expressed in heterologous cellular expression systems, and explant cultures of amphid sensory neurons are not functional.

Mammalian TRPV4 was then targeted transgenically to the ASH neurons of *osm-9* mutants. Surprisingly, TRPV4 expression in *C. elegans* ASH sensory neurons rescues *osm-9* animals’ defects in the avoidance of hyperosmotic stimuli and nose-touch [16, 17]. In contrast, mammalian TRPV4 is unable to rescue the odorant avoidance defect of *osm-9* mutants, suggesting that this specific function of TRPV channels differs between vertebrates and invertebrates. TRPV4 appears to be integrated in the normal ASH sensory neuron signalling machinery, since the transgene fails 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, markedly reduces complementation, indicating that TRPV4 very probably 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 stimuli and the effect of temperature on the avoidance responses of *osm-9ash::trpv4* worms more closely resemble the known functional properties of mammalian TRPV4 than that of normal worms. These data suggest that TRPV4 functions as an osmotically gated channel and that, in this model, TRPV4 directs the osmotic avoidance behaviour of the worm. It appears unlikely that these fundamental properties of the response of *osm-9ash::trpv4* worms would resemble that of mammalian TRPV4, if the latter were downstream from the sensor. Moreover, TRPV4 does not complement the odorant avoidance deficit of *osm 9* worms, in which G protein-coupled receptors function as the sensors. In aggregate, these data and considerations suggest that mammalian

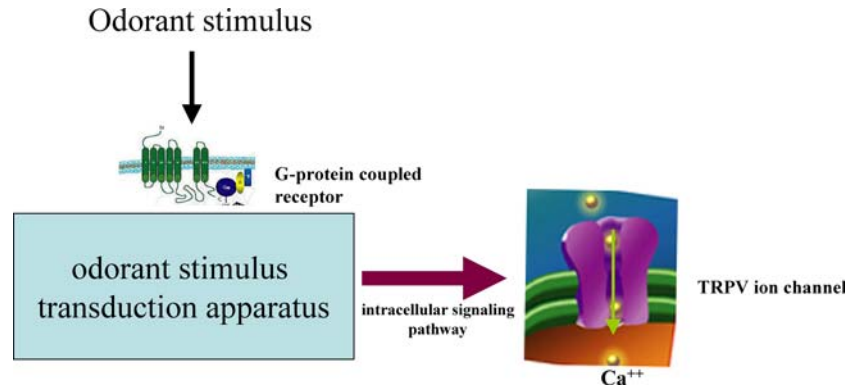


Fig. 1 Scheme indicating the specifics of signal transduction in sensory (nerve) cells in response to odorant. The odorant cue activated the transient receptor potential vanilloid (*TRPV*) ion channel via a G protein-coupled receptor mechanism. This happens

in the ASH sensory neuron of *Caenorhabditis elegans* in response to 8-octanone. The TRPV channel, OSM-9 or OCR-2, is down-stream of the G-Protein coupled receptor. Calcium influx is an amplification mechanism which is vital for this signalling pathway

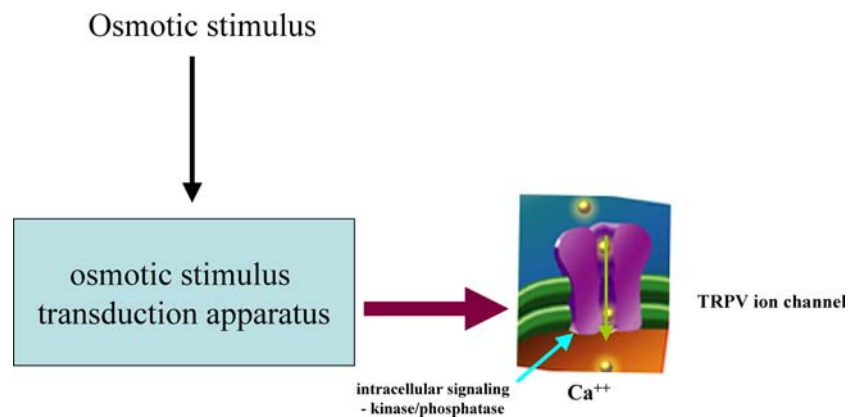


Fig. 2 Scheme indicating the specifics of signal transduction in sensory (nerve) cells in response to osmotic stimuli. This drawing represents one hypothetical scenario (I) in which, analogous to Fig. 1, the TRPV channel functions down-stream of a—yet unknown—osmotic stimulus transduction apparatus. Intracellular

signalling via phosphorylation (de-phosphorylation) dependent pathways activates the channel. For heterologous cellular expression systems, two groups have obtained contradictory data that suggest phosphorylation of TRPV4 is of relevance [30, 33]

