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The TRPV4 channel: structure-function relationship and promiscuous gating behaviour

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Abstract Transient receptor potential (TRP) channels provide an enormous variability of Ca^{2+} influx mechanisms triggered by a wide range of stimuli. In this review, we discuss the activation properties of the Ca^{2+}- and Mg^{2+}-permeable TRP channel of the vanilloid subfamily TRPV4. This channel is activated by various physical and chemical stimuli, such as cell swelling, heat, phorbols and, probably, by endogenous ligands, which are able to induce Ca^{2+} entry. Not much is known about the regulation of this channel. We will refer only to a mechanism of Ca^{2+}-dependent inhibition of TRPV4. Possible functional roles of this channel will be correlated with its observed expression pattern. Finally, we discuss the structural determinants of TRPV4 channel function.

Keywords Ca^{2+} entry channels · TRP channels · Structure-function relationship · Mechano-sensors · Temperature sensors

Introduction

The regulation of Ca^{2+} entry in mammalian cells is one of the most important mechanisms for controlling not only short-term cell functions, such as contraction, secretion, exocytosis and sensory signal transduction, but also long-term responses such as cell growth, proliferation and cell death. Novel players regulating Ca^{2+} entry have been found recently in the still-growing family of so-called transient receptor potential (TRP) cation channels. A mutation in a *Drosophila* gene was found that resulted in impaired vision due to the lack of a specific Ca^{2+} influx pathway in the photoreceptors (the pioneering work of Montell, Hardie, Rubin, Minke and colleagues, for reviews see [7, 8, 13]). Because of its electrical phenotype, this gene was called *trp* from "transient receptor potential" and the mammalian *trp* gene-related family of membrane proteins was baptized the TRP superfamily and comprises various cation channels. More than 20 mammalian members of the TRP family have been identified. Based on their homology, the following nomenclature has been accepted for three subfamilies [16]: TRPC (C for canonical), TRPV (V for vanilloid) and TRPM (M from the tumour suppressor melastatin).

TRP membrane proteins consist of six transmembrane spanning helices (TM1–6), with cytoplasmic N- and C-termini, and a pore region between TM5 and TM6 [2, 15] (see also Fig. 2). TM4 is not positively charged. The N-termini of TRPV and TRPC, but not those of TRPM, contain multiple ankyrin-binding repeats. The C-terminal part of TM6 of TRPCs and TRPMs, but not that of TRPVs, includes the "TRP domain", a conserved stretch of 25 amino acids starting with the nearly invariant "TRP box". In addition, all TRPs have multiple regulatory and protein interaction sites. Multiple putative protein kinase A (PKA), and C (PKC) phosphorylation sites have been identified and partially tested functionally, phosphatidylinositide 3-kinase (PI3K) and Src-homology-2 (SH2)-recognition domains (YXXM motifs) have also been identified in several TRPs. Putative PDZ interaction domains have been identified in many TRPs, but have not yet been explored functionally. Also, most of the binding partners for the mammalian TRPs are not known.

An abundant variety of stimuli activate members of the various TRPV subfamilies. Vanilloid compounds such as capsaicin, the spicy compound of hot chilli peppers, but also moderate heat and protons activate TRPV1 channels, which function as an integrator of diverse painful stimuli. TRPV2 is sensitive to noxious heat and can be activated constitutively by growth factors. Heat is so far the only known activator of TRPV3 [24, 25, 34]. TRPV5 and TRPV6 do apparently not require an activating stimulus, but are tightly controlled by the membrane potential, including a voltage-dependent open-pore block by Mg^{2+} and a Ca^{2+}-dependent feedback mechanism [9, 17, 18, 20, 28]. This review focuses on a special member of the

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TRPV family, TRPV4. We discuss gating mechanisms of TRPV4 and try to link some of its functional properties to the channel structure.

TRPV4: a mechano-sensory channel?

