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# TRPC3: A Multifunctional, Pore-Forming Signalling Molecule

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**Abstract** TRPC3 represents one of the first identified mammalian relatives of the *Drosophila trp* gene product. Despite intensive biochemical and biophysical characterization as well as numerous attempts to uncover its physiological role in native cell systems, this channel protein still represents one of the most enigmatic members of the transient receptor potential (TRP) superfamily. TRPC3 is significantly expressed in brain and heart and likely to play a role in both non-excitable as well as excitable cells, being potentially involved in a wide spectrum of $Ca^{2+}$ signalling mechanisms. Its ability to associate with a variety of partner proteins apparently enables TRPC3 to form different cation channels in native cells. TRPC3 cation channels display unique gating and regulatory properties that allow for recognition and integration of multiple input stimuli including lipid mediators and cellular $Ca^{2+}$ gradients as well as redox signals. The physiological/pathophysiological functions of this highly versatile cation channel protein are as yet barely delineated. Here we summarize current knowledge on properties and possible signalling functions of TRPC3 and discuss the potential biological relevance of this signalling molecule.

**Keywords** TRPC3 · Cation channel subunit · Cellular regulation · $Ca^{2+}$ signalling · Lipid sensor

# 1
# TRPC3 Basic Features

## 1.1
## Gene Products and Expression Pattern

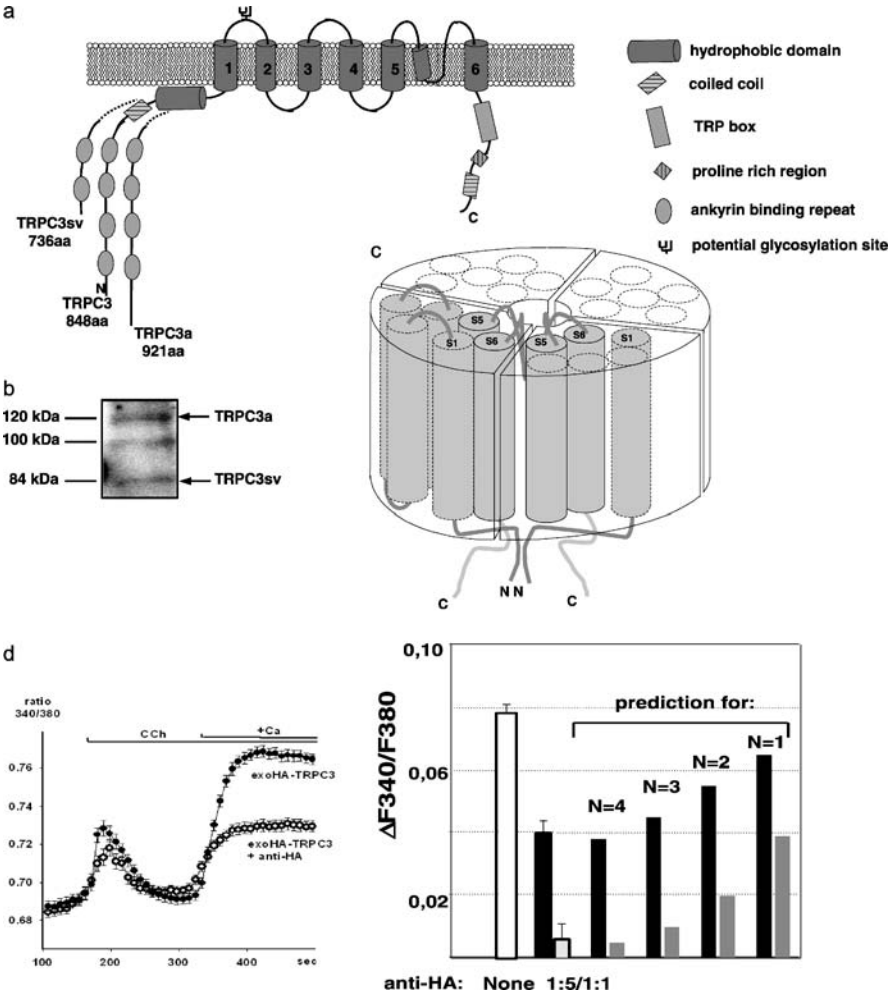
Human TRPC3 (hTRPC3) cDNA was originally cloned using a library derived from HEK293 cells and the expressed sequence tag (EST) sequence R34716 as a probe (Zhu et al. 1996). The human TRPC3 gene consists of 11 exons located on chromosome 4. TRPC3 messenger RNA (mRNA) comprises roughly 4 kb and was found abundantly expressed in brain (Riccio et al. 2002). In peripheral tissues, substantial expression of TRPC3 mRNA has been detected in pituitary gland, and somewhat lower levels in heart and lung (Riccio et al. 2002). Notably, TRPC3 appears to be expressed predominantly in embryonic and developing tissues (Strubing et al. 2003). The human TRPC3 protein comprises 848 amino acids (aa) and shares 96.41% homology with mouse TRPC3 (mTRPC3; 836 aa) and 94% with rat TRPC3 rTRPC3 828 aa; Preuss et al. 1997). Human, mouse and rat genomes contain one additional exon that gives rise to the expression of an N-terminally extended splice variant of TRPC3 (Yildirim et al. 2005; hTRPC3a: 921 aa; mTRPC3a: 911 aa; rTRPC3a: 910 aa). A short splice variant

