J Pharmacol Sci **91**, 271 – 276 (2003)

Journal of Pharmacological Sciences

Current Perspective

Transient Receptor Potential Protein as a Novel Non-Voltage-Gated Ca^{2+} Entry Channel Involved in Diverse Pathophysiological Functions

Ryuji Inoue[1,*], Toyohisa Hanano[1], Juan Shi[1], Yasuo Mori[2] and Yushi Ito[1]

[1]*Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[2]*Center for Integrative Bioscience, National Institute for Physiological Sciences, Okazaki 444-8585, Japan*

Received January 22, 2003

Abstract. In both excitable and non-excitable cells, many chemical and physical stimuli elicit continuous Ca^{2+} influx through yet poorly understood pathways distinct from voltage-gated Ca^{2+} channels, leading to activation and modulation of various cellular functions. The molecular entities of these pathways have long been enigmatic, but important clues have been obtained from recent investigations on the *Drosophila* transient receptor potential (TRP) protein and its mammalian homologues. TRP proteins function as non-voltage-gated Ca^{2+} channels that are constitutively active or gated by a multitude of stimuli including light, pheromones, lipids, temperature, acid, osmolarity, and oxidative stress; and thus they may play divergent roles in cell pathophysiology. This short paper briefly overviews the current knowledge about these channels with a main focus on their possible linkage with in vivo function.

Keywords: calcium mobilization, cation channel, cell cycle, pain, phospholipid

General features of TRP channels

The transient receptor potential (TRP) proteins are a novel and rapidly expanding superfamily that encompass non-voltage-gated Ca^{2+} channels involved in long-lasting Ca^{2+} entry. Since the first identification of its archetypal member in the investigation of *Drosophila* visual transduction (dTRP, Ref. 1), the number of proteins assigned to this superfamily has increased explosively leading to subclassification into three homologous (TRPC, TRPV, and TRPM; Fig. 1) and three distantly related (TRPP, TRPML, and TRPN) subfamilies (2 – 5). The predicted membrane topology of TRP channels is six transmembrane domains flanked by variable cytosolic N- and C-termini, which contain some of several putative protein-protein interaction sites such as ankyrin-like repeats, proline-rich motifs, calmodulin /inositol-1,4,5-trisphosphate receptor (IP_3R) binding sites, PDZ binding motifs, and consensus phosphorylation motifs for protein kinases. Because of this, membrane topology, these channels are categorized into the voltage-dependent cation channel superfamily, but the positively charged amino acid residues in the fourth transmembrane segment characteristic of voltage-dependent gating are absent (1 – 4). In notable contrast to the voltage-gated Ca^{2+} channels and fast ligand-gated Ca^{2+}-permeable channels that participate in rather specialized cellular functions, the fascinating aspect of TRP proteins is their potentially ubiquitous and divergent roles in cell pathophysiology. The genes encoding TRP proteins are conserved the length of the phylogenic tree, being found from microorganisms to higher vertebrates including yeast, fly, worm (*C. elegans*), and mammals (6). Besides their relative abundance in the central nervous system, TRP proteins show broad tissue distribution; e.g., in sensory organs and cardiovascular, gastrointestinal, respiratory, urogenital, hematopoietic, and immune systems (2 – 4). There is now a growing body of evidence supporting the possibility that these proteins may mediate the visual, auditory, taste, and pain signal transductions; and they may regulate the blood pressure, gut motility, mineral absorption, body fluid balance,

*Corresponding author. FAX: +81-92-642-6079
E-mail: inouery@pharmaco.med.kyushu-u.ac.jp

Invited article

Throughout this review, a unified TRP nomenclature (TRPC*, TRPV*, and TRPM*) rather than their old names (in brackets; VR1, OTRPC4, ECaC1, CaT1, and MLSN-S, etc.) is used. For details, refer to Ref. 5.