The first description of TRPV4 [originally called osmosensitive, transient receptor potential channel-4 (OTRPC4), TRP12 or vanilloid receptor-related, osmotically activated channel (VR-OAC)] and its functional expression were linked to vertebrate mechanosensation [3, 12, 19, 26, 33]. TRPV4 is an outwardly rectifying cation channel with a single-channel conductance of approximately 60 pS at negative and 90–100 pS at positive potentials [26, 30, 32]. TRPV4 is undoubtedly activated by hypotonic cell swelling (Fig. 1A), which, however, does not necessarily mean that this channel acts as a cellular osmosensor per se. The activation mechanism of TRPV4 is clearly different from that of other swelling-

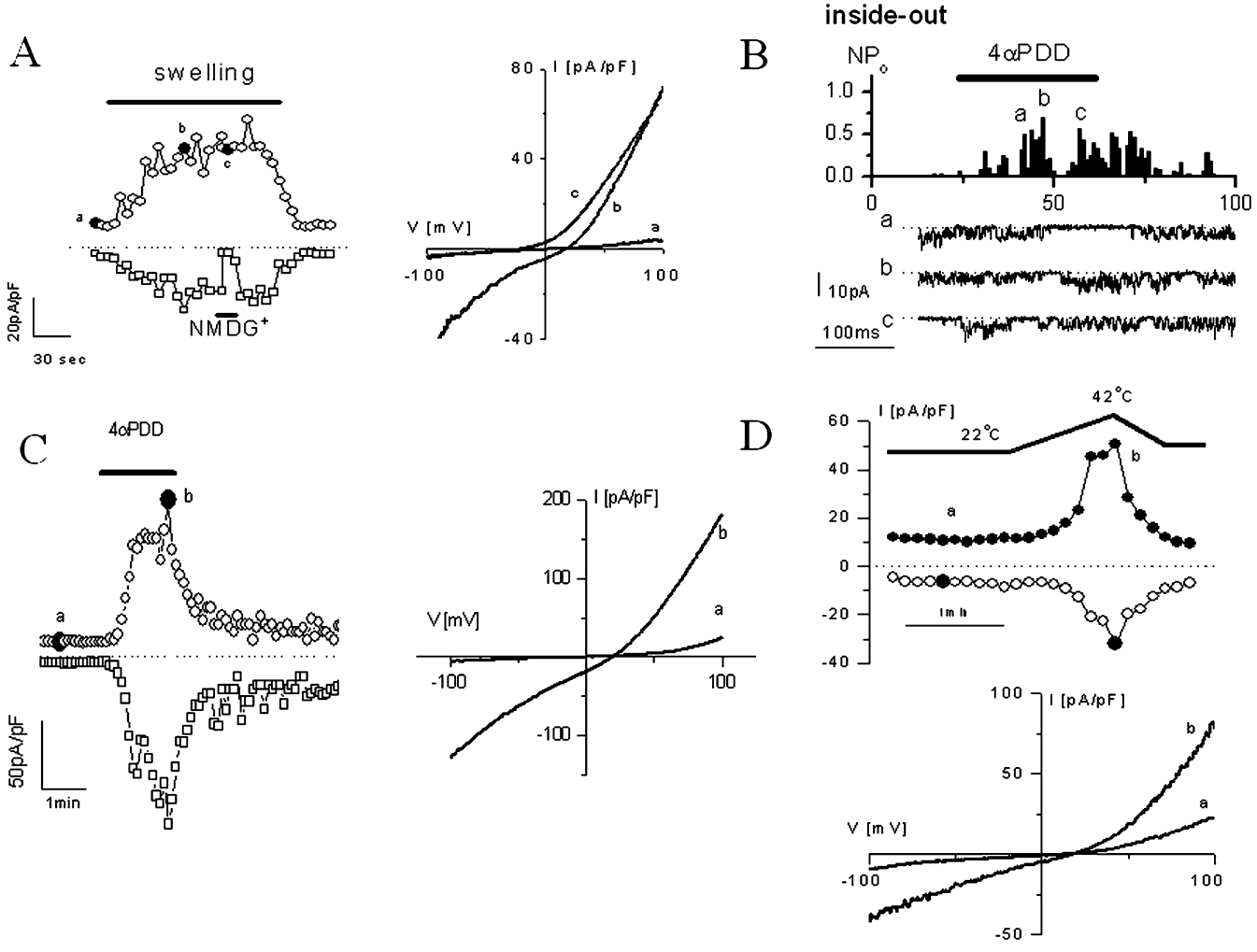

Fig. 1A–D Activation of the transient receptor potential-vanilloid channel TRPV4. **A** *Left*: cell swelling activates a cation current in TRPV4-transfected, but not in non-transfected cells (latter not shown). Dialysing the cells with a pipette solution containing 10 mM BAPTA prevents activation of the volume-regulated anion current VRAC (latter not shown). Currents, measured from voltage ramps, were monitored at +80 (*circles*) and −80 mV (*squares*). Note the disappearance of the inwards current when Na^+ is replaced by *N*-methyl-D-glucamine ($NMDG^+$). *Right*: current/voltage (*I*/*V*) relationships from the same experiment. The letters *a–c* (*closed circles*) indicate the times at which the *I*/*V* curves were measured. Note the shift of the swelling-activated current (*b*, *c*) towards positive potentials compared with the current before swelling (*a*). **B** Activation of TRPV4 in inside-out patches by application of 1 μM 4*α*-phorbol 12,13-didecanoate (*4αPDD*). This activation was never observed in non-transfected cells. Single channel conductance is ~60 pS (−60 mV). *Upper panel*: NP_o as a function of time (*N* number of channels, P_o single-channel open probability); *lower