**Fig. 1 a** Proposed membrane topology and domain structure of TRPC3. The N-terminus of TRPC3 contains four ankyrin domains, a coiled-coil domain and a hydrophobic domain preceding the first transmembrane segment. A second coiled-coil domain is located in the C-terminus downstream of the TRP box and the proline-rich region. A putative glycosylation site is located in the first extracellular loop. Transmembrane 5 (TM5), TM6 and the connecting pore loop are proposed to form the central cation-conducting pore. TRPC3 splice variants exhibiting different N-termini, TRPC3sv (short variant) lacking two ankyrin domains (736 aa) and TRPC3a with an extended N-terminus (921 aa) are shown. **b** Detection of three TRPC3 splice variants in rat heart by immunoblotting. Rat cardiomyocytes were lysed and 150 μg of the resulting protein was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. A custom made TRPC3-specific antibody detected three proteins (~84 kDa, ~100 kDa, ~120 kDa) potentially corresponding to the TRPC3 splice forms reported so far. It is of note that the 84 kDa protein was detected only occasionally. **c** Hypothetical organization of a tetrameric TRPC3 pore structure. **d** Inhibition of exo HA-tagged TRPC3 channels by an anti-HA antibody demonstrates the existence of homotetramers. *Left*: Time course of FURA-2 $Ca^{2+}$-measurement in HEK293 cells co-transfected with YFP-TRPC3 and exo HA-TRPC3 (DNA ratio 1/1). Incubation with HA-antibody significantly inhibits carbachol (CCh)-induced $Ca^{2+}$ entry in a $Ca^{2+}$ re-addition protocol. *Right*: Maximum $Ca^{2+}$ entry derived from $Ca^{2+}$ re-addition experiments as described above (average values±SEM, $n$=40). Dependency of inhibition on cDNA ratio (tagged/non-tagged=1/5, *black columns* and 1/1, *grey columns*) is displayed. Expected values for different order multimers ($n$=1–4), based on the concept that incorporation of a single HA-tagged protein is sufficient to confer antibody sensitivity, are shown

of TRPC3, termed Trp3sv, has been isolated from a rat heart complementary DNA cDNA) library encoding a 736-aa protein with a truncated N-terminus (Ohki et al. 2000; Fig. 1a, b).

## 1.2 Domain Structure and Membrane Topology of TRPC3

TRPC3 has been supposed as an integral membrane protein with seven membrane-spanning hydrophobic regions (H1–H7), of which six helical stretches (H2–H7) form the transmembrane core domain (TM1–TM6), which is flanked by an intracellular N- and C-terminal domain (Vannier et al. 1998; Zhu et al.



1996). The first hydrophobic region, H1, has been proposed as an intracellular, membrane-associated segment, and based on the similarity of the TRPC membrane topology to that of voltage-gated $K^+$ channels ($K_V$), transmembrane segments TM5, TM6 and their connecting loop were designated as the putative pore region (Vannier et al. 1998; Fig. 1a).

### 1.3 Subunit Assembly and Multimerization

According to the general concept of a tetrameric pore structure in cation channels with a 6TM membrane topology, TRPC3 is assumed to form tetrameric channel complexes as illustrated in Fig. 1c. It is important to note that the existence of native homotetrameric TRPC3 channels has not definitely been proved and the subunit composition and stoichiometry of native TRPC3 heterotetramers remains elusive. Nonetheless, heterologous overexpression of TRPC3 is likely to generate homomultimeric channels. The stoichiometry of pore complexes may be tested in cells expressing defined mixtures of blocking sensitive mutants and wild-type proteins (Kosari et al. 1998). Characterization of a TRPC3 mutant that contains an exohemagglutinin (exo-HA) tag, which confers sensitivity to block by anti-HA antibody (Poteser et al. 2006), substantiated the concept of TRPC3 being able to assemble in homotetramers (Fig. 1d). Inhibition was consistent with multimers comprising four TRPC3 molecules ($n = 4$) but significantly different from the inhibition predicted for $n = 1$ or 2.