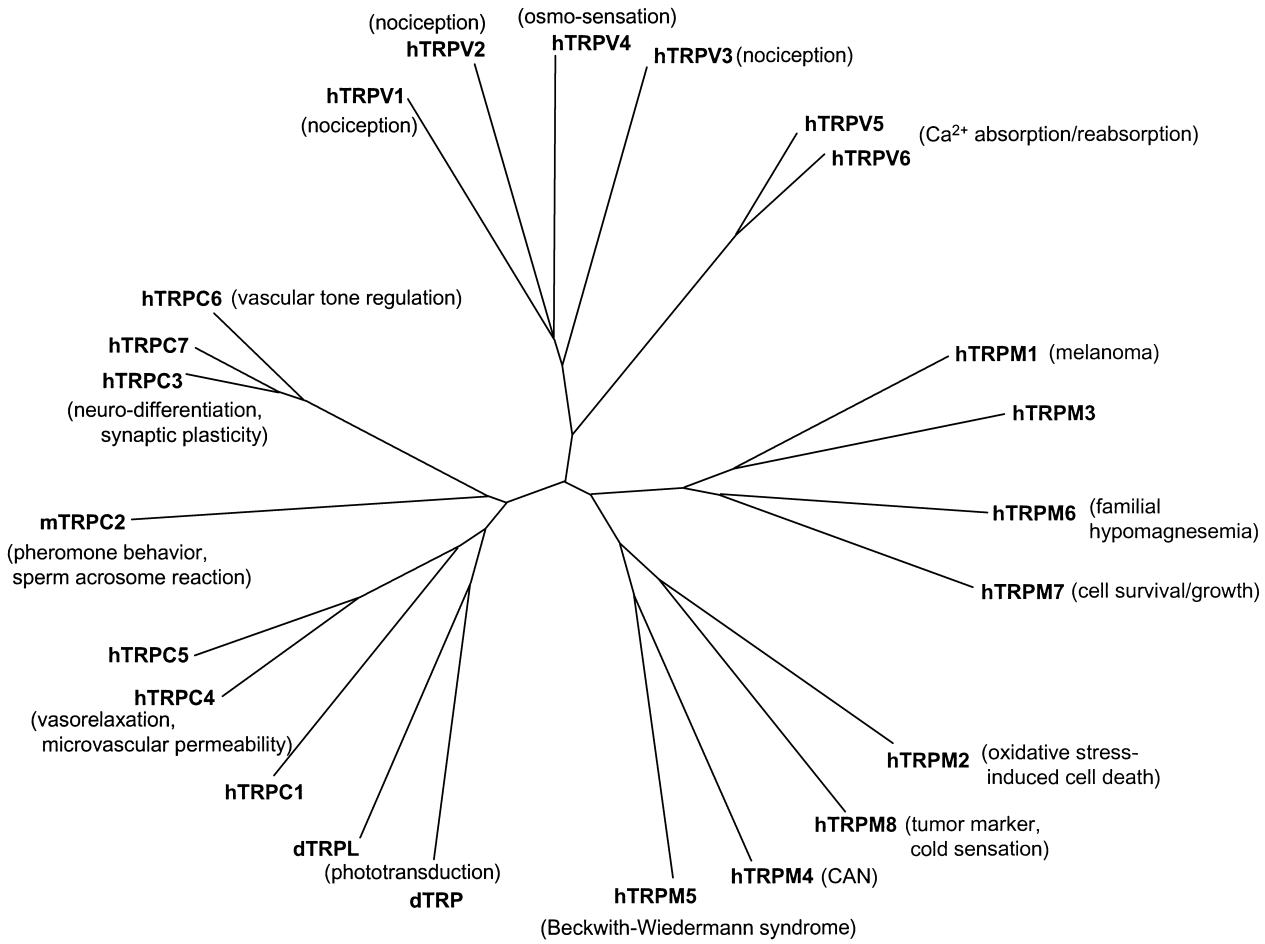

Fig. 1. Human TRP dendrogram. The results of CLUSTALW alignment of whole amino acid sequence. For TRPC2, the murine sequence was used. Postulated pathophysiological functions are shown in brackets. For further details, see the text. Affixes 'd', 'h' and 'm' denote *Drosophila*, human and mouse, respectively.

cell survival/growth/death, and pheromone behaviors (1 – 4). Such enormous functional diversity of TRP proteins may arise from their capability of sensing a wide array of extracellular and intracellular signals (cell surface receptor stimulation, temperature, acidity, mechanical stress, redox state, etc.), and from homo- and hetero-philic interactions occurring between different TRP isoforms/splice variants and other accessory proteins expressed in a cell-specific manner that are co-assembled into large signaling complexes (2 – 4).

