HEP (2007) 179:575–591
© Springer-Verlag Berlin Heidelberg 2007

TRPC Channels: Integrators of Multiple Cellular Signals

J. Soboloff[1] · M. Spassova[1] · T. Hewavitharana[1] · L.-P. He[1] · P. Luncsford[1] · W. Xu[1] · K. Venkatachalam[2] · D. van Rossum[3] · R. L. Patterson[4] · D. L. Gill[1] (✉)

[1]Department of Biochemistry and Molecular Biology,
University of Maryland School of Medicine, 108 North Greene Street,
Baltimore MD, 21201, USA
dgill@umaryland.edu

[2]Department of Biological Chemistry, Johns Hopkins School of Medicine, ,
Baltimore MD, 21205, USA

[3]Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore MD, 21205, USA

[4]Department of Biology, Pennsylvania State University, University Park, PA, 16801, USA

1 Introduction 576

2 TRPC Ion Channel Properties and Cellular Role 577

3 Modes of Activation: Are TRPC Channels SOCs? 577

4 Role of Phospholipase C in TRPC Function 579

5 TRPC Channel Modification by Protein Kinase C 580

6 Role of the $InsP_3$ Receptor in TRPC Activation 582

7 Pharmacology of TRPC Channels 583

8 Biological Relevance and Emerging Roles for TRPC Channels 585

References 587

Abstract TRPC channels are ubiquitously expressed among cell types and mediate signals in response to phospholipase C (PLC)-coupled receptors. TRPC channels function as integrators of multiple signals resulting from receptor-induced PLC activation, which catalyzes the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG). $InsP_3$ depletes Ca^{2+} stores and TRPC3 channels can be activated by store-depletion. $InsP_3$ also activates the $InsP_3$ receptor, which may undergo direct interactions with the TRPC3 channel, perhaps mediating store-dependence. The other PLC product, DAG, has a direct non-PKC-dependent activating role on TRPC3 channels likely by direct binding. DAG also has profound effects on the TRPC3 channel through PKC. Thus PKC is a powerful inhibitor of most TRPC channels and DAG is a dual regulator of the TRPC3 channel. PLC-mediated DAG results in rapid channel opening followed later by a slower DAG-induced PKC-mediated deactivation of the channel. The

decreased level of PIP_2 from PLC activation also has an important modifying action on TRPC3 channels. Thus, the TRPC3 channel and PLCγ form an intermolecular PH domain that has high specificity for binding PIP_2. This interaction allows the channel to be retained within the plasma membrane, a further operational control factor for TRPC3. As nonselective cation channels, TRPC channel opening results in the entry of both Na^+ and Ca^{2+} ions. Thus, while they may mediate Ca^{2+} entry signals, TRPC channels are also powerful modifiers of membrane potential.

Keywords Calcium signaling · TRPC channels · Store-operated channels

1 Introduction

Ca^{2+} signals in response to receptors mediate and control countless cellular functions ranging from short-term responses such as secretion and contraction to longer term control of growth, cell division, and apoptosis. TRPC channels are ubiquitously expressed among cell types and mediate important signals in response to phospholipase C (PLC)-coupled receptors. TRPC channels function as integrators of multiple signals resulting from receptor-induced PLC activation, which catalyzes the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG). $InsP_3$ depletes Ca^{2+} stores and evidence indicates TRPC3 channels are store-operated. $InsP_3$ also activates the $InsP_3$ receptor, which is able to undergo direct interactions with the TRPC3 channel, perhaps mediating store-dependence. The other PLC product, DAG, has a direct non-protein kinase C (non-PKC)-dependent activating role on TRPC3 channels likely as a direct result of binding to the TRPC3 molecule itself. DAG also has profound effects on the TRPC3 channel through PKC. Thus PKC is a powerful inhibitor of most TRPC channels and DAG is a dual regulator of the TRPC3 channel. PLC-mediated DAG results in rapid channel opening followed later by a slower DAG-induced PKC-mediated deactivation of the channel. The decreased level of PIP_2 as a result of PLC activation also has an important modifying action on TRPC3 channels. Thus, the TRPC3 channel and PLCγ form an intermolecular pleckstrin homology (PH) domain that has high specificity for binding PIP_2. This interaction with the TRPC3 channel allows the channel to be retained within the plasma membrane, a further operational control factor determining channel function in the plasma membrane. As nonspecific cation channels, TRPC channel opening results in the entry of both Na^+ and Ca^{2+} ions. Thus, while they may mediate Ca^{2+} entry signals, TRPC channels are also powerful modifiers of membrane potential. Here we discuss recent information on the control and significance of the operation of TRPC channels' coupling properties, TRPC pharmacology, and new insights regarding the physiological roles of these cation channels.