Analysis of the potential of other TRPC isoforms to associate with TRPC3 upon heterologous overexpression suggested a preference of TRPC3 to associate with its closer relatives TRPC6 and TRPC7 (Hofmann et al. 2002), while its ability to associate with more distant relatives was controversially interpreted (Lintschinger et al. 2000; Liu et al. 2005). In HEK293 cells, TRPC3 has been demonstrated to interact with TRPC6 and TRPC7 to form receptor-operated channels. Co-transfection of a dominant-negative mutant of TRPC6 (dnTRPC) and TRPC3 resulted in a decreased receptor activated $Mn^{2+}$ influx. Moreover, association of TRPC3 and TRPC6 fusion proteins was clearly demonstrated by fluorescence resonance energy transfer (FRET) experiments, while minimal FRET signals were detected in cells co-expressing fluorescent protein fusions of TRPC3 and TRPC1, TRPC4 or TRPC5 (Hofmann et al. 2002). In epithelial cells, the existence of TRPC3/6 complexes associated with $Ca^{2+}$ signalling proteins of the $G_q$-PI-PLC pathway has been demonstrated (Bandyopadhyay et al. 2004). Similarly, in brain synaptosomes, TRPC3 has been shown to interact with TRPC6 and TRPC7, but not with other members of the TRPC family (Goel et al. 2002). By contrast, Lintschinger et al. observed functional interactions between TRPC1 and TRPC3 in the HEK293 expression system, and such interactions have recently been confirmed in hippocampal neurons (Wu et al. 2004) as well as in human salivary gland cells in which TRPC3 and TRPC1 were shown to associate via N-terminal interactions to form heteromeric store-operated

channels (Liu et al. 2005). Moreover, the association of TRPC3 with TRPC4 was recently demonstrated in a study using HEK293 as well as native vascular endothelial cells (Poteser et al. 2006).

In summary, TRPC3 displays a remarkable potential to form divergent types of cation channels by multimerization with other TRPC proteins. Assembly of TRP homo- and heterotetramers is likely based on interactions between the N-terminal domains of the pore-forming subunits as illustrated in Fig. 1c. Assembly of TRP pore complexes via N-terminal interactions was suggested by demonstration of the ability of N-terminal domains to associate as well as by marked dominant-negative effects of N-terminal fragments (Engelke et al. 2002; Groschner et al. 1998; Liu et al. 2005). Nonetheless, stable assembly of tetramers is likely to involve additional interactions between other domains in hydrophobic segments (Xu et al. 1997) or in the C-terminus (Poteser et al. 2006).

## 2
## Channel Properties

### 2.1
### The Pore

In analogy to $K_V$ channels, the short hydrophobic segments located between transmembrane segments five and six of the individual subunits are considered to line the central ion conducting pathway of TRP channels (see Clapham et al. 2001). Evidence for the contribution of a membrane protein or a protein domain to an ion channel pore may be obtained by different experiments, with the most convincing proof coming from mutation of the putative pore-forming region that results in altered pore properties (Hofmann et al. 2002; Strubing et al. 2003). Alternatively, the blocking activity of antibodies directed against epitopes within motives that putatively contribute to the outer vestibule of the channel pore may be taken as evidence in support of a particular pore structure concept. To date, such evidence is sparse for TRPC channels, and particular information on the structure of the pore region and the selectivity filter of TRPC3 channels is currently lacking. Nonetheless, both approaches yielded evidence in support of a role of the S5–S6 linker in the permeation pathway of a related protein, TRPC1 (Liu et al. 2003). Hence, it appears reasonable to consider this structure essential for the formation of the ion-conducting pathway of TRPC3 channels.