TRPC subfamily as receptor-operated Ca^{2+} entry channels

The members of the TRPC subfamily ('C' denotes classical or canonical) are activated by stimulation of G-protein-coupled or tyrosine kinase receptors linked to phosphoinositide hydrolysis via phospholipase C (PLC), thus being an interesting experimental model or promising molecular candidates for native 'receptor-operated Ca^{2+} channels'. TRPCs can further be subdivided by their sequence homology into three subgroups, i.e., TRPC1/TRPC4/TRPC5, TRPC2, and TRPC3/TRPC6/TRPC7; and heteromultimeric association seems to occur between the subgroups to create further divergent channel properties. All TRPC isoforms possess putative calmodulin/IP_3R binding sites on the C-terminus, and in some, a site that may be associated with activation by diacylgycerol (DAG) on the N-terminus. The mechanism of activation of the TRPC members is controversial-activation (or modulation) by both store-depletion and lipid second messengers (DAG and metabolites) has been proposed (2 – 4, 6).

Recent investigations using genetically engineered mice deficient of TRPC4 have revealed the unequivocal importance of this protein in vascular endothelial function. In aortic endothelial cells of TRPC4 knock-out mice, Ca^{2+} entry evoked by store depletion (SOC) was

substantially abolished, and this was paralleled by impaired nitric oxide synthesis and selective attenuation of a component of agonist-induced vasorelaxation that is sensitive to micromolar concentration of La^{3+} and L-nitroarginine (7). In lung vascular endothelial cells of the same mice, the normal thrombin-induced increased microvascular permeability was found to be greatly attenuated and accompanied by a drastic decrease of SOC and a reduced endothelial cell retraction response with the lack of actin stress fiber formation (8). These results strongly indicate the essential role of TRPC4 as a Ca^{2+} providing pathway in regulating the vascular tone via endothelium-dependent NO production and its possible implications in pathological changes during vascular injury and inflammation. Different lines of evidence suggest that TRPC6 may also serve as a key component regulating the vascular tone and thus the blood pressure. Using the antisense knock-out strategy combined with immunohistochemical and electrophysiological techniques, two recent reports have demonstrated that TRPC6 is essential for activation of Ca^{2+}-permeable nonselective channels and consequent Ca^{2+} entry evoked by both sympathetic α_1-adrenoceptor stimulation via PLC activation (9) and intravascular pressure elevation (10).

Several interesting cues linking TRPC isoforms with other native functions have also been obtained by heterologous expression experiments and tissue distribution patterns. Recent notable examples include the involvement of TRPC3 in brain-derived nerve growth factor-induced neuronal differentiation and plasticity (3) and involvement of TRPC4 in histamine-induced exocytotic responses of chromaffin and PC12 cells (11) and gut pace-making activities of ICCs (interstitial cells of Cajal) (12).

Although non-functional in humans as a pseudogene, TRPC2 receives special attention because of its unique impact on sexual and social animal behaviours. TRPC2 mRNA and protein are enriched in the vomeronasal regions of rat and mouse that participate in pheromone sensory signaling, and targeted disruption of this gene causes the loss of sex discrimination and male-male aggression (13). TRPC2 has also been implicated in mice in the initiation of the acrosome reaction as a Ca^{2+} entry pathway activated by the contact of sperm with the egg cell surface glycoprotein ZP3 (2 – 4). Heterologous expression of a long splice variant of murine TRPC2, which is expressed in erythroid cells, produces a sustained Ca^{2+} influx in response to erythropoietin receptor stimulation, thus implying its potential role in regulating hematopoiesis (14).

TRPV subfamily as Ca^{2+} channels activated by diverse physical and chemical stimuli

