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## TRPC3: a versatile transducer molecule that serves integration and diversification of cellular signals

Published online: 12 May 2005  
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**Abstract** Transient receptor potential (TRP) proteins have been recognized as sensors for a wide variety of external and internal signals involved in maintenance of cellular homeostasis and control of physiological functions. Evidence of a striking versatility in terms of signal integration and transduction has been reported for members of the canonical (or classical) TRP subfamily (TRPCs). TRPC species are cation channel subunits and emerge as multifunctional signal transduction molecules that are able to function as components of divergent signalplexes. Results obtained in heterologous expression systems suggest TRPC3 as a paradigm of multifunctional signal transduction by a cation channel protein. TRPC3 serves cellular  $\text{Ca}^{2+}$  signaling by multiple mechanisms and may control a variety of distinct physiological functions. In this review, we summarize current knowledge on the properties and possible signaling partners of TRPC3, and discuss the role of TRPC3 channel proteins in cellular signaling networks.

**Keywords** TRPC proteins · Non-selective cation channels · Capacitative  $\text{Ca}^{2+}$  entry channels · Heteromultimerization · Phospholipase C ·  $\text{Na}^+/\text{Ca}^{2+}$  exchange · Redox signaling · Lipid rafts

### Introduction

Based on the pivotal role of the *Drosophila* *trp* gene product in light-induced  $\text{Ca}^{2+}$  entry into invertebrate photoreceptors (Montell and Rubin 1989) and the striking sequence similarities between transient receptor potential (TRP) proteins and classical ion channel proteins, analysis of the function of mammalian TRP species has strongly been fo-

cused on a possible role as  $\text{Ca}^{2+}$  entry channels. The initial hypothesis of TRPC species (canonical mammalian TRP homologs) representing the structural basis of the ubiquitous  $\text{Ca}^{2+}$  entry pathway termed capacitative- (CCE) or store-operated  $\text{Ca}^{2+}$  entry (SOCE), which replenishes cellular  $\text{Ca}^{2+}$  stores after intracellular  $\text{Ca}^{2+}$  mobilization, (Birnbaumer et al. 1996; Zhu et al. 1996; Zitt et al. 1996; Philipp et al. 1998), was questioned or contradicted in many follow-up studies (Zitt et al. 1997; Sinkins et al. 1998; Zhu et al. 1998; McKay et al. 2000; Schaefer et al. 2000). The prominent approach used in these investigations was heterologous expression of a single TRPC species in cellular systems that have proven suitable for analysis of ion channel function. However, the established heterologous expression systems turned out largely inappropriate in terms of reconstitution of TRPC channels with well-defined properties corresponding to a native  $\text{Ca}^{2+}$  entry system. Highly discrepant results obtained in heterologous expression studies and the inability to reproducibly reconstitute native capacitative  $\text{Ca}^{2+}$  entry channels by overexpression of a TRPC species led to the hypothesis that TRPC proteins are pore-forming subunits, which may require association with unidentified endogenous proteins to form a SOCE (or CCE) channel (Sinkins et al. 1998; McKay et al. 2000). This concept was later on continuously supported by studies using antisense and siRNA knock-down strategies or expression of dominant negative TRP species to suppress or modify endogenous SOCE (Groschner et al. 1998; Liu et al. 2000; Philipp et al. 2000; Wu et al. 2000; Baldi et al. 2003; Liu et al. 2003; Wu et al. 2004) and appears reasonable and sufficient to explain the phenomenon that expression of a TRPC species generates divergently regulated  $\text{Ca}^{2+}$  entry pathways in different cell systems or even in one host cell type at different levels of expression (Vazquez et al. 2003). The concept implies that a given TRPC species is able to associate with other channel and auxiliary subunits to form distinct signaling complexes with a composition dependent on the availability of the complex partners. Potential signaling partners, as identified in functional and protein–protein interaction assays, are other TRPC species (Lintschinger et al. 2000;