2 TRPC Ion Channel Properties and Cellular Role

Although often studied in the context of Ca^{2+} entry, all of the TRPC channels are nonselective cation channels, with no selective preference for Ca^{2+} over other divalent or monovalent cations (Clapham 2003; Spassova et al. 2004). This is in distinct contrast to known store-operated Ca^{2+} channels (SOCs), which show remarkable selectivity for Ca^{2+} (Parekh and Penner 1997; Parekh and Putney 2005). Thus, Sr^{2+}, which has similar properties to the Ca^{2+} ion including the ability to alter the fluorescence properties of indicator dyes such as fura-2, enters cells poorly in response to store-depletion. However, Sr^{2+} readily enter cells transfected with TRPC channels in response to either receptor activation or increases in DAG concentrations (Ma et al. 2000; Venkatachalam et al. 2003; He et al. 2005). While this has provided a useful tool to distinguish between TRPC channels and SOCs, it does not address the physiological role of TRPC channels. Considering the relatively small magnitude of the Ca^{2+} ion difference across the plasma membrane compared with that for Na^{+}, it is likely that the opening of TRPC channels has a greater effect upon membrane potential than on Ca^{2+} concentration. Recent studies provide strong support for this concept (Welsh et al. 2002; Beech et al. 2004; Beech 2005; Reading et al. 2005; Soboloff et al. 2005). After careful analysis of the expression patterns of TRPC channels in A7r5 vascular smooth muscle cells using real-time PCR and Western blot, we established that TRPC6 was the only member of the DAG-responsive TRPC3/6/7 subfamily of channels expressed in this system. Hence, responses to the soluble DAG analog 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) appear primarily to be mediated by TRPC6. However, TRPC6 short interfering RNA (siRNA), which decreased TRPC6 levels without significant compensation by other channels, decreased the OAG-induced current, but not OAG-induced Ca^{2+} entry (Soboloff et al. 2005). This was apparently because the Ca^{2+} entry was primarily mediated by voltage-operated Ca^{2+} channels rather than TRPC6 channels. Moreover, we determined that, even after TRPC6 knockdown, the response of residual TRPC6 channels was more than sufficient to depolarize the cells. Hence, the primary role of TRPC6, at least in this system, was depolarization rather than Ca^{2+} entry.

3 Modes of Activation: Are TRPC Channels SOCs?

There is controversy concerning the role of TRP channels in mediating store-operated Ca^{2+} entry. TRP channels appear ubiquitously among cell types, and multiple TRP channel subtypes are expressed in most cells (Venkatachalam et al. 2002; Clapham 2003). One problem with assessing the physiological role of TRP channels is that functional analysis of overexpressed channels is usually

against a background of endogenous SOCs and TRP channels. Similarly, reduction of TRP channel expression by antisense, siRNA, or knockout approaches can be confused by the functional overlap and redundancy with multiple endogenous TRP channel subtypes. One unifying characteristic among the *Drosophila* TRP and most members of the mammalian TRPC family, is their response to receptor-activated PLC (Montell 2001). Thus, these channels can be classified as "receptor-operated" channels (ROCs). However, PLC activation and concomitant $InsP_3$ production ensures at least some Ca^{2+} store release in most cell types. Therefore, the question of whether the TRPC channels function as SOCs as opposed to ROCs is difficult to answer, and there is obvious overlap in these modes of entry. The "hallmark" that has been used to define store-operated Ca^{2+} entry is a response to store-depletion alone using Ca^{2+} pump blockers such as thapsigargin, or ionophores such as ionomycin (Parekh and Penner 1997). However, store-emptying with these agents is not a physiological process per se. Thus, physiologically, stores are released following PLC-coupled receptor activation, and the consequences of PLC activation are not limited to store-release alone.

There seems little disagreement that members of the quite diverse TRP family of channels do not yet fulfill the criteria of being authentic Ca^{2+} release-activated Ca^{2+} (CRAC) channels. The Ca^{2+}-selective TRPV5 and TRPV6 channels may represent the closest match to characteristics of CRAC channels (Yue et al. 2001), but it is clear that there are a number of channel distinctions from CRAC channels (Voets et al. 2001; Kahr et al. 2004). Whether or not exogenously expressed TRP channels operate in a "store-dependent" mode is controversial, with studies claiming a number of TRPC, TRPM, and TRPV channels are activated when stores are emptied, and as many studies suggesting they are not (extensively reviewed in Venkatachalam et al. 2002; Spassova et al. 2004).