The founding member of this subfamily TRPV1 (the vanilloid receptor, VR1) was originally isolated from rat sensory neurons by an expression cloning assay as a protein responsive to capsaicin, a pungent ingredient of chili pepper. Overall similarity in membrane topology and partial sequence homology particularly in the putative pore loop and the sixth transmembrane domain suggest that TRPV1 is a homologue of dTRP and TRPC channels (2 – 4, 15). TRPV1 codes for Ca^{2+}-permeable cation channels activated not only by vanilloids, but also by endogenous lipids (endocannabinoids, eicosanoids) and pathologically increased acidity (pH <approx. 5.9) and temperature (>approx. 43°C). The threshold for heat activation is lowered by decreased pH and inflammatory or algoneic agents such as bradykinin, ATP, and nerve growth factor. This occurs through protein kinase C-dependent and -independent (phosphatitidylinositol bisphosphate; MAP kinase) mechanisms. Such polymodal gating to noxious stimuli, and inflammation-induced hypersensitivity ('hyperalgia') together with the preponderant expression of the channels in small-diameter sensory neurons of dorsal root and trigeminal ganglia and the markedly reduced thermal sensitivity of TRPV1 knock-out mice, endows this protein with a central importance in peripheral nociception (15). Interestingly, recently cloned TRPV1 homologues TRPV2 (VRL-1) and TRPV3 (VRL-3) have also been shown to be thermosensitive (but vanilloid-insensitive) Ca^{2+}-permeable cation channels and may mediate different ranges of heat-evoked pain sensation (apparent threshold: approx. 52°C and 36 – 39°C, respectively). TRPV2 is preferentially expressed in medium to large sensory neurons in dorsal root ganglia (DRG), whereas TRPV3 colocalizes in DRG with TRPV1 to presumably form a heteromultimeric channel exhibiting varying sensitivities to noxious stimuli (15, 16). In addition, it has been found that in TRPV1 knockout mice, the reflex urine voiding induced by bladder distention, which is likely to be mediated by stretch-evoked ATP release from the urothelium, is greatly reduced. This observation raises the possibility that TRPV1 may also function as a mechanosensor in the urinary bladder (17).

TRPV4 (OTRPC4/VR-OAC/TRP12/VRL-2) is a mammalian homologue of the *C. elegans* chemo- and osmo-protein OSM-9, and it is activated in response to reduction in extracellular osmolarity by as little as approx. 10% and conducts both monovalent and divalent cations with a moderate preference for Ca^{2+} (P_{Ca}/P_{Na} = 6.3) (2 – 4, 18). TRPV4 is abundantly expressed in renal epithelia (especially in the distal convoluted tubule), but

also at lower levels in heart, lung, liver, spleen, testis, and many other tissues including ear hair cells, auditory ganglia, and other sensory neurons in the brain (15). Although the exact in vivo function of TRPV4 remains unclear, it could be involved as an osmo- or mechanosensor in mineral reabsoption/secretion in the kidney, regulatory volume decrease, and neurosensory functions such as hearing. A recent investigation has disclosed an unexpected feature of TRPV4 as a thermosensitive channel activated by a near-physiological range of temperature. This finding, together with its immunohistochemical localization in the anterior hypothalamus, suggests its integrative role for thermal and osmotic information in the brain (19).

TRPV5 and TRPV6 (ECaC1, ECaC2/CaT1) constitute a separate subgroup that is less homologous to the other TRPV isoforms (20). Heterologously expressed TRPV5 and TRPV6 channels are constitutively active (however, at a low expression level, the latter shows SOC properties; Ref. 2), and exhibit high Ca^{2+} selectivity (P_{Ca}/P_{Na} >approx. 100) and Ca^{2+}-dependent inactivation reminiscent of voltage-gated Ca^{2+} channels. Tissue expression of TRPV5 and TRPV6 is most abundant in renal and duodenal epithelia, respectively, and seems to be transcriptionally controlled by 1,25-dihydroxyvitamin D_3, plasma Ca^{2+} level and estrogen (15, 20). These features point to the physiological importance of these proteins as transcellular Ca^{2+} transporting pathways in the kidney and intestine and also their potential involvement in Ca^{2+} absorption and reabsorption disorders (15, 20).