Overexpression of TRPC3 in classical expression systems generates a cation-conducting pathway, which is most likely based on the formation of multimeric pore complexes that allow cation permeation without appreciable selectivity (Hurst et al. 1998; Lintschinger et al. 2000). A thorough investigation of TRPC3 channels in the specific environment of endothelial cells was performed by Kamouchi et al. (1999) and revealed a permeability ratio (pCa/pNa) of about 1.6 (Kamouchi et al. 1999). As TRPC3 is likely to associate with other TRPC

species and/or other not-yet-identified transmembrane proteins, multiple populations of channels containing TRPC3 as pore-forming subunits may coexist at variable ratios depending on TRPC expression levels. This concept has been put forward to explain TRPC3-dependent divalent entry phenomena that displayed divergent regulatory properties and different sensitivity to block by the lanthanide $Gd^{3+}$, indicating the existence of TRPC3 channels with different pore structures (Trebak et al. 2002). Single TRPC3 channels generated by heterologous overexpression have repeatedly been characterized, and a unitary conductance of 60–66 pS (Hurst et al. 1998; Kamouchi et al. 1999; Kiselyov et al. 1998; Poteser et al. 2006; Zitt et al. 1997), as well as a 17-pS sub-conductance (Kiselyov et al. 1999), has been reported.

As $Ca^{2+}$ permeation through TRPC3 has been demonstrated in classical ion substitution experiments (Kamouchi et al. 1999), promotion of cellular $Ca^{2+}$ entry in response to overexpression of TRPC3 has been generally attributed to $Ca^{2+}$ permeation through the TRPC3 pore. However, recent evidence indicates a potential role of TRPC3-mediated $Na^{+}$ entry and membrane depolarization as essential determinants of cellular $Ca^{2+}$ homeostasis, and it points to a more complex and indirect link between TRPC3 channel activity and cellular $Ca^{2+}$ signals (Eder et al. 2005; Rosker et al. 2004).

## 2.2 The Gating: Primary Modes of Activation

Several studies suggest that TRPC3 channels display significant constitutive activity (Dietrich et al. 2003; Hurst et al. 1998) exceeding that of other related TRPC species such as TRPC6. Basal activity of TRPC channels, specifically of TRPC3/6/7 channels, has been related to the glycosylation status, mimicking the higher glycosylation state of TRPC6 and resulting in a reduced constitutive activity (Dietrich et al. 2003). Typically, TRPC3 currents display only little voltage dependence as illustrated in Fig. 2a.

There is general agreement in that cellular TRPC3 channel activity is enhanced in response to stimulation of cellular phospholipase C (PLC) activity (Fig. 2a), and solid evidence has been presented for a rather direct activation of TRPC3 currents by the lipid mediator diacylglycerol (DAG) (Hofmann et al. 1999; Lintschinger et al. 2000; McKay et al. 2000). Hence, TRPC3 as well as its closest relatives TRPC6 and TRPC7 are considered as a unique family of lipid-sensitive cation channels. Besides direct activation of TRPC3 currents by PLC-derived DAG, control of TRPC3 channels by the filling state of intracellular $Ca^{2+}$ stores has repeatedly been suggested (Kiselyov et al. 1998; Vazquez et al. 2003). The ability of TRPC3 to contribute to store-operated $Ca^{2+}$ entry is likely dependent on the expression pattern of $Ca^{2+}$ signalling molecules such as TRPC heteromerization partners or regulatory channel subunits. TRPC1 has recently been identified as one potential heteromerization partner that