This abundance of apparently conflicting information on the coupling of TRPC channels to store-emptying is overwhelming at first sight. However, in heterologous expression systems in which the TRP channels are expressed alongside endogenous SOCs, the determination of whether TRP channels are directly activated by store-depletion as opposed to modifying or being modified by existing SOCs is difficult. Thus, it appears very hard to truly assess the TRPC channel phenotype in overexpression systems. In certain cell types, the high expression levels of specific TRPC channel subtypes have allowed more definitive analysis of endogenously expressed TRPC channels. However, in such cases the channels are generally not store-operated. For example, TRPC3 channels are highly expressed in pontine neurons and allow passage of Na^{+} and Ca^{2+} ions in response to PLC-coupled receptors, but are not activated by store-emptying (Li et al. 1999). We have made similar observations in vascular smooth muscle cells regarding the mechanism of activation of TRPC6 (Soboloff et al. 2005). However, the variation in the observed activation of TRPC channels may result from their assembly into multimeric structures (Montell 2001). Thus, it was originally observed that coexpression of the *Drosophila*

TRP and TRPL channels in mammalian cells resulted in a store-operated phenotype, whereas when they were singly expressed the channels were store-independent (Xu et al. 1997). A heteromer comprising TRPC1, TRPC3, and TRPC7 was recently reported to mediate SOC activity in human embryonic kidney (HEK)-293 cells (Zagranichnaya et al. 2005); however, the entry of Ba^{2+} mediated by this combination of TRPC channels suggests a divergence from endogenous SOCs. The overall conclusion from many studies is that it is possible that TRPC channels may be activated in response to store depletion, but it is unproved whether they actually contribute to endogenously operating SOCs or CRAC channels.

The questionable physiological relevance of artificially emptying stores (Gill and Patterson 2004) and the uncertainty of overexpressing TRP channels makes it difficult to draw conclusions on their store-dependence. Moreover, since many of the TRP channels are widely expressed, it is difficult to assess whether using knockout or knockdown approaches has led to a definitive identification of the function of natively expressed TRP channels (Gill and Patterson 2004). In some cases, Ca^{2+} signaling has been affected by such deletion approaches, but curiously, the "lost" currents do not correspond to those attributed to the overexpression of the corresponding channel. The properties of channels may be influenced not only by heteromeric makeup, but also by any one of the many proteins suggested to be in tight proximity in the molecular domains or "signalplexes" (Montell et al. 2002a). When overexpressed, particularly by transient overexpression, which yields far higher channel levels than endogenous expression, it is very likely that channels are not assembled and organized as in native cells. We should also consider the importance of other classes of "receptor-activated" or "store-dependent" channels that, unlike CRAC, are not selective for Ca^{2+} ions. This is an increasingly voiced perspective (Gill and Patterson 2004), and it is likely that nonselective cation channels play crucial roles in mediating depolarization responses and hence contributing to the modification of a number of other voltage-dependent channels or electrogenic transporters (for example, the K^+ channels, Cl^- channels, or the Na^+-Ca^{2+} exchanger).

4
Role of Phospholipase C in TRPC Function

In the fly retina, TRP channels appear to be organized by the PDZ-containing scaffold protein INAD, and they are likely in intimate contact with the rhodopsin-triggered phototransduction machinery (Montell 1999). Less is known about the organization of TRPC channels in mammalian cells, but there are indications that the PDZ-containing Na^+/H^+ exchanger regulatory factor (NHERF) protein interacts with TRPC channels (Tang et al. 2000) and may organize a spatial relationship with G protein-coupled receptors (GPCRs), PLC, and the cytoskeleton in an analogous fashion (Suh et al. 2001).