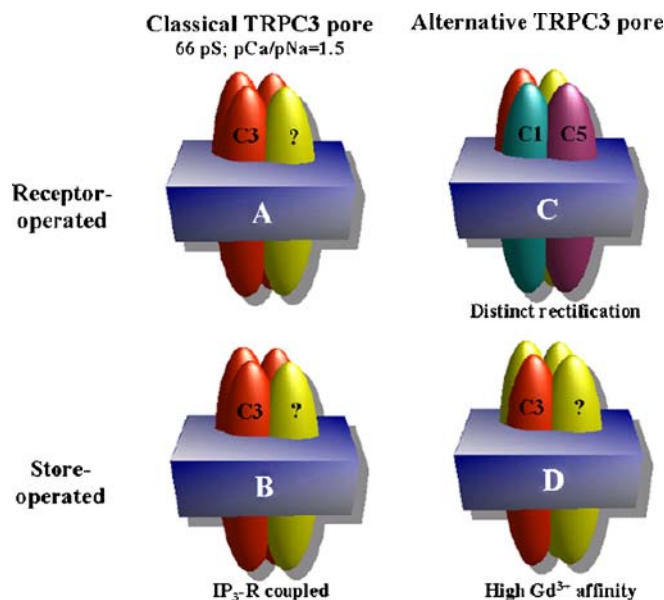
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Goel et al. 2002; Hofmann et al. 2002) as well as scaffold (Lockwich et al. 2000) and ion transport proteins (Kiselyov et al. 1999; Rosker et al. 2004). It is expected that the oligomerization with pore-forming proteins, e.g., other TRPC species, will change the biophysical and pharmacological properties of the permeation pathway, while association with scaffolds and signaling partners that do not contribute to the TRPC pore may alter other features such as activation and coupling to specific cellular functions. According to this hypothesis, multiple oligomeric complexes containing a specific TRPC isoform may co-exist in native cells, thereby TRPC species are likely to serve control of distinct functions within the cellular signaling network. These TRPC signalplexes may be able to sense multiple input signals and translate the integrated information into specific physiological effects, depending on the cellular localization of the signalplex and the involved cellular signaling proteins. The picture of a multifunctional signal transducer molecule has recently emerged for TRPC3. The physiological significance of this multifunctionality, precisely the functional role of individual TRPC3 complexes, is so far elusive.

### The pore: is TRPC3 able to form multiple cation channel structures?

TRPC3 is an 848-amino acid protein that shares with other TRPCs a common domain layout with a cytosolic N-terminal domain containing multiple ankyrin-like repeats, a transmembrane domain that comprises six transmembrane spanning segments and a cytosolic C-terminus (Vannier et al. 1998; Clapham et al. 2001). In analogy to the architecture of voltage-gated  $K^+$  channels, formation of a pore structure is assumed to require assembly of TRPC3 in a homo- or heterotetrameric complex with four short hydrophobic segments each located between transmembrane segments five and six of the individual subunits lining the central ion conducting pathway (reviewed in Clapham et al. 2001). Overexpression of this protein in classical expression systems such as HEK293 and CHO cells was found to generate a cation conductance with unobtrusive biophysical properties being essentially nonselective ( $pCa/pNa$  about 1.5; Kamouchi et al. 1999), and fairly voltage independent (Zitt et al. 1997; Hurst et al. 1998; Lintschinger et al. 2000; Philipp et al. 2003). One glimpse of a signature detectable in whole cell TRPC3 currents is the rather noisy appearance at negative membrane potentials (Hurst et al. 1998; Lintschinger et al. 2000), indicative of a rather large unitary conductance of these channels. Indeed, single TRPC3 channels generated by heterologous overexpression have repeatedly been characterized and a unitary conductance of 60–66 pS (Zitt et al. 1997; Hurst et al. 1998; Kamouchi et al. 1999) as well as a 17 pS subconductance (Kiselyov et al. 1999) have been reported. Several studies suggest that TRPC3 channels display some constitutive activity (Hurst et al. 1998; Dietrich et al. 2003) and are activated in response to stimulation of cellular phospholipase C activity, involving the lipid

mediator diacylglycerol but not depletion of intracellular  $Ca^{2+}$  stores (Hofmann et al. 1999; Lintschinger et al. 2000; McKay et al. 2000). However, another report demonstrates activation of this conductance in response to thapsigargin, a classical tool to deplete cellular  $Ca^{2+}$  stores (Kiselyov et al. 1999). Albeit this study did not conclusively demonstrate that the channels were activated at low endoplasmic reticulum  $Ca^{2+}$  and in the absence of elevations in cytoplasmic  $Ca^{2+}$ , as stipulated for a classical CCE channel, thapsigargin-induced activation of these TRPC3 channels was demonstrated to require  $IP_3$  production and coupling of TRPC3 channels to  $IP_3$  receptors (Kiselyov et al. 1999). The fairly well defined (classical) TRPC3 pore properties may therefore correspond to a TRPC channel that is receptor operated (Fig. 1a), in terms of being activated in response to stimulation of phospholipase C-coupled receptors by the lipid mediator diacylglycerol, and to a similar pore structure that is controlled by interaction with  $IP_3$  receptors in the membrane of the endoplasmic reticulum (store-operated) (Fig. 1b). Since the classical TRPC3 conductance is typically observed in cells that display high levels of TRPC3 expression (in expression systems) one might speculate that the underlying pore-forming complexes contain TRPC3 as the prominent species as depicted in Fig. 1a, b. The direct role of TRPC3 as a pore forming subunit of these channels appears likely as the observed membrane conductance is



