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Review

The negative feedback regulation of TRPV4 Ca^{2+} ion channel function by its C-terminal cytoplasmic domain

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ABSTRACT

The transient receptor potential vanilloid 4 (TRPV4) cation channel, a member of the TRP vanilloid subfamily, is expressed in a broad range of tissues where it participates in the generation of a Ca^{2+} signal and/or depolarization of the membrane potential. Regulation of the abundance of TRPV4 at the cell surface is critical in osmo- and mechanotransduction.

In this review, we discussed that the potential effect of Ca^{2+} occurs via its action at an intracellular site in the C-terminus of the channel protein by the effect of the modulation on TRPV4 (such as 824 Ser residue phosphorylation), and its regulation for TRPV4 functions related with cell surface spread, wound healing or its polarity reorientation through its differential affinity with actin or tubulin.

Contents

1. Introduction

The transient receptor potential vanilloid 4 (TRPV4) cation channel, a member of the TRP vanilloid subfamily, is expressed in a broad range of tissues, in which it contributes to the generation of a Ca^{2+} signal and/or the depolarization of membrane potential (see the reviews: [1–3]). The predicted TRPV4 structure harbors six membrane-spanning domains with a pore loop, an N-terminal domain with at least three ankyrin repeats, and a C-terminal domain residue within the cytoplasm (Fig. 1).

The participation of TRPV4 in osmo- and mechanotransduction is relevant to several important functions, including cellular and systemic volume homeostasis, arterial dilation, nociception, bladder voiding, and the regulation of ciliary beat frequency [4–8]. TRPV4 channel activity can be sensitized via the co-application of a variety of stimuli and by the participation of a number of cell signaling pathways, which suggests the presence of different regulatory sites [9,10]. In this regard, several proteins have been proposed to modulate TRPV4 subcellular localization and/or function: microtubule-associated protein 7, calmodulin, F-actin, and pacsin3 [11–16]. Other researchers have demonstrated a functional and physical interaction between inositol trisphosphate receptor 3 and TRPV4, which sensitizes the latter to the mechano- and osmotransducing messenger, 5′-6′-epoxieicosatrienoic acid [17,18]. TRPV4 is also responsive to temperature, endogenous arachidonic acid metabolites, and phorbol esters, including the 4-α phorbol 12, 13-didecanoate (4-αPDD), and participates in receptor-operated Ca^{2+} entry, thus evidencing multiple activation modes [19–22]. However, the precise manner in which TRPV4 is regulated in the cell by these protein interactions, chemicals, and stimuli remains to be clearly elucidated. In Table 1, we summarized the proteins which interact with the cytoplasmic C-terminal domain of TRPV4.

Many mammalian cell types, including renal tubule cells, bronchial epithelia, keratinocytes, spermatocytes, or erythrocytes, encounter varying osmolarities in their environment [23–27]. The ability of

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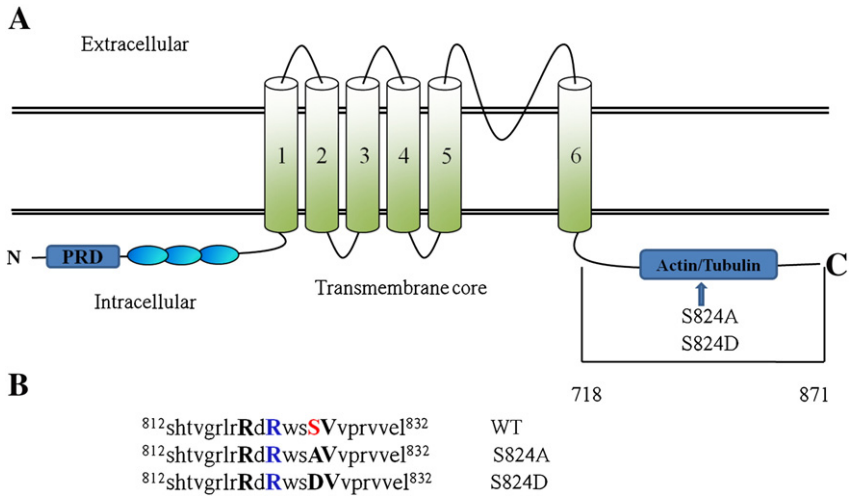

Fig. 1. The putative phosphorylation site on TRPV4 and its F-actin/tubulin binding domain. A) Transmembrane topology of the mouse TRPV4 (871aa length). Indicated are the three ankyrin-binding repeats (ANK; as gray circles), the six trans-membrane regions (TM1–TM6), the Ca^{2+}/F-actin- or microtubule-binding site (F-actin or microtubule), and the putative SGK1 phosphorylation site (S824) which is indicated by the arrow type (WT; Gene Bank # BC127052). The putative cytoplasmic region of TRPV4 (718–871aa) is also indicated by underlining. The binding proteins which interact with the region are summarized in Table 1. The putative SGK1 phosphorylation site (Ser 824) is located in the F-actin, microtubule or CaM (812–832aa) binding region of the TRPV4 channel (Table 2). The line indicates the GST C-terminal cytoplasmic region of TRPV4 (718–871aa) or mutant (S824A or S824D) fusion protein expressed in *E. coli*. With the site-directed mutagenesis, S824A and S824D were constructed and aligned the WT and SGK1 substrate-specific motif. B) The alignment of TRPV4 WT, S824A, and S824D with the consensus SGK1 substrate motif. The putative SGK1 phosphorylation site (Ser 824) of TRPV4 is located in the specific conserved SGK1 substrate [R-X-R-X-X-(S/T)Φ; Φ is a hydrophobic amino acid].