It is likely that many different structural and adaptor proteins contribute to such a coupling complex. In recent studies, we determined that PLC-γ plays an adaptor-role independent of its enzymic activity in the coupling between receptors and the activation of Ca^{2+} entry (Patterson et al. 2002). Thus, experiments revealed that knocking down of PLC-γ1 or PLC-γ2 isoforms prevented GPCRs (which activate PLC-β) from activating Ca^{2+} entry, even though there was still $InsP_3$-mediated Ca^{2+} release. In contrast, thapsigargin (TG)-induced Ca^{2+} entry was not affected. Using DT40 B cells in which PLC-γ2 (the only isoform in B cells) was knocked out, GPCR-induced Ca^{2+} entry was prevented without an effect on purely store-operated Ca^{2+} entry induced by TG. The B cell receptor (BCR) in these cells activates the enzymic activity of PLC-γ2, and as expected, the $InsP_3$-mediated Ca^{2+} release is restored in the PLC-γ2-knockout cells by expression of wildtype PLC-γ2 but not by the lipase-inactive mutant. Whereas the PLC-γ2 knockout cells were devoid of Ca^{2+} entry in response to the muscarinic receptor (a GPCR), both wildtype and LIM PLC-γ2 restored Ca^{2+} entry in response to activation of the muscarinic receptor. The results indicate that PLC-γ2 plays a permissive role that is independent of its enzymic activity in mediating receptor- but not TG-induced Ca^{2+} release. We have since determined that this occurs as a result of intermolecular PH domains that form when PLC-γ and TRPC channels interact (van Rossum et al. 2005).

Hence, whereas both TRPC channels and PLC-γ lack complete PH domains along with the corresponding capacity to bind to lipids (in particular, PIP_2), they can interact with each other, causing the formation of a functional PH domain containing portions of each molecule. The resulting increase in affinity for lipids in the plasma membrane causes the TRPC channels to be retained at the plasma membrane, rather than recycled into the cell. Therefore, PLC-γ plays a dual role (1) mediating production of DAG and $InsP_3$, and (2) providing a permissive adaptor that is required for GPCRs to activate Ca^{2+} entry channels. The implication from this is that PLC-γ allows coupling to channels without necessarily requiring release of Ca^{2+} from stores.

5 TRPC Channel Modification by Protein Kinase C

For the TRPC family of proteins, it seems that their primary mechanism of activation is through PLC activation, making them functionally analogous to their fly homologs. While $InsP_3$ clearly causes Ca^{2+} release, the other product of PLC, DAG, has a direct role in TRPC activation. The closely related TRPC3, TRPC6, and TRPC7 subgroup of channels, therefore, is directly activated by DAG and its cell-permeant analogs (Hofmann et al. 1999; Okada et al. 1999; Ma et al. 2000; Venkatachalam et al. 2001; Zitt et al. 2002). This action of DAG is independent of PKC (Hofmann et al. 1999; Okada et al. 1999) and may be mediated by an N-terminal domain in the TRPC6 channel (Zhang

and Saffen 2001). However, the response to DAG is subgroup-specific. Thus, overexpressed TRPC1, TRPC4, and TRPC5 channels do not respond to DAG (Hofmann et al. 1999; Schaefer et al. 2000; Venkatachalam et al. 2003). DAG also has an important dual role in controlling TRPC channels (Venkatachalam et al. 2003; Venkatachalam et al. 2004). Hence, while not activating TRPC4 or TRPC5 channels, DAG in fact has a profound inhibitory effect on both channels.