**Fig. 1** Hypothetical heterogeneity of TRPC3 channels based on divergent composition of pore-forming tetramers, as indicated by electrophysiological and pharmacological investigations. **a** TRPC3 (C3) channel structure with fairly well defined biophysical properties (classical pore; **a**, **b**) has been postulated to be gated in response to phospholipase C stimulation by diacylglycerol (receptor-operated) as well as by interaction with  $IP_3$  receptors ( $IP_3R$ ) in the endoplasmic reticulum, which may confer sensitivity to the filling state of the intracellular  $Ca^{2+}$  pool (store-operated). In addition, distinctly different pore structures (alternative pores; **c**, **d**) have been postulated, e.g., tetramers containing other TRPC such as TRPC1 (C1) and TRPC5 (C5) as additional subunits. Alternative TRPC3 pore complexes may also be gated in a receptor- or store-dependent manner.

strictly dependent on TRPC3 expression and inhibited in a dominant negative manner by TRPC3 mutants which lack channel function (Rosker and Groschner, unpublished data). Importantly, mutations in the putative pore region of a closely related species, TRPC6, were found to result in a correctly membrane targeted protein that lacks channel function and exerts a dominant negative effect on the classical TRPC3 conductance due to hetero-oligomerization (Hofmann et al. 2002). Nonetheless, detailed mutagenesis studies demonstrating that alterations in the putative pore structure are associated with distinct changes in permeability properties are so far lacking. The stoichiometry of channel complexes underlying the classical TRPC3 conductance has so far not been determined, and the contribution of additional as yet unidentified proteins to the pore structure of these channels cannot be excluded at present. Members of other TRP subfamilies as well as non-TRP cation channel proteins may well be considered as potential subunits of TRPC3 channels. Divergent regulation of such channels may arise from differences in the channel's subunit composition that affect interactions with signaling partners such as IP<sub>3</sub> receptors but not the permeation pathway. Striking evidence for heterogeneity of TRPC3 cation channels came from investigations that utilized mainly fluorometric measurement of intracellular Ca<sup>2+</sup> levels as a measure of divalent entry combined with pharmacological strategies to isolate specific divalent entry pathways. It was recognized that TRPC3 when expressed at a limited level in DT40 avian B lymphocytes generates a store-operated divalent entry pathway that displays pharmacological properties distinctly different from the receptor/phospholipase C-dependent divalent Ca<sup>2+</sup> entry generated in the same expression system at high levels of TRPC3 expression or in HEK293 cells (Trebak et al. 2002). Unfortunately, the store-operated Ca<sup>2+</sup> entry pathway formed by TRPC3 was not characterized electrophysiologically and pore properties of the underlying channels still wait to be determined. This TRPC3-mediated Ca<sup>2+</sup> entry pathway (Fig. 1d) displayed a higher sensitivity to block by Gd<sup>3+</sup> than the classical receptor-operated Ca<sup>2+</sup> entry pathway, thereby resembling the endogenous store-operated Ca<sup>2+</sup> entry channels of HEK293 cells, for which TRPC3 overexpression was actually reported to modulate regulatory properties (Thyagarajan et al. 2001). Thus, TRPC3-containing channel complexes may exist, which do not match the classical TRPC3 pore properties and are activated by store depletion (Fig. 1d). Further support for this hypothesis was provided by experiments with human T cell mutants that display impaired TRPC3 expression and suppressed store-operated Ca<sup>2+</sup> entry, demonstrating a role of TRPC3 in a Ca<sup>2+</sup> entry pathway with biophysical properties strikingly different from that of classical, overexpression-generated TRPC3 channels (Philipp et al. 2003). It appears tempting to speculate that TRPC3 is a rather minor determinant of the permeation pathway of such channels (Fig. 1d). The channel proteins which serve as interaction partners of TRPC3 in these store-operated channels as well as the subunit stoichiometry remain unclear. Obvious candidates in terms of supplementary subunits for TRPC3 are other TRPC species. Heteromulti-