panel*: three individual recordings at times corresponding to the letters *a–c* in the *upper panel*. **C** *left*: time course of the whole-cell current activated by 4*α*PDD. Currents were measured at +80 (*closed circles*) and −80 mV (*open circles*); *right*: I/V curves at times indicated by the corresponding *letters* in the time course (holding potential 0 mV, steps from −100 to +100 mV). **D** *Upper panel*: time course of current activation by heat measured from voltage ramps at +80 (*closed circles*) and −80 mV (*open circles*). The *uppermost* trace shows the temperature. *Lower panel*: *I*/*V* curves at the times indicated by the *letters a* and *b* in the time course. Holding potential was 0 mV. Ramps were applied from −100 to +100 mV

activated channels, like the volume-regulated anion channel VRAC [19], but its expression pattern is in agreement with a possible role as osmo- or volume sensor. TRPV4 has been identified in epithelial cells of kidney tubules, in sweat glands, in the stria vascularis of the cochlea and in osmosensory cells of the circumventricular organs in the brain [3, 4, 12, 26]. TRPV4 is also expressed in heart and in vascular endothelial cells. It has been shown to function as a Ca^{2+} entry channel in native endothelium [30, 31, 33]. TRPV4 might function as a mechano-sensing Ca^{2+} entry channel in vascular endothelial cells and may even be a candidate channel for shear stress-induced Ca^{2+} entry. The distribution of TRPV4 transcripts in a variety of other tissues, like fat tissue, lung, trachea, submucosal glands, spleen, sympathetic ganglia, dorsal root and trigeminal ganglia, suggests a more polymodal role in diverse cell functions not restricted to osmosensation [1, 3, 4, 5, 12]. It is of particular interest that TRPV4 is expressed in the cochlear hair cells, the vibrissal Merkel cells that form synaptic contacts with sensory nerve endings and in sensory ganglia [12].