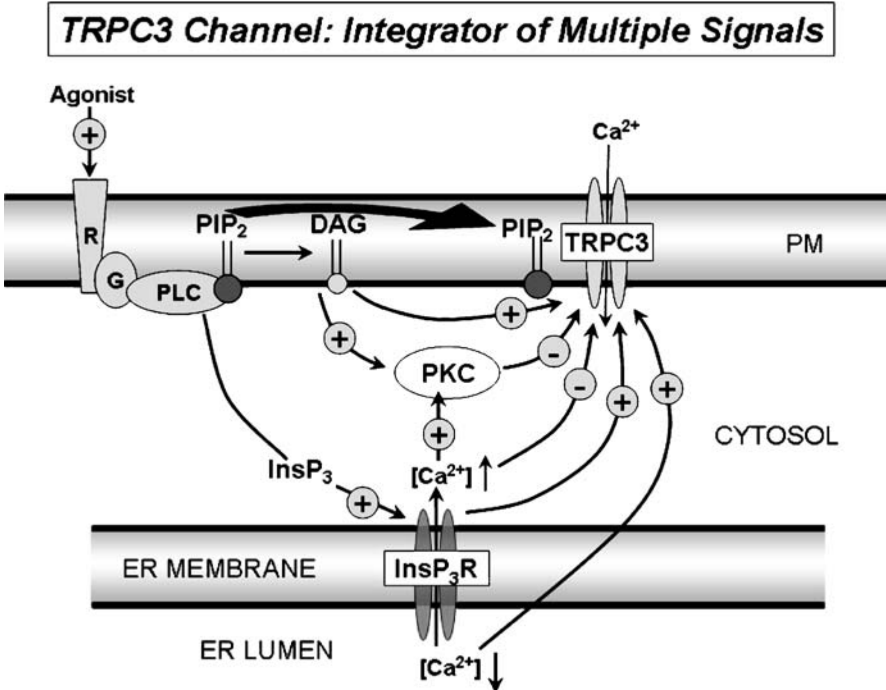

Fig. 1 Model depicting the different signals that activate or modify the TRPC3 channel (from Soboloff et al. 2006). PLC has multiple effects on the channel. Thus, PLC enzymatically breaks down PIP_2 into DAG and $InsP_3$. $InsP_3$ depletes stores of Ca^{2+} and much information indicates TRPC3 channels are store-operated. $InsP_3$ also activates the $InsP_3$ receptor, which is able to undergo direct interactions with the TRPC3 channel, perhaps mediating store-dependence. The other PLC product, DAG, has a direct non-PKC-dependent activating role on TRPC3 channels likely as a direct result of binding to the TRPC3 molecule itself. DAG also has profound effects of the TRPC3 channel through PKC. PKC is therefore a powerful inhibitor of most TRPC channels and DAG is a dual regulator of the TRPC3 channel. PLC-mediated DAG results in rapid channel opening followed later by a slower PKC-mediated deactivation of the channel. The decreased level of PIP_2 level as a result of PLC activation also has an important modifying action on TRPC3 channels. Hence, the TRPC3 channel and PLCγ form an intermolecular PH domain that has high specificity for binding PIP_2. This interaction with the TRPC3 channel allows the channel to be retained within the plasma membrane, a further operational control factor determining channel function in the plasma membrane. Details of these mechanisms are given in the text

Moreover, this inhibitory effect is clearly mediated through PKC (in contrast to the stimulatory action on TRPC3 channels). The effect was seen not only with exogenously added DAG. Thus, we could modify endogenously produced DAG by manipulating both DAG kinase and DAG lipase the two major metabolizing enzymes controlling turnover of DAG (Venkatachalam et al. 2003).

We were also able to show that this same PKC-mediated inhibition functions on TRPC3 channels that are activated by DAG. Treatment with the PKC activator, PMA, blocks the stimulation by subsequent addition of DAG. The action of PMA, however, is completely prevented by GF 109203X, a reliable aminoalkyl bisindolylmaleimide PKC blocker. In this case, the action of DAG is not only rapid, but longer lasting, indicating that endogenously activated PKC likely results in the fast turnoff of TRPC3 channels. With TRPC3 channels, therefore, the single molecule has two opposing actions on the same channel. DAG clearly activates the channel through a PKC-independent mechanism. Thereafter, there appears to be a slower DAG-induced PKC-mediated inhibition of the channel, suggesting that this is an important feedback mechanism. This feedback mechanism takes on yet added significance considering that the Ca^{2+} entering the cytoplasm through the TRP channels will enhance the activation of PKC and hence the turning off of the channel. Interestingly, we were able to show that Ca^{2+} entry induced by receptors in nontransfected HEK-293 cells was partially (50%) blocked by PKC activation (Fig. 1), suggesting that endogenous TRPC-like channel activity may account for some of the entry.

Despite all this information, a question that remains is: What mediates the receptor-induced activation of TRPC4 and TRPC5 channels? Thus, while the two channels are receptor-activated just like members of the TRPC3/6/7 group of channels, they are not activated by DAG (Venkatachalam et al. 2002). We and others have speculated that, in addition to DAG and $InsP_3$ production, another consequence of receptor-induced PLC activation is a decrease in PIP_2. Hence, the possibility that the activation of TRPC channels may also reflect decreased PIP_2 levels is an area of investigation worthy of considerable attention.

6
Role of the $InsP_3$ Receptor in TRPC Activation

There have been a number of studies indicating that TRPC3 channels can specifically interact with $InsP_3$ receptors. $InsP_3R$ constructs containing the N-terminal $InsP_3$ binding domain were sufficient to couple to TRPC3 channels and render them activatable by $InsP_3$ (Kiselyov et al. 1999). It was revealed that a relatively short segment within the C-terminus of TRPC3 was required for interaction with the $InsP_3R$, and two specific $InsP_3R$ sequences close to the $InsP_3$ binding domain interacted with TRPC3 (Boulay et al. 1999). Studies have shown that $InsP_3$ receptors interact with an inhibitory calmodulin-binding site

on TRPC3, displacing calmodulin and thereby activating the channel (Tang et al. 2001; Zhang et al. 2001).