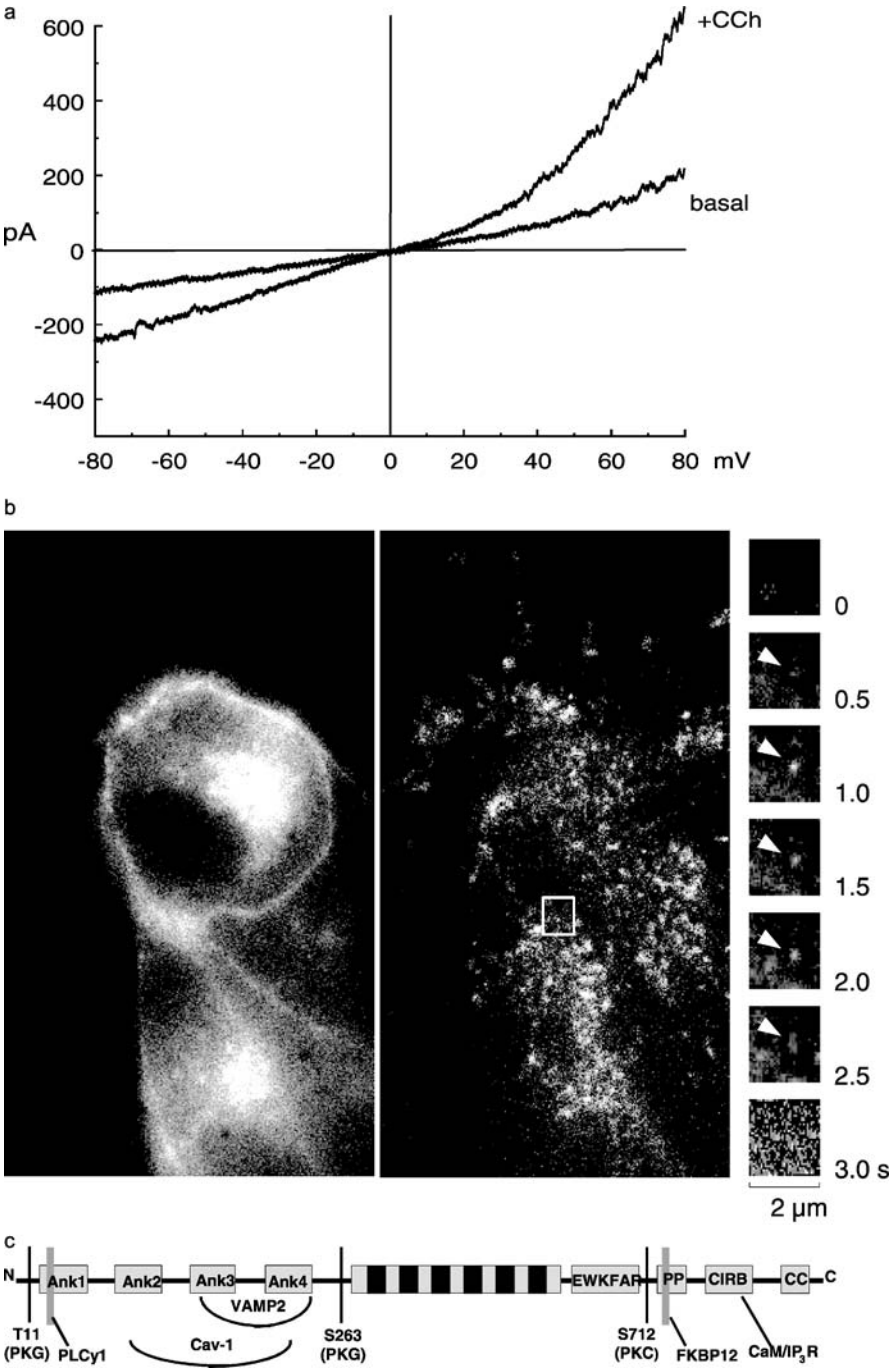
enables formation of store-operated TRPC3 cation channels (Liu et al. 2005). In essence, both the binding of lipid mediators to TRPC3 channels complexes and an ill-defined stimulus generated by reduction of the filling state of intracellular $Ca^{2+}$ stores are considered as gating mechanisms for TRPC3 channels. Recently, channels generated by the long TRPC3 splice variant TRPC3a were found sensitive to both activation via the $G_q$–PLC pathway and to direct store depletion (Yildirim et al. 2005). Signalling proteins potentially involved in gating of TRPC3 channels are the inositol 1,4,5-trisphosphate ($IP_3$) receptor ($IP_3R$) and calmodulin (CaM) (Zhang et al. 2001), which bind to a combined interaction domain in the C-terminus of TRPC3 that has been termed CIRB (CaM–$IP_3$ receptor-binding site) (Fig. 2c; Tang et al. 2001). $IP_3R$-mediated gating of TRPC3 channels was suggested as a mechanism that confers sensitivity of TRPC3 channels to the filling state of intracellular $Ca^{2+}$ stores in terms of a "conformational coupling model" (Berridge 1995; Irvine 1990). This concept was furthered along by the identification of another integral membrane protein resident in the membrane of the endoplasmic reticulum, junctate, which was found to associate with both TRPC3 and the $IP_3R$ (Treves et al. 2004). However, a key role of $IP_3Rs$ was questioned by other studies demonstrating the presence of PLC-dependent as well as store-operated function of TRPC3 in expression systems lacking all three isoforms of the $IP_3R$ (Wedel et al. 2003). Nonetheless, $Ca^{2+}$ -dependent binding of CaM to the CIRB region was shown to interfere with the $IP_3R$–TRPC3 interaction and to inhibit TRPC channel activity (Zhang et al. 2001). Intracellular $Ca^{2+}$ has repeatedly been demonstrated as a key regulator of TRPC3 channels, and it governs TRPC3 conductances in a rather complex manner, as both permissive (Zitt et al. 1997) and inhibitory effects of intracellular $Ca^{2+}$ have been demonstrated (Lintschinger et al. 2000).