TRPV4: activation by ligands and modulation by Ca^{2+}

We have shown that cell swelling and heat are by far the most potent activators of TRPV4. The non-PKC-activating synthetic phorbol ester 4α-phorbol 12,13-didecanoate (4αPDD) is a very robust and potent activator of TRPV4 (Fig. 1B, C and see in detail [30]). Activation by 4αPDD is slow, suggesting that diffusion into the cell might be rate limiting. In line with an intracellular mode of action is the observation that 4αPDD, applied from the cytoplasmic side in inside-out patches, activates TRPV4. Interestingly, a very potent activator of TRPV1, phorbol-12,13-didecanoate 20-homovanillate (PDDHV), which is closely related to 4αPDD (a vanillyl moiety is present), is not able to activate TRPV4 channels in inside-out patches, possible due to its inability to bind directly to TRPV4. PDDHV is however an activator in whole-cell recordings, probably because the vanillyl moiety is cleaved under these conditions by intracellular esterases (H. Watanabe, B. Nilius, unpublished observations). Activation by 4αPDD is transient, and repetitive application results in a decreased response, indicative of desensitization.

An increase in intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) inhibits the response to 4αPDD with an IC_{50} of approximately 400 nM [31]. Similarly, inactivation of TRPV4 is very slow if activated in Ca^{2+}-free extracellular solution, but fast and nearly complete if Ca^{2+} influx via TRPV4 is present. The mechanism of TRPV4 modulation by Ca^{2+} is not yet known.

The potent activation by 4αPDD and the fact that several endogenous ligands have been described for TRPV1 suggest the existence of endogenous ligands for TRPV4. We also have obtained evidence that compounds with amphiphilic side chains, similar to the decanoic acid of 4αPDD, might activate TRPV4. Such compounds might be similar to endocannabinoids. Another candidate could be arachidonic acid (AA). Such a mechanism could nicely explain hypo-osmotic activation of TRPV4 and the observed AA-dependent increase of $[Ca^{2+}]_i$ during cell swelling [21]. Nevertheless, this is not the only mechanism for swelling-induced TRPV4 activation (see below).

TRPV4: activation by heat

TRPV4 can, like its close relatives TRPV1, 2 and 3, also be activated by heat (Fig. 1 and for details [4, 31]). However, in contrast to TRPV1, 2 and 3, which are activated above 30 °C and even at noxious temperatures, TRPV4 is activated at temperatures below 30 °C. TRPV4 responds to heat within the physiological range [31]. Activation of TRPV4 by cell swelling is modulated by heat and, vice versa, TRPV4 activation by heat can be changed by osmolarity [4, 12]. This feature might point to a change of the set-point for volume/heat activation or even to different mechanisms of activation. As reported for TRPV1 [27], heat activation of TRPV4 might also be sensitized by application of ethanol (H. Watanabe, B. Nilius, unpublished observations). Importantly, heat does not activate TRPV4 in cell-free inside-out patches, but phorbol derivatives can activate the channel in the same patch-clamp configuration (see Fig. 1B and [31]). A possible explanation might be temperature-dependent production or enhancement of a yet-unknown ligand, rather than direct activation of TRPV4 by heat. The physiological function of TRPV4 as a temperature sensor is supported by its expression in the thermosensory regions of the hypothalamus, i.e. the anterior and medial preoptic areas but also peripheral in suprabasal keratinocytes of the plantar skin in rat [4]. TRPV4 is also expressed in vascular endothelium and plays a role in temperature-dependent Ca^{2+} homeostasis [31]. We have observed that $[Ca^{2+}]_i$ is elevated at 37 °C in mouse aortic endothelial cells that express TRPV4. Such an elevation could have important consequences, e.g. for the steady-state production of NO. TRPV4 could therefore act as both a cold and a warm receptor by inducing either vasoconstriction or vasodilatation of peripheral blood vessels [14]. In addition, the temperature sensitivity of TRPV4 in vascular endothelium may play a role in mediating inflammatory pathophysiological effects in fever, e.g. by changing the endothelial barrier properties that depend on Ca^{2+} influx.

TRPV4: mapping structure-function

The availability of 4αPDD provided a very robust tool, which has been successfully used in structure-function studies of this channel. Figure 2 shows the topology of TRPV4.