While the evidence for TRPC–$InsP_3R$ interactions is quite compelling, the question of the role of $InsP_3Rs$ in physiological store-operated Ca^{2+} entry is less clear. The actions of the two $InsP_3R$ antagonists, 2-aminoethoxydiphenyl borate (2-APB) and xestospongin C, to block both endogenous store-operated Ca^{2+} entry and receptor-induced TRPC3 activation was considered evidence for a mediating role of $InsP_3Rs$ in the activation of both channels (Ma et al. 2000). More compelling, however, were studies revealing that in the DT40 B lymphocyte triple $InsP_3R$ knockout variant line, store-operated Ca^{2+} entry was entirely identical to that observed in wildtype cells (Sugawara et al. 1997; Broad et al. 2001; Ma et al. 2001, 2002; Venkatachalam et al. 2001). Exhaustive searching for the presence of $InsP_3Rs$ in these knockout cells by examination of transcripts, full-length proteins or fragments thereof, $InsP_3$-binding activity, or physiological $InsP_3R$-mediated Ca^{2+} release in intact or permeabilized cells confirmed in all cases the absence of all $InsP_3Rs$ in these cells (Sugawara et al. 1997; Broad et al. 2001; Ma et al. 2001; Venkatachalam et al. 2001). Moreover, the blocking action of 2-APB on capacitative Ca^{2+} entry (CCE) was unaltered by $InsP_3R$ elimination (Broad et al. 2001; Ma et al. 2001; Ma et al. 2002). These and further studies indicated that its action was either upon the channel (Bakowski et al. 2001; Broad et al. 2001; Prakriya and Lewis 2001) or, more interestingly, upon the coupling machinery for SOCs (Ma et al. 2002; Schindl et al. 2002). However, TRPC3 channels expressed rapidly and with high efficiency in DT40 cells were shown to be activated by PLC-coupled receptors independently of $InsP_3R$ expression and independently of store-emptying (Venkatachalam et al. 2001). On the other hand, it was observed that TRPC3 channels expressed more slowly and at lower efficiency in the same cells were partially-dependent on $InsP_3R$ expression and wholly-dependent on store-emptying (Vazquez et al. 2001).

While apparently enigmatic, the coupling phenotype could rest on expression conditions (Venkatachalam et al. 2002; Vazquez et al. 2003; Putney 2004). Cells may have a limited quantity of the machinery required for store-coupling (be it junctions, $InsP_3Rs$, or linking proteins); thereby the level of expression of channels determines whether store-coupling occurs (Venkatachalam et al. 2002; Putney 2004).

7
Pharmacology of TRPC Channels

Recent studies have revealed the 3,5-bistrifluoromethyl pyrazole derivative BTP2 inhibits SOC/CRAC channels with approximately 100-fold greater potency than econazole or SKF (Ishikawa et al. 2003; Zitt et al. 2004; He et al. 2005). This has since been followed with the discovery that BTP2 also inhibits

TRPC channels with a similar potency (He et al. 2005). However, BTP2's actions are not nonspecific. It does not affect the properties of K^+ channels or voltage-gated Ca^{2+} channels, nor does it alter the Ca^{2+} permeability of mitochondria or ER (Ishikawa et al. 2003; He et al. 2005). Moreover, TRPV6 channels, with some properties similar to SOCs, are unaffected by BTP2 (He et al. 2005). Although the mechanism of this inhibition is not clear, we recently established that it decreases the open probability of the TRPC3 channels, without affecting amplitude, using single channel measurements of TRPC3 (He et al. 2005). Hence, rather than inhibiting conductance, BTP2 may target the process of opening or closing (or both the opening and closing) of the channel.

The observation that BTP2 appears to selectively inhibit SOC and TRPC channels indicates that TRPC channels share some common properties with SOC channels. This action is not limited to the TRPC3/6/7 channel family, since BTP2 also blocked TRPC5 with similar sensitivity as TRPC3 (He et al. 2005). While BTP2 does not appear to discriminate between TRPC channels, recent studies have revealed some specific pharmacological modifiers of TRPC channels. For example, flufenamate, a somewhat nonspecific cation channel blocker, reversibly enhances TRPC6 channel activity (Inoue et al. 2001), while inhibiting all other members of the TRPC family. Although this is the only published example of an agent that is specific for a single TRPC channel type, other agents are somewhat effective in differentiating between different TRPC subfamilies. Thus, Hisatsune et al. (2004) revealed a requirement for TRPC6 phosphorylation by Fyn, a member of the Src family of protein tyrosine kinases (PTKs). Specifically, Fyn enhanced DAG- and epidermal growth factor-induced TRPC6 activation by increasing Ca^{2+} conductance (Hisatsune et al. 2004). In a separate study, Vazquez et al. (2004) showed that the Src kinase phosphorylates TRPC3, and that the Src kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2) inhibits TRPC3 channel activation. Importantly, they also found that TRPC5 was not sensitive to PP2. Hence, it appears that this Src kinase sensitivity is unique to the TRPC3/6/7 subfamily of channels.