Importantly, classical gating processes, which determine open probability, as well as processes that control plasma membrane presentation of channels, are considered essential for cellular regulation of TRPC3 conductances. Rapid enhancement of TRPC3 channel density in the plasma membrane may explain activation of a TRPC3 conductance without a classical gating process. As recently reported for TRPC5 (Bezzerides et al. 2004), a substantial fraction of TRPC3 appears to be targeted to a pool of highly mobile, plasma membrane-associated vesicles as evident from high-resolution fluorescence microscopy experiments (TIRFM, Fig. 2b). Rapid insertion and retrieval of TRPC3 channels via regulated exo- and endocytosis may therefore be considered as one mechanism regulating cellular TRPC3 conductances. However, a recent study suggests that activation of TRPC3 channels via the $G_q$–PLC pathway involves a process that is independent of membrane recruitment of channels (Smyth et al. 2006). Further analysis of the contribution of gating mechanisms and mechanisms that merely govern membrane insertion/retrieval of TRPC3 channels in conductance activation will require a thorough analysis at the single channel level.

## 2.3 Subcellular Targeting and Cellular Regulation

TRPC3 has been suggested as part of a multimolecular signalling complex containing proteins of the Gq–PLC pathway, proteins of the endoplasmic reticulum (ER) membrane and scaffolds and adaptor proteins such as ezrin and caveolin-1 (Cav-1) (Lockwich et al. 2001). Interaction between TRPC3 and other proteins have been identified that may as well be essential for correct targeting or activation of TRPC3 conductances, such as immunophilins, which interact with TRPC3 via the C-terminal proline-rich region (Sinkins et al. 2004). Appropriate assembly of TRPC3 pore complexes with scaffolds and adaptor proteins is likely to enable particular mechanisms of cellular regulation of TRPC3 conductances. Structural motifs in TRPC3 that are involved in such interactions are highlighted in Fig. 2c. Cytoskeletal rearrangements were found to trigger internalization of TRPC3 complexes, reminiscent of the internalization of caveolae, and Cav-1 was demonstrated to co-localize and associate with TRPC3 (Lockwich et al. 2001). Consequently, a caveolin-binding motif has been identified between aa 324–351 (Brazer et al. 2003) in the N-terminus

**Fig. 2 a** Current-to-voltage relation of basal and carbachol-stimulated TRPC3 conductances in HEK293 cells. Typical whole-cell membrane currents recorded during voltage-ramp protocols in TRPC3-expressing HEK293 cells (T3–9) under basal conditions and after challenge with carbachol (CCh, 200 μM) are shown. Bath solution contained 137 mM NaCl, 5.4 mM KCl, 10 mM hydroxyethylpiperazine ethanesulphonic acid (HEPES), 10 mM glucose, 1 mM MgCl (nominally $Ca^{2+}$ free). Patch pipettes contained 120 mM Cs-methanesulphonate, 20 mM CsCl, 10 mM HEPES, 1 mM $MgCl_2$, 1 mM ethyleneglycoltetraacetic acid (EGTA; pH adjusted to 7.4). Voltage-clamp protocols (depolarizing ramps from −100 to +80 mV/0.6 V/s, 0.2 Hz, holding potential −70 mV) were controlled by pClamp software (Axon Instruments, Foster City). Experiments were performed at room temperature. **b** Rapid membrane insertion and retrieval of TRPC3 in HEK293 cells visualized by total internal reflection fluorescent microscopy (TIRFM). *Left*: Epifluorescence image illustrating the cellular distribution of YFP-TRPC3 in HEK293 cells. *Right*: TIRFM images illustrating clustered localization of YFP-TRPC3 in the cell membrane, with a series of small images representing a sequence illustrating the time course of the fluorescence within a defined area, as indicated (*white square*). The *arrow* highlights the appearance of a TRPC3 cluster probably due to a vesicle fusion event, and its disappearance within 3 s. **c** Putative sites relevant for regulatory phosphorylation of TRPC3 and for protein–protein interactions. Sites relevant for regulatory phosphorylation by the cyclic guanosine monophosphate (cGMP)-dependent kinase protein kinase G (*PKG*) and *PKC* are indicated along with domains involved in the interaction of TRPC3 with phospholipase Cγ1 (*PLCγ1*), caveolin-1 (*Cav-1*), vesicle-associated membrane protein (*VAMP*), FK506 binding protein 12 (*FKBP12*), calmodulin (*CaM*), and inositol-tris-phosphate receptor (*$IP_3R$*). The domain for mutually exclusive binding of CaM or the $IP_3R$ designated as CIRB is shown