merization among TRP proteins has repeatedly been demonstrated (Xu et al. 1997; Goel et al. 2002; Hofmann et al. 2002; Strubing et al. 2003) and a highly restrictive oligomerization among closely related TRPC species has been suggested (Goel et al. 2002; Hofmann et al. 2002). One subunit composition that is likely to exist in native tissues is TRPC3/TRPC6 (Dietrich et al. 2005). Heteromultimerization among these close TRPC relatives may be of particular physiological significance since these two proteins have been shown to generate channels that display divergent basal activity (Dietrich et al. 2003). Thus, variation of the TRPC3/TRPC6 expression ratio may result in profound modification of basal cation conductance and resting membrane potential, which is a key determinant of cell functions. Recent studies demonstrated that the formation of specific heteromeric channel structures composed of TRPC3 and the more distant relatives TRPC1 and TRPC5 or TRPC4 (Fig. 1c) are possible (Strubing et al. 2003). Such TRPC3 channel complexes appear to require the TRPC1 protein as key structure, are activated in a receptor/phospholipase C-dependent manner and display distinct biophysical properties with a more prominent outward rectification than classical overexpression-generated TRPC3 conductances.

In aggregate, TRPC3 is likely to serve cellular signal transduction by formation of divergent cation channel complexes. It is of note, that the exact subunit composition of these pore forming TRPC3 complexes is not clearly defined at present. As depicted in Fig. 1, it appears possible that TRPC3 channels with divergent pore structure (classical or alternative) that are gated by different stimuli such as lipid messengers (receptor-operated) and coupling to the endoplasmic reticulum (store-operated) may exist in native tissues.

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### The gating: are TRPC3 channels able to sense multiple input stimuli?

Stimulation of phospholipase C is unequivocally the key trigger for activation of TRPC3 channels as substantiated by the observation that suppression of phospholipase C activity or inhibition of phospholipase C-derived signals inhibits TRPC3 channels. Some reports indicate that phospholipase C-signaling and the ill-defined mechanism that communicates reductions in the endoplasmic reticulum Ca<sup>2+</sup> content to the plasma membrane converge at TRPC3 channels due to a physical coupling between TRPC3 channels and IP<sub>3</sub> or ryanodine receptors in the membrane of cellular Ca<sup>2+</sup> stores (Kiselyov et al. 1998; Kiselyov et al. 2001; Tang et al. 2001; Vazquez et al. 2001; Zhang et al. 2001). Nonetheless, evidence has been presented demonstrating that TRPC3 is able to form phospholipase C-regulated cation channels that are gated by diacylglycerol and independently of IP<sub>3</sub>, indicating a prominent role for the lipid messenger (Hofmann et al. 1999; Trebak et al. 2003). It is tempting to speculate about a direct interaction between the lipid species and channel structures, but the molecular mechanism by which diacylglycerol activates TRPC3 channels remains to be clarified. The rather unique