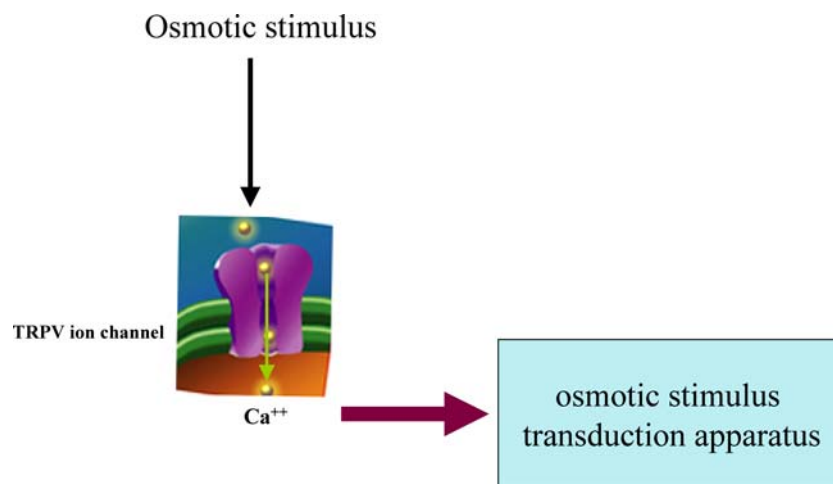


Fig. 3 Scheme indicating the specifics of signal transduction in sensory (nerve) cells in response to osmotic stimuli. This drawing represents another hypothetical scenario (II) where the TRPV channel is at the start of the signalling cascade. Scenarios I and II need not be mutually exclusive. Apart from phosphorylation of the

TRPV channel, which could possibly be of relevance in vivo, we also have to bear in mind the possible significance of 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

TRPV4 functions as the osmotic and mechanical sensor, or at least as a component thereof. It should be reiterated that TRPV4 was expressed practically only in ASH, a single sensory neuron, in which 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 *ocr-2*, not by mammalian TRPV1), and it respected genetically defined pathways for osmotic avoidance. Thus, this approach has considerable impact on the understanding of the functioning of TRPV4 and on TRPV channel functioning in general. Figures 1–3 highlight current concepts for signal transduction involving TRPV channels in sensory cells in response to odorant and osmotic stimuli. In this respect, stimulating questions have to be tendered. While TRPV4 restores responsiveness to hyper-osmotic stimuli in *C. elegans osm-9* mutants, it is only gated by hypoosmotic stimuli in transfected mammalian cells. The basis for this difference is not known. One possibility has been suggested by the results of a recent study in which 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 [18]. Lastly, it should not be forgotten that this animal model allows the exploration of the medically relevant TRPV4 channel in a genetic model organism, which is nevertheless a multicellular organism with a nervous system that can execute a set of behavioural responses, amongst them avoidance behaviour in response to noxious stimuli.

Related to this investigation, it has been reported recently that mammalian TRPV2 can rescue a particular deficit in the *ocr-2* mutant, namely the dramatic down-regulation of serotonin biosynthesis in the sensory ADF neuron [27, 34]. For 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 [16, 17]. A very recent landmark paper reported that specific polyunsaturated fatty acids drive TRPV-dependent avoidance reactions in *C. elegans*, and the molecular identity of some of these lipids was defined for the first time [13].

Thus, almost 5 years after its first description [15, 29, 32], it has become clear that TRPV4 functions in the transduction of osmotic stimuli in live animals. It is necessary for the maintenance of osmotic equilibrium in mammals, and it can drive osmotic avoidance responses in *C. elegans*. The molecular mechanisms of these in-vivo functions remain to be determined. Beyond this, other in-vivo functions of the TRPV4 channel await discovery. This hope is based firmly on exciting recent observations [31].

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