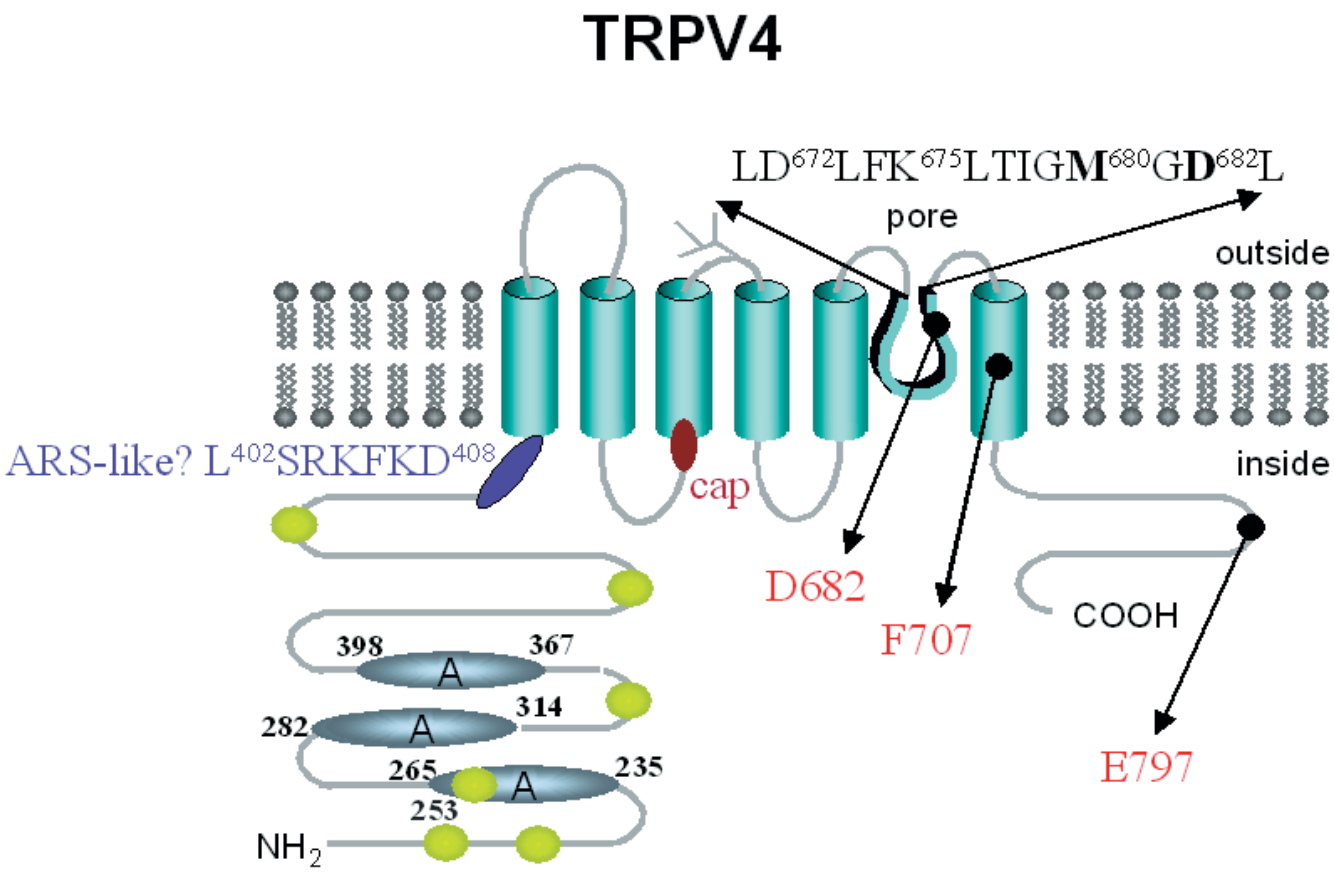

Fig. 2 A tentative structure-function scheme of TRPV4. For detailed explanation see text (*ARS* arachidonate recognition sequence, *A* ankyrin binding sequence, *cap* Tyr/Ser motif)