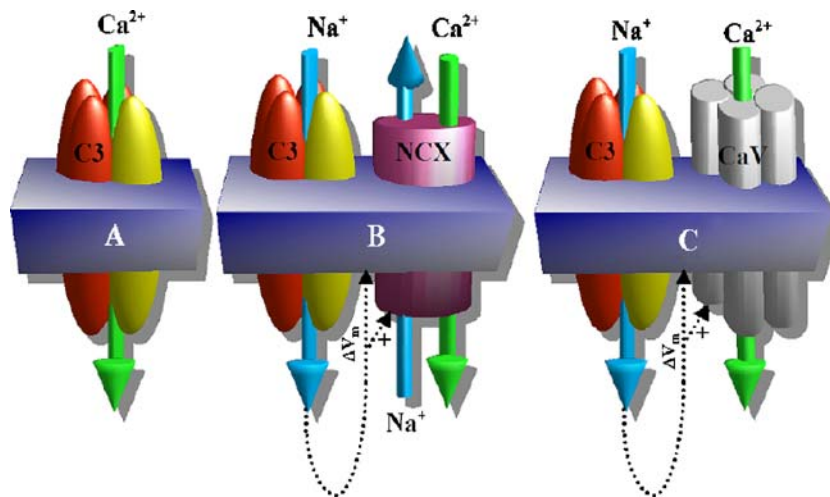


lipid sensitivity may be considered as one fingerprint feature of TRPC3 and its close relatives TRPC6 and TRPC7. Additional forms of lipid sensitivity of TRPC3 have as well been suggested. TRPC3 was found to aggregate in specific cholesterol-rich microdomains, a type lipid rafts termed caveolae and to associate in signalplexes that contain the cholesterol-binding scaffold protein caveolin-1 (Lockwich et al. 2001). The function of TRPC3 channels was found sensitive to selective oxidation of membrane cholesterol by use of cholesterol oxidase (Groschner et al. 2004). Thus, TRPC3 may display particular sensitivity to its lipid environment and serve integrative transduction of stimuli derived by activation of phospholipase C-coupled receptors as well as by changes in the cells, lipid metabolism or oxidative stress signals. Cellular activity of the non-receptor tyrosine kinase src was recently identified as a further cellular parameter that may affect the gating of classical TRPC3 channels (Vazquez et al. 2004). Moreover, input stimuli that affect membrane fusion events may be transduced by TRPC3, as regulation of cellular TRPC3 conductance via rapid recruitment of TRPC3 channel complexes from membrane associated vesicles has been demonstrated (Singh et al. 2004). The TRPC3 protein therefore represents a signal transduction molecule that is highly versatile not only in terms of forming various pore structures but also as a multifunctional sensor molecule. TRPC3 containing sensor complexes may control key cellular functions predominantly by generating specific types of cellular  $\text{Ca}^{2+}$  signals.

### The physiological role: how does TRPC3 govern $\text{Ca}^{2+}$ homeostasis and cellular functions?

TRPC3 channels have been postulated to associate with a variety of signaling partners to form larger signalplexes. The channel underlying the classical TRPC3 conductance,

may generate divergent types of  $\text{Ca}^{2+}$  signals depending on the availability of  $\text{Ca}^{2+}$ -handling partner proteins in a particular tissue. Association of various  $\text{Ca}^{2+}$  transport systems and  $\text{Ca}^{2+}$  binding proteins with TRPC3 has been demonstrated (Kiselyov et al. 1999; Tang et al. 2001; Rosker et al. 2004; Treves et al. 2004) and it appears reasonable to speculate that TRPC3-associated  $\text{Ca}^{2+}$  transport systems function as signaling partners of TRPC3 channels. Such signaling partners are considered as determinants of signal input as well as of signal output in terms of generation and tailoring the TRPC3-mediated  $\text{Ca}^{2+}$  signals. One example for such a signaling partnership is the recently reported association of classical, overexpression-generated TRPC3 channels to the cardiac type  $\text{Na}^+/\text{Ca}^{2+}$  exchanger NCX1 (Rosker et al. 2004). Experiments in the HEK293 expression system demonstrated a tight functional and physical coupling of the two-ion transport systems. Functional interaction was suggested to involve TRPC3-mediated  $\text{Na}^+$  loading and membrane depolarization which inhibits the forward-mode  $\text{Ca}^{2+}$  extrusion efficiency of the exchanger or may even shift its operation to reversed mode resulting in an additional  $\text{Ca}^{2+}$  entry component. According to this concept, coupling of classical TRPC3 channels to NCX1 (Fig. 2b) generates a signalplex that provides an efficient mechanism for signal amplification at high levels of TRPC3 activity. Hence, TRPC3-mediated  $\text{Ca}^{2+}$  signals may be based not only on  $\text{Ca}^{2+}$  permeation through its pore structure (Fig. 2a) but also by translation of TRPC3-mediated  $\text{Na}^+$  entry into  $\text{Ca}^{2+}$  signals. A similar, signal modifying partnership may exist in excitable tissue in terms of a functional coupling between nonselective TRPC3 cation channels and voltage-gated  $\text{Ca}^{2+}$  channels. Cation inward currents through TRPC3 channels are most likely an essential determinant of membrane potential and expected to govern the function of voltage-gated  $\text{Ca}^{2+}$  channels. Depending on the activity of TRPC3 channels and the activation and inactivation properties of the voltage-gated  $\text{Ca}^{2+}$



**Fig. 2** Generation of cellular  $\text{Ca}^{2+}$  signals by TRPC3 channels may involve functional interaction with specific signaling partners. **a** TRPC3-mediated  $\text{Ca}^{2+}$  signaling may not simply involve permeation of  $\text{Ca}^{2+}$  through the TRPC3 pore, but also changes in the function of associated  $\text{Ca}^{2+}$  transport systems such as **b** the  $\text{Na}^+$ /

$\text{Ca}^{2+}$  exchanger (NCX) or **c** voltage-gated  $\text{Ca}^{2+}$  channels (CaV), which are expected to sense cation entry through TRPC3 channels in terms of membrane depolarization and local  $\text{Na}^+$  loading, and convert this signal into a specific  $\text{Ca}^{2+}$  signaling pattern.

channels present in excitable tissues, TRPC3-mediated  $\text{Ca}^{2+}$  signals may be strongly amplified at a certain TRPC channel activity due to concomitant activation of voltage-gated  $\text{Ca}^{2+}$  channels as depicted in Fig. 2c. Overexpression of TRPC3 as recently demonstrated for pathophysiological situations such as pulmonary arterial hypertension (Yu et al. 2004) may cause membrane depolarization of vascular smooth muscle due to enhanced constitutive TRPC3 activity, leading to increased  $\text{Ca}^{2+}$  entry through  $\text{CaV}1.2$  (L-type)  $\text{Ca}^{2+}$  channels.