The lanthanides La^{3+} and Gd^{3+} also have differential effectiveness across TRPC channel function. Whereas SOC-mediated Ca^{2+} entry is sensitive to concentrations as lower than 1 μM, TRPC3/6/7 channels are only modified at 10- to 50-fold higher levels (Ma et al. 2000; Putney 2001). In contrast, TRPC4 and -5 are activated by lanthanides (Jung et al. 2003). Single channel measurements revealed that this activation resulted from a substantial increase in the open probability of TRPC5 channels due to three negatively charged residues close to the extracellular mouth of the channel pore (Jung et al. 2003). These residues are absent in other channel members, providing a clear explanation for why this response is unique to the TRPC4/5 subfamily (Jung et al. 2003). Based upon these studies, while TRPC channels have some similarities in their activation and channel properties, they appear to have some unique pharmacological properties.

8
Biological Relevance and Emerging Roles for TRPC Channels

Although the TRPC channels are similar proteins, both in their modes of activation (PLC-mediated) and channel properties (slow nonselective cation channels), differences in the expression patterns of the different proteins along with the phenotype of a number of different knockout models have revealed distinct biological roles for the different channels. Perhaps the clearest example of this is TRPC2. A pseudogene in humans and higher primates (Liman and Innan 2003), this protein is primarily expressed in the vomeronasal organ, where it mediates the pheromone response. Elimination of this gene leads to a clear change in behavioral responses of mice, indicating that TRPC2 is required for this biological function (Leypold et al. 2002; Stowers et al. 2002). In contrast, the physiological impact of elimination of the TRPC6 gene, which has a well-established role in vascular smooth muscle contraction (Inoue et al. 2001; Jung et al. 2002; Welsh et al. 2002; Soboloff et al. 2005), has been far less straightforward, due primarily to compensation by expression of TRPC3, a closely related channel (Dietrich et al. 2005). However, since, unlike TRPC6, TRPC3 exhibits significant constitutive activity, there was a significant increase in blood pressure and agonist-induced contractility. Hence, despite the considerable homology that exists between different TRPC channels, TRPC channels have distinct and likely irreplaceable biological functions.

Whereas knockout of TRPC6 in the whole animal was complicated by the upregulation of related proteins, TRPC6 RNA interference (RNAi) was achieved in cultured cells without significant compensation by other channels. Thus, in a recent study Soboloff et al. (2005), targeted RNAi was combined with a rigorous assessment of both message and protein, to provide new information on the presence and function of *endogenously* expressed TRPC6 channels in A7r5 smooth muscle cells. TRPC6 knockdown experiments revealed that an OAG-activated nonselective cation current with a current–voltage relationship close to that of known TRPC6 channels was substantially reduced (Soboloff et al. 2005). This reduction in current mirrored the reduction in TRPC6 protein. However, the corresponding TRPC6-mediated OAG-dependent entry of Ca^{2+} was not significantly altered by TRPC6 knockdown. Yet the OAG-induced Ca^{2+} entry was almost completely inhibited by L-type Ca^{2+} channel blockers, indicating Ca^{2+} was entering through L-type voltage-dependent Ca^{2+} channels. However, pharmacological characterization of this current revealed TRPC6-like characteristics. Thus, it was sensitive to inhibitors of Src kinase and it was strongly inhibited by activation of PKC known to inhibit TRPC channels (Venkatachalam et al. 2002, 2003). The explanation for these results is that the TRPC6 channel, as a nonselective cation channel, is predominantly mediating the entry of Na^{+} as opposed to Ca^{2+} ions, resulting in depolarization and the opening of L-type channels. Hence the TRPC6 channel is a mediator between

PLC-generated DAG and the activation of Ca^{2+} entry through L-type channels. A scheme depicting this signaling process is shown in Fig. 2.