Pore

We first identified the functionally important determinants for Ca^{2+} permeation in the putative pore region of TRPV4. TRPV4 is a Ca^{2+} entry channel with permeability ratios for Ca^{2+}:Na^{+} of 6.9 and for Mg^{2+}:Na^{+} of 2.5. We have shown that the permeability of TRPV4 for Ca^{2+} is mainly determined by Asp^{682} at the C-terminal part of the pore and the methionine residue Met^{680} located at the centre of a putative selectivity filter [29]. The positively charged Lys^{675} and the negatively charged Asp^{672} have only a minor influence on permeation.

Ligand activation

We searched for a possible site for 4αPDD activation. Deletion of the ankyrin-binding repeats attenuated the responses to 4αPDD and heat [31], but not activation by cell swelling [12], indicating that these deletion mutants are still functional. This might indicate that cell swelling and 4αPDD act at different sites and that the ankyrin repeats might be important for forming the ligand-binding pocket. A Tyr/Ser (YS) motif in the C-terminal region of the intracellular linker between the transmembrane helix 2 and 3 ("cap" in Fig. 2) might, in analogy with TRPV1 [10], also be essential.

Activation by heat and cell swelling?

It has been recently shown that AA also potently activates TRPM2, another widely expressed Ca^{2+}-permeable channel from the TRPM branch of the TRP family. This activation was dependent on an ISXXTKE archidonate recognition sequence (ARS) [6], a sequence that was first shown to be important for AA signalling in the two-pore-domain K^{+} channel TREK-1 [22]. However, an ARS-like sequence, LSRKFKD, is present in TRPV4 but only at the C-terminal end of the N-terminus (aa402–408 in mTRPV4). Activation of TRPV4 by AA is therefore probably distinct from its action on TREK-1 and TRPM2. Interestingly, it has recently been shown that the tyrosine phosphorylation of TRPV4 induced by hypotonic cell swelling is sensitive to inhibitors of the Src family tyrosine kinases. Association of TRPV4 with Src family kinases, probably Lyn, requires an intact Src family kinase SH2 domain, since the mutation of a putative tonicity-dependent tyrosine phosphorylation site, Y253, abolishes hypotonicity-dependent channel activity. It has therefore been suggested that hypotonic stress results in

Src family tyrosine kinase-dependent tyrosine phosphorylation of a "tonicity sensor" of TRPV4 at residue Y253 [35]. However, deletion of the first ankyrin-binding repeat (aa235–265), does not impair activation of TRPV4 by cell swelling in our hands (J. Vriens, B. Nilius, unpublished observations). More experimental work seems to be necessary to identify probably different activation sites for 4αPDD, heat and cell swelling.

Modulation by Ca^{2+}

Surprisingly, a mutation in the 6th transmembrane helix delays Ca^{2+}-dependent inactivation [32]. The mechanism of TRPV inactivation by Ca^{2+} in general is not yet solved. Mutant channels, containing a single mutation in the C-terminus of TRPV4 (E797), are constitutively open. Such mutants increase $[Ca^{2+}]_i$ in non-stimulated cells. It is likely that this constitutive activation reflects a defect in Ca^{2+} inactivation. This C-terminal residue has been previously identified as part of a site for capsaicin binding on TRPV1 [11]. The summary scheme in Fig. 2 sketches some of the putative important sites of TRPV4 structure-function determinants.

Conclusion

Excitingly, TRPV4 is a channel activated by diverse stimuli, but the corresponding gating mechanisms are poorly understood. Its functional significance is probably relates to the induced Ca^{2+} influx, which integrates the various physical and chemical stimuli. TRPV4 currents can be fine-tuned by a variety of modulators, among which extra- and intracellular $[Ca^{2+}]$ play a dominant role. This polymodal function of TRPV4 makes it especially attractive as target for future research in transgenic models and native cells.

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