In summary, our current knowledge on the function of TRPC3 suggests this protein as a uniquely multifunctional signal transducer that may govern a large number of physiological functions. The predicted existence of multiple TRPC3 channel structures opens the view on possible selective pharmacological interventions and highlights the potential of TRPC3 as a potential therapeutic target.

**Acknowledgements** Supported by FWF, SFB BIOMEMBRANES project F715.

## References

- Baldi C, Vazquez G, Calvo JC, Boland R (2003) TRPC3-like protein is involved in the capacitative cation entry induced by 1 $\alpha$ , 25-dihydroxy-vitamin D3 in ROS 17/2.8 osteoblastic cells. *J Cell Biochem* 90:197–205
- Birbaumer L, Zhu X, Jiang M, Boulay G, Peyton M, Vannier B, Brown D, Platano D, Sadeghi H, Stefani E, Birbaumer M (1996) On the molecular basis and regulation of cellular capacitative calcium entry: roles for Trp proteins. *Proc Natl Acad Sci USA* 93:15195–15202
- Clapham DE, Runnels LW, Strubing C (2001) The TRP ion channel family. *Nat Rev Neurosci* 2:387–396
- Dietrich A, Mederos y Schnitzler M, Emmel J, Kalwa H, Hofmann T, Gudermann T (2003) N-linked protein glycosylation is a major determinant for basal TRPC3 and TRPC6 channel activity. *J Biol Chem* 278:47842–47852
- Dietrich A, Mederos y Schnitzler M, Kalwa H, Storch U, Gudermann T (2005) Functional characterization and physiological relevance of the TRPC3/6/7 subfamily of cation channels. *Naunyn-Schmiedeberg's Arch Pharmacol* (in press)
- Goel M, Sinkins WG, Schilling WP (2002) Selective association of TRPC channel subunits in rat brain synaptosomes. *J Biol Chem* 277:48303–48310
- Groschner K, Hingel S, Lintschinger B, Balzer M, Romanin C, Zhu X, Schreibmayer W (1998) Trp proteins form store-operated cation channels in human vascular endothelial cells. *FEBS Lett* 437:101–106
- Groschner K, Rosker C, Lukas M (2004) Role of TRP channels in oxidative stress. *Novartis Found Symp* 258:222–230; discussion 231–235, 263–266
- Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G (1999) Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 397:259–263
- Hofmann T, Schaefer M, Schultz G, Gudermann T (2002) Subunit composition of mammalian transient receptor potential channels in living cells. *Proc Natl Acad Sci USA* 99:7461–7466
- Hurst RS, Zhu X, Boulay G, Birbaumer L, Stefani E (1998) Ionic currents underlying HTRP3 mediated agonist-dependent  $\text{Ca}^{2+}$  influx in stably transfected HEK293 cells. *FEBS Lett* 422:333–338
- Kamouchi M, Philipp S, Flockerzi V, Wissenbach U, Mamin A, Raeymaekers L, Eggermont J, Droogmans G, Nilius B (1999) Properties of heterologously expressed hTRP3 channels in bovine pulmonary artery endothelial cells. *J Physiol* 518:345–358
- Kiselyov K, Xu X, Mozhayeva G, Kuo T, Pessah I, Mignery G, Zhu X, Birbaumer L, Muallem S (1998) Functional interaction between InsP3 receptors and store-operated Htrp3 channels. *Nature* 396:478–482
- Kiselyov K, Mignery GA, Zhu MX, Muallem S (1999) The N-terminal domain of the IP3 receptor gates store-operated hTrp3 channels. *Mol Cell* 4:423–429