TRPM subfamily as cell growth/death regulator

The members of TRPM subfamily (or melastatin family, TRPM1 – 8) have a long N-terminus lacking ankyrin-like repeats and a variable C-terminus (2 – 4), many of which exhibit intriguing features implying their close relationship with cell proliferative potential and its abnormalities. The first TRPM member melastatin (TRPM1/LTRPC1) was identified (MLSN-S) as a tumor suppressor protein in malignant melanoma (21), which is devoid of typical ion channel structure and later turned out to be a short N-terminal splice variant of its full-length form (MLSN-L). Heterologous expression of MLSN-L can produce a constitutive Ca^{2+} entry and this is suppressed by coexpression of MLSN-S via inhibition of translocation to the plasma membrane (22), thus suggesting a crucial role of this protein in regulating cell proliferative potential. Similar roles in aberrant cell growth have been suggested for TRPM8 (TRP-p8), which was detected as a novel tumor marker gene up-regulated in prostate cancer and other malignant tissues (23), and for TRPM5 (MTR1/LTRPC5), which is mapped to a chromosomal region associated with the Beckwith-Wiedermann syndrome and predisposition to a variety of neoplasias (24). Other three TRPM members, TRPM2, TRPM6, and TRPM7 are unique in possessing a kinase domain in their long C-termini, and they are thus regarded as 'bifunctional' proteins (2 – 4). Recombinant characterization has shown that TRPM2 (LTRPC2) is likely to function as a cell death-mediating Ca^{2+}-permeable cation channel activated by reactive oxygen species such as hydrogen peroxide, and this is in part mediated by NAD^+ and/or ADP ribose via the Nudix motif. Expression of TRPM2 is abundant in the brain, but also its mRNA transcripts can be detected in many other tissues such as heart, liver, lung, and hematopoietic and immune organs and cells (25, 26). TRPM7 (TRP-PLIK/Chak1/LTRPC7) is more ubiquitously expressed and encodes constitutively active cation channels regulated by the cytosolic MgATP level, reduction of which to a submillimolar range causes its dramatic enhancement. Although the estimated Ca^{2+} selectivity is low ($P_{Ca}/P_{Cs} = 0.34$), a significant amount of Ca^{2+} sufficient to elevate $[Ca^{2+}]_i$ seems to permeate through this channel (27 – 29). Targeted deletion of TRPM7 in DT-40 B-lymphocytes caused an immediate cell growth arrest and death, suggesting that Ca^{2+} entry through TRPM7 may be essential for cell viability (28). We have confirmed this possibility in human retinoblastoma cells, where elimination of extracellular Ca^{2+} and down-regulation of endogenously expressed TRPM7 by serum deprivation depresses $[Ca^{2+}]_i$ and arrests cell growth. Furthermore, partial inhibition of endogenous TRPM7-mediated Ca^{2+} entry by pharmacological interventions results in a graded retardation of cell growth rather than death (unpublished data), while overexpression of TRPM7 in HEK293 cells causes rapid cell deterioration and death (28). These results suggest that the cell status may be finely tuned by the quantity of continuous Ca^{2+} entry (and presumably Na^+) via TRPM7 between the maintenance of basal cellular conditions (i.e., cell viability), growth, and death. In this respect, it is interesting to note that a non-voltage-gated, receptor-operated Ca channel antagonist, carboxyamido-triazole, significantly inhibits the Ca^{2+} influx, in vitro growth of tumor cells, and cancer progression in clinical trials (30).

In addition to the role in cell viability/growth/death regulation, many TRPM members may fulfill more divergent cellular functions; a long splice variant of TRPM4 (TRPM4b) displays 25pS Ca^{2+} activated, monovalent cation-selective channel (CAN) activity reminiscent of native CANs identified from various secretion epithelia (31); TRPM5 is specifically ex-

pressed in taste cells with other taste-signaling molecules (32); TRPM6 is enriched in renal and intestinal epithelia and its mutations are associated with familial hypomagnesemia (33); TRPM7 allows permeation of trace metal ions including both essential and toxic divalent cations (29); TRPM8 functions as cold activated and menthol-activated Ca^{2+} entry channels, thus likely mediating cold sensation (34).

Perspectives

The efforts to link TRP proteins with particular in vivo functions have just started, and most of the plausible examples that have emerged remain largely speculative. Nevertheless, in some, compelling evidence has been obtained by targeted gene disruption (e.g., TRPC2, TRPC4, and TRPV1) or by genetic analyses of hereditary diseases such as polycystic kidney disease (TRPP) and mucolipidosis (TRPML), which are not described here. One intractable feature of TRP proteins is their seemingly inconsistent properties that depend on the experimental conditions (e.g., expression system and level, cellular localization, coexpressed proteins, etc.). However, this could alternatively be interpreted as a reflection of the potential of TRP proteins to create channels with an amazing diversity and complexity of properties and consequent functions in vivo. Thus, in the future, in addition to thorough molecular elucidation of native non-voltage-gated Ca^{2+} entry mechanisms in light of TRP proteins, the attempts to discover selective drugs targeting the respective TRP isoforms ('reversed pharmacological' approach) will be of promising importance in developing an entirely new category of drugs with high therapeutic potential; i.e., the 'non-voltage-gated Ca^{2+} channel antagonist'.

Acknowledgment

We thank Prof. A.F. Brading, Department of Pharmacology, Oxford University, for critical reading of our manuscript.

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