a
600
400
200
pA
0
-200
-400
+CCh
basal
-80 -60 -40 -20 0 20 40 60 80 mV
b
0
0.5
1.0
1.5
2.0
2.5
3.0 s
2 µm
c
N
Ank1
Ank2
Ank3
Ank4
VAMP2
EWKFAR
PP
CIRB
CC
C
T11 (PKG)
PLCγ1
Cav-1
S263 (PKG)
S712 (PKC)
FKBP12
CaM/IP3R

of TRPC3. It remains to be clarified if targeting of TRPC3 to the specific membrane environment of caveolae is essential for channel gating by lipids, or if caveolin-TRPC3 association is involved in cellular trafficking of TRPC3 complexes. Importantly, oxidative modification of membrane lipids, specifically of cholesterol, was found to markedly promote TRPC3 activity, a mechanism that may enable TRPC3 to serve as a sensor for the cellular redox state as proposed for channels in vascular endothelial cells (Balzer et al. 1999; Groschner et al. 2004). Such redox-dependent lipid regulation of TRPC3 may well be related to targeting of TRPC3 channels to the cholesterol-rich environment of caveolae. Consistently, cholesterol has recently been found to promote membrane presentation of TRPC3 (Graziani et al. 2006).

The cytoplasmic N-terminus of TRPC3 (aa 123–221) contains a site for interaction with a protein that most likely governs vesicular trafficking of TRPC3, the vesicle-associated membrane protein VAMP2 (Singh et al. 2004). Moreover, correct plasma membrane targeting of TRPC3 has been shown to involve the ankyrin domains in the N-terminus of TRPC3 (Wedel et al. 2003), as well as a unique interaction between TRPC3 and PLCγ1, which generates a composite PH (pleckstrin homology) domain (van Rossum et al. 2005). This bimolecular domain comprising two incomplete lipid binding structures represented by the very N-terminus of TRPC3 and PH-c of PLCγ1 was found to bind $PIP_2$ and sphingosine-1-phosphate (van Rossum et al. 2005) and has been suggested as a structure essential for plasma membrane targeting and the function of TRPC3 channel complexes. Plasma membrane targeting appears to be governed in addition by the CIRB (761–795 aa) region in the C-terminus of TRPC3 (Wedel et al. 2003).

## 2.4 Regulatory Phosphorylation

TRPC3 displays several potential sites for regulatory phosphorylation in both the N-and C-terminal cytoplasmic domain (Fig. 2c). Protein kinase C (PKC) has been implicated in down-regulation of TRPC3 activity via phosphorylation of S712 (Trebak et al. 2005), and cyclic GMP (cGMP)-dependent phosphorylation has been shown to suppress TRPC3-mediated store-operated $Ca^{2+}$ entry in HEK293 cells mediated by phosphorylation of TRPC3 at positions T11 and S263 (Kwan et al. 2004). Notably, suppression of TRPC3 activity in response to PKC activation was suggested to involve PKG and a cross-talk between these phosphorylation pathways in PKG-expressing cell systems and in endothelial cells (Kwan et al. 2005). Moreover, PLC-dependent activation of TRPC3 has been demonstrated to require regulatory phosphorylation involving the non-receptor tyrosine kinase Src. Pharmacological inhibition of Src, as well as a dominant negative Src, suppressed TRPC3 activation (Vazquez et al. 2004).

## 3 Pharmacology

Useful blockers with appreciable selectivity for TRPC3 channels are still missing. Nonetheless, a number of non-selective $Ca^{2+}$ entry blockers were identified as inhibitors of TRPC3 channel activity. Such non-selective inhibitors include the organoborane, 2-aminoethoxydiphenyl borate (2APB), and the imidazole derivative SKF96365, as well as flufenamate and lanthanides. Since 2APB was initially described as a membrane-permeant inhibitor of $IP_3$Rs, the observed suppression of TRPC3-mediated $Ca^{2+}$ entry has been attributed to effects on $IP_3$Rs (Ma et al. 2000). However, a more recent report demonstrated direct inhibitory effects of 2APB on TRPC3 channels. $IP_3$-independent, DAG-triggered TRPC3 activity was inhibited by about 50% with 2APB (30 μM) in HEK293 cells expressing TRPC3 (Lievremont et al. 2005). The apparent incomplete inhibition of lipid mediator-induced TRPC3 channel activity is in contrast to the reported complete block of store-operated channel activity. This discrepancy may be taken as support for the concept of multiple TRPC3 pore structures. A similar phenomenon was reported for the sensitivity of TRPC3 to the lanthanides $Gd^{3+}$ and $La^{3+}$. When expressed in HEK293 cells, TRPC3 channels were completely inhibited by 150 μM $La^{3+}$ (Zhu et al. 1998), while for channels expressed in COS-1 cells complete block required 10 μM of $La^{3+}$ (Preuss et al. 1997). $Gd^{3+}$ was found to inhibit receptor/PLC-regulated TRPC3 in HEK293 cells completely at 200 μM but lacked any effect on this TRPC3 pathway up to 10 μM concentrations, which suppressed an endogenous $Ca^{2+}$ entry pathway in this cell line (Zhu et al. 1998). TRPC3, expressed at limited levels in DT40 chicken B lymphocytes, was reported to generate a store-operated $Ca^{2+}$ entry pathway that is sensitive to $Gd^{3+}$ in the low micromolar range (Vazquez et al. 2003), again indicating that lanthanides may discriminate different TRPC3 containing pore structures.