these cells to adapt to changing osmotic conditions is critical for cellular homeostasis, and disequilibria can result in dramatic events, including apoptosis and necrosis [28,29]. Therefore, a variety of cell types have evolved specialized mechanisms of volume regulation to counteract the damage induced by either cell swelling or shrinking [30,31]. Under hypotonic conditions, cells increase their volume via water uptake mediated by the osmotic gradient [32,33]. The reduction of the volume to its former value is achieved by a process referred to as regulatory volume decrease (RVD), which allows these cells to survive in a hypotonic environment [34,35]. The direct participation of TRPV4 in RVD at the cellular level and systemic osmosensing in organisms has been demonstrated [36]. Abnormal osmotic regulation in TRPV4−/− mice has also been previously reported [33,37]. Under hypotonic conditions, several researchers have demonstrated that the swelling of HaCaT keratinocytes, human airway epithelia, and TRPV4-transfected CHO cells leads to the activation of TRPV4, thereby resulting in an influx of Ca^{2+} followed by RVD [10,34,38–40]. Whereas the roles of the channels in RVD have been fairly definitively determined, the nature of the signal that leads to the initiation of RVD remains unclear.

Recently we also observed that an interaction between TRPV4 and F-actin, tubulin, or CaM regulates cell surface area in TRPV4-expressing cells [13,15]. Thus in this review, we discuss the C-terminus role of the channel protein which is related with the effect of the modulation on its regulation for TRPV4 functions (Fig. 1).

2. The regulation mechanism of TRPV4 by its C-terminal domain

Because the Ser824 residue of TRPV4 has been detected also in the consensus SGK1 substrate sequences, it was attempted to ascertain whether or not SGK1 phosphorylates the Ser 824 residue of TRPV4, as one of its specific substrate proteins [15,41]. Previously, we observed that the SGK1 mediated phosphorylation of the TRPV4 Ser 824 residue exerts a synergistic effect on its functional Ca^{2+} entry, as well as its reactivity to 4-αPDD, interactions with CaM, subcellular localization, and cell survival [13,15,41].

TRPV4 channel activation and serine phosphorylation were enhanced by exposure to the non-PKC activator, phorbol 12-myristate 13-acetate (4-αPDD), or by the application of bradykinin, which activates PKC via a G-protein-coupled mechanism or PKA [42,43]. This enhancement was inhibited by the PKC inhibitors staurosporine, BIM, and rottlerin, and by mutation of the serine/threonine residues S162, T175 and S189 [44,45]. In the lines of these reports, we also expected that TRPV4 activity enhancement by S824 phosphorylation is agonized or antagonized by the selective SGK1 activity modulators (IGF2 and wortmannin). With the treatment of wortmannin or IGF2, we observed TRPV4 WT single channel property change, expectedly [13,15,41]. However, we observed that the basal activity and sensitivity to 4-α PDD of TRPV4 S824A (an analog of unphosphorylated TRPV4 by SGK1) were higher than those of TRPV4 WT. This observation suggested that the C-terminal domain of TRPV4 near serine residue 824 seems to be assigned to regulate its function by the unknown controlling mechanism beyond the phosphorylation modification, such as the protein–protein interaction with CaM [13].

Recently our observations indicated that TRPV4 is modulated by the phosphorylation of Ser824 residue negative/positive feedback regulation loop [13,41]. In a short-term regulation loop, TRPV4 WT appears to be dually modulated by the association of regulatory proteins (such as Ca^{2+} bound CaM) on its C-terminal cytoplasmic domain. At first, the naive TRPV4 begins to open as a response to several over-threshold environmental signals (e.g. mechanical chemical temperature, osmolality). The TRPV4 is positively activated by the dissociation with regulatory proteins such as CaM, at low levels of Ca^{2+} ion concentration. However, at high Ca^{2+} ion levels by the fully active TRPV4, the channel is negatively feedback-inhibited and returned to the inactive form—these mechanisms constitute the short-term negative/positive feedback regulation loop (Fig. 2). This phenomenon also explains our observation that, after activation with 4-α PDD or heat, TRPV4 WT evidenced oscillations of Ca^{2+} ion concentration in the cytoplasm. On the other hand, after TRPV4 WT is phosphorylated on its Ser824 residue by SGK1, the prolonged active

Table 1
The binding site in TRPV4 C-terminal domain for the various interacting proteins.

Protein	Binding site (aa # in 718–871aa)	Reference
MAP7	789–809	[11]
Actin/tubulin	798–809	[14,15]
IP3 receptor	811–840	[17,18]
Calmodulin	811–830	[12,18,47–49,52]