Calculations reveal that even 90% reduction of TRPC6 channels would still allow depolarization sufficient to activate L-type channels. Thus, under conditions of RNAi resulting in approximately 90% reduction of TRPC6 protein and current carried by TRPC6 channel, there was still substantial depolarization-mediated activation of Ca^{2+} entry through L-type channels (Soboloff et al. 2005). The function of TRPC channels mediating depolarization and activation of L-type channels has also been indicated in other studies. Thus, in cerebral arteries, TRPC6 antisense treatment reduced pressure-induced depolarization and arterial constriction, suggesting that TRPC6 channels are activated as a result of pressure and may play an important role in the control of myogenic tone (Welsh et al. 2002). Recently, the TRPC3 channel, which is also expressed in cerebral arteries, was shown to mediate purinergic receptor-induced depolarization and contraction (Reading et al. 2005). Thus, members of the TRPC3/6/7 subfamily of nonselective cation channels may play an important role in the control of smooth muscle cell membrane potential to effect control over voltage-operated Ca^{2+} entry and muscle contraction.

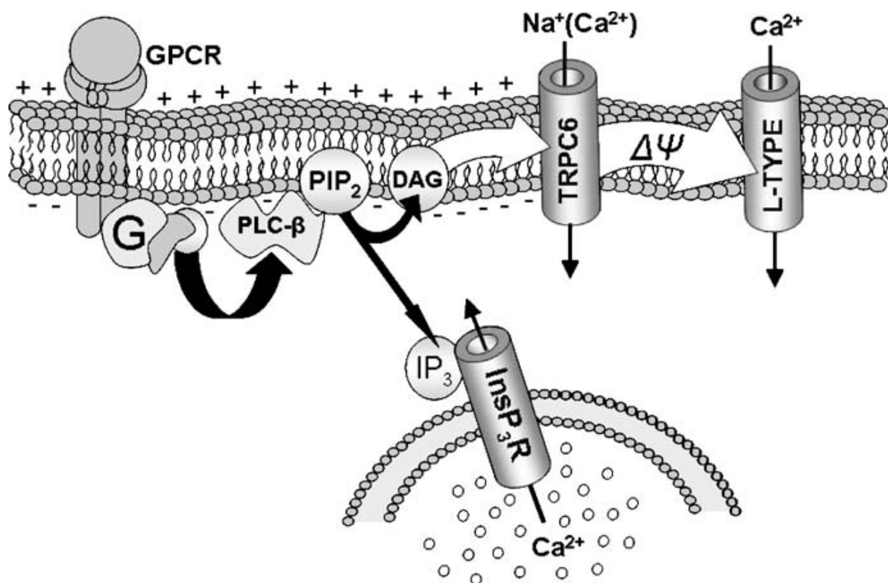

Fig. 2 Model to depict the role of TRPC6 channels in the coupling between receptor-induced PLC stimulation and L-type Ca^{2+} channel activation (from Soboloff et al. 2006). The G protein-coupled receptor (*GPCR*) activates PLC-β via G protein (*G*) and results in the formation of DAG and $InsP_3$. While $InsP_3$ induces Ca^{2+} release from stores, DAG activates the nonselective cation channel TRPC6. The predominant entry of Na^{+} ions (in addition to Ca^{2+} ions) results in depolarization of the membrane and the activation of voltage-sensitive L-type Ca^{2+} channels

Overall, rather than a group of putative SOCs, TRPC channels should be viewed as a group of receptor-operated nonselective cation channels, with distinct cell type-specific roles. Hence, while the low basal activity of TRPC6 is a required characteristic in vascular smooth muscle, the leakiness of TRPC3, dangerous in the circulatory system, likely serves an important functional role in pontine neurons, where it is highly expressed. In addition, it has become increasingly clear that the nonselective cation properties of TRPC channels are important in their biological function. As regulators of membrane potential, their activation has an impact on many different cellular functions including Ca^{2+} entry. Recent information reveals the TRPC1 channel as a required component of the mechanosensitive cation channel in *Xenopus* oocytes (Maroto et al. 2005), indicating it opens in response to shape or pressure changes. Earlier, the TRPA1 channel was suggested to be the mechanosensitive channel that transduces cation flux in cochlear hair cells (Corey et al. 2004). Based on the profound actions of DAG and PIP_2 on TRPC channels, we are considering a model in which TRPC channels are primarily "lipid sensors." We consider that their sensing of membrane stretch may be another manifestation of this lipid-sensing role. Indeed, it is possible that on a broader scale, many other TRP channel subtypes may be sensing mechanical and thermal changes by detecting changes in the local protein-lipid environment.

Acknowledgements This work was supported by NIH grants HL55426 and AI058173, and the Interdisciplinary Training Program in Muscle Biology, University of Maryland School of Medicine.

References

Bakowski D, Glitsch MD, Parekh AB (2001