Lanthanides have been shown to block TRPC3 currents in CHO cells at rather low levels from the cytoplasmic side ($EC_{50}$ 0.02 μM; Halaszovich et al. 2000). Thus intracellular accumulation of lanthanides may contribute at a variable degree to block of TRPC3 channels, depending on the experimental conditions.

SKF96365 (1-(β-[3-(4-methoxy-phenyl)propoxy]-4-methoxyphenethyl)-1H-imidazole hydrochloride) and flufenamate represent inhibitors that have been widely employed for inhibition of TRPC cation channels. Like 2APB and lanthanides, these compounds effectively suppress membrane transport pathways in a rather non-selective manner with remarkable inhibitory potency for channels outside the TRP superfamily, such as voltage-gated $Ca^{2+}$ channels (Merritt et al. 1990) as well as $Cl^-$ channels (Sergeant et al. 2001). Thus, none of the currently available inhibitors is suitable as a tool to identify native TRPC conductances. The mechanisms by which the organic compounds suppress TRPC3 are largely elusive, and indirect mechanisms such as interference with regulatory components of TRPC3 complex may be considered.

## 4 Biological Relevance and Emerging Biological Roles of TRPC3

Considering the predominant expression of TRPC3 in specific regions of the brain (Li et al. 2005; Riccio et al. 2002) and in the heart (Eder et al. 2006; Goel et al. 2006), along with particular high expression in embryonic tissues (Strubing et al. 2003), one might speculate about a function of TRPC3 in development of neuronal and cardiac tissue, including a possible role as a determinant of cell proliferation and differentiation. So far, information from a knock-out animal model is lacking, and alternative approaches in cellular model systems—such as dominant-negative suppression, small interfering RNA (siRNA)-mediated knock-down of expression or selective block of channels by isoform specific antibodies—have so far barely been exploited to investigate the role of TRPC3 in neuronal or cardiac cells. Nonetheless, such experiments have provided evidence for functional expression and physiological significance of TRPC3 channels in classical non-excitable tissues such as epithelial and endothelial cells (Bandyopadhyay et al. 2004; Groschner et al. 1998; Liu et al. 2005) as well as immune cells (Philipp et al. 2003).

The role of TRPC3 channels in excitable tissues is so far barely understood. Importantly, the function of TRPC3 channels in excitable cells may critically depend on the functional link of TRPC3 to voltage-gated channels and other prominent ion transport systems in these cells. One example for such a signalling partnership is the recently reported association of TRPC3 channels to the cardiac-type $Na^+/Ca^{2+}$ exchanger NCX1 (Rosker et al. 2004; Eder et al. 2006). TRPC3 may govern $Ca^{2+}$ homeostasis in NCX1-expressing cells, not only via $Ca^{2+}$ permeation through its pore structure but also by translation of TRPC3-mediated $Na^+$ entry into $Ca^{2+}$ signals. A similar partnership may exist between TRPC3 and voltage-gated $Ca^{2+}$ channels. In view of the significant basal activity of TRPC3 channels, which may be a property of native TRPC3 channels (Albert et al. 2006), it appears reasonable to speculate that enhanced expression of TRPC3 by itself might result in relevant reduction of cell membrane potential and profound alterations of the function of excitable cells. An interesting observation, with respect to this concept, is the recently observed association of pulmonary artery smooth muscle dysfunction with increased expression of TRPC3 (Yu et al. 2004). Elevated density of TRPC3 is expected to cause membrane depolarization of vascular smooth muscle due to enhanced constitutive TRPC3 activity, leading to increased, pathophysiologically relevant $Ca^{2+}$ entry through CaV1.2 (L-type) $Ca^{2+}$ channels.

In summary, our current knowledge suggests TRPC3 as a multifunctional and versatile sensor molecule of particular physiological and pathophysiological relevance.

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