- Kiselyov K, Shin DM, Shcheynikov N, Kuroski T, Muallem S (2001) Regulation of  $\text{Ca}^{2+}$ -release-activated  $\text{Ca}^{2+}$  current (I<sub>crac</sub>) by ryanodine receptors in inositol 1,4,5-trisphosphate-receptor-deficient DT40 cells. *Biochem J* 360:17–22
- Lintschinger B, Balzer-Geldsetzer M, Baskaran T, Graier WF, Romanin C, Zhu MX, Groschner K (2000) Coassembly of Trp1 and Trp3 proteins generates diacylglycerol- and  $\text{Ca}^{2+}$ -sensitive cation channels. *J Biol Chem* 275:27799–27805
- Liu X, Wang W, Singh BB, Lockwich T, Jadlovec J, O'Connell B, Wellner R, Zhu MX, Ambudkar IS (2000) Trp1, a candidate protein for the store-operated  $\text{Ca}^{2+}$  influx mechanism in salivary gland cells. *J Biol Chem* 275:3403–3411
- Liu X, Singh BB, Ambudkar IS (2003) TRPC1 is required for functional store-operated  $\text{Ca}^{2+}$  channels. Role of acidic amino acid residues in the S5-S6 region. *J Biol Chem* 278:11337–11343
- Lockwich TP, Liu X, Singh BB, Jadlovec J, Weiland S, Ambudkar IS (2000) Assembly of Trp1 in a signaling complex associated with caveolin-scaffolding lipid raft domains. *J Biol Chem* 275:11934–11942
- Lockwich T, Singh BB, Liu X, Ambudkar IS (2001) Stabilization of cortical actin induces internalization of transient receptor potential 3 (Trp3)-associated caveolar  $\text{Ca}^{2+}$  signaling complex and loss of  $\text{Ca}^{2+}$  influx without disruption of Trp3-inositol trisphosphate receptor association. *J Biol Chem* 276:42401–42408
- McKay RR, Szymeczek-Seay CL, Lievreumont JP, Bird GS, Zitt C, Jungling E, Luckhoff A, Putney JW Jr (2000) Cloning and expression of the human transient receptor potential 4 (TRP4) gene: localization and functional expression of human TRP4 and TRP3. *Biochem J* 351:735–746
- Montell C, Rubin GM (1989) Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. *Neuron* 2:1313–1323
- Philipp S, Hambrecht J, Braslavski L, Schroth G, Freichel M, Murakami M, Cavalie A, Flockerzi V (1998) A novel capacitative calcium entry channel expressed in excitable cells. *EMBO J* 17:4274–4282
- Philipp S, Trost C, Wornat J, Rautmann J, Himmerkus N, Schroth G, Kretz O, Nastainczyk W, Cavalie A, Hoth M, Flockerzi V (2000) TRP4 (CCE1) protein is part of native calcium release-activated  $\text{Ca}^{2+}$ -like channels in adrenal cells. *J Biol Chem* 275:23965–23972
- Philipp S, Strauss B, Hirnet D, Wissenbach U, Mery L, Flockerzi V, Hoth M (2003) TRPC3 mediates T-cell receptor-dependent calcium entry in human T-lymphocytes. *J Biol Chem* 278:26629–26638
- Rosker C, Graziani A, Lukas M, Eder P, Zhu MX, Romanin C, Groschner K (2004)  $\text{Ca}^{2+}$  signaling by TRPC3 involves  $\text{Na}^{+}$  entry and local coupling to the  $\text{Na}^{+}/\text{Ca}^{2+}$  exchanger. *J Biol Chem* 279:13696–13704
- Schaefer M, Plant TD, Obukhov AG, Hofmann T, Gudermann T, Schultz G (2000) Receptor-mediated regulation of the nonselective cation channels TRPC4 and TRPC5. *J Biol Chem* 275:17517–17526

- Singh BB, Lockwich TP, Bandyopadhyay BC, Liu X, Bollimuntha S, Brazer SC, Combs C, Das S, Leenders AG, Sheng ZH, Knepper MA, Ambudkar SV, Ambudkar IS (2004) VAMP2-dependent exocytosis regulates plasma membrane insertion of TRPC3 channels and contributes to agonist-stimulated Ca<sup>2+</sup> influx. *Mol Cell* 15:635–646
- Sinkins WG, Estacion M, Schilling WP (1998) Functional expression of TrpC1: a human homologue of the *Drosophila* Trp channel. *Biochem J* 331:331–339
- Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE (2003) Formation of novel TRPC channels by complex subunit interactions in embryonic brain. *J Biol Chem* 278:39014–39019
- Tang J, Lin Y, Zhang Z, Tikunova S, Birnbaumer L, Zhu MX (2001) Identification of common binding sites for calmodulin and inositol 1,4,5-trisphosphate receptors on the carboxyl termini of trp channels. *J Biol Chem* 276:21303–21310
- Thyagarajan B, Poteser M, Romanin C, Kahr H, Zhu MX, Groschner K (2001) Expression of Trp3 determines sensitivity of capacitative Ca<sup>2+</sup> entry to nitric oxide and mitochondrial Ca<sup>2+</sup> handling: evidence for a role of Trp3 as a subunit of capacitative Ca<sup>2+</sup> entry channels. *J Biol Chem* 276:48149–48158
- Trebak M, Bird GS, McKay RR, Putney JW Jr (2002) Comparison of human TRPC3 channels in receptor-activated and store-operated modes. Differential sensitivity to channel blockers suggests fundamental differences in channel composition. *J Biol Chem* 277:21617–21623
- Trebak M, St John Bird G, McKay RR, Birnbaumer L, Putney JW Jr (2003) Signaling mechanism for receptor-activated canonical transient receptor potential 3 (TRPC3) channels. *J Biol Chem* 278:16244–16252
- Treves S, Franzini-Armstrong C, Moccagatta L, Arnoult C, Grasso C, Schrum A, Ducreux S, Zhu MX, Mikoshiba K, Girard T, Smida-Rezgui S, Ronjat M, Zorzato F (2004) Junction is a key element in calcium entry induced by activation of InsP<sub>3</sub> receptors and/or calcium store depletion. *J Cell Biol* 166:537–548
- Vannier B, Zhu X, Brown D, Birnbaumer L (1998) The membrane topology of human transient receptor potential 3 as inferred from glycosylation-scanning mutagenesis and epitope immunocytochemistry. *J Biol Chem* 273:8675–8679
- Vazquez G, Lievreumont JP, St JBG, Putney JW Jr (2001) Human Trp3 forms both inositol trisphosphate receptor-dependent and receptor-independent store-operated cation channels in DT40 avian B lymphocytes. *Proc Natl Acad Sci USA* 98:11777–11782
- Vazquez G, Wedel BJ, Trebak M, St John Bird G, Putney JW Jr (2003) Expression level of the canonical transient receptor potential 3 (TRPC3) channel determines its mechanism of activation. *J Biol Chem* 278:21649–21654
- Vazquez G, Wedel BJ, Kawasaki BT, Bird GS, Putney JW Jr (2004) Obligatory role of Src kinase in the signaling mechanism for TRPC3 cation channels. *J Biol Chem* 279:40521–40528
- Wu X, Babnigg G, Villereal ML (2000) Functional significance of human trp1 and trp3 in store-operated Ca(2+) entry in HEK-293 cells. *Am J Physiol Cell Physiol* 278:C526–C536
- Wu X, Zagranichnaya TK, Gurda GT, Eves EM, Villereal ML (2004) A TRPC1/TRPC3-mediated increase in store-operated calcium entry is required for differentiation of H19-7 hippocampal neuronal cells. *J Biol Chem* 279:43392–43402
- Xu XZ, Li HS, Guggino WB, Montell C (1997) Coassembly of TRP and TRPL produces a distinct store-operated conductance. *Cell* 89:1155–1164
- Yu Y, Fantozzi I, Remillard CV, Landsberg JW, Kunichika N, Platoshyn O, Tigno DD, Thistlethwaite PA, Rubin LJ, Yuan JX (2004) Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proc Natl Acad Sci USA* 101:13861–13866
- Zhang Z, Tang J, Tikunova S, Johnson JD, Chen Z, Qin N, Dietrich A, Stefani E, Birnbaumer L, Zhu MX (2001) Activation of Trp3 by inositol 1,4,5-trisphosphate receptors through displacement of inhibitory calmodulin from a common binding domain. *Proc Natl Acad Sci USA* 98:3168–31673
- Zhu X, Jiang M, Peyton M, Boulay G, Hurst R, Stefani E, Birnbaumer L (1996) Trp, a novel mammalian gene family essential for agonist-activated capacitative Ca<sup>2+</sup> entry. *Cell* 85:661–671
- Zhu X, Jiang M, Birnbaumer L (1998) Receptor-activated Ca<sup>2+</sup> influx via human Trp3 stably expressed in human embryonic kidney (HEK)293 cells. Evidence for a non-capacitative Ca<sup>2+</sup> entry. *J Biol Chem* 273:133–142
- Zitt C, Zobel A, Obukhov AG, Harteneck C, Kalkbrenner F, Luckhoff A, Schultz G (1996) Cloning and functional expression of a human Ca<sup>2+</sup>-permeable cation channel activated by calcium store depletion. *Neuron* 16:1189–1196
- Zitt C, Obukhov AG, Strubing C, Zobel A, Kalkbrenner F, Luckhoff A, Schultz G (1997) Expression of TRPC3 in Chinese hamster ovary cells results in calcium-activated cation currents not related to store depletion. *J Cell Biol* 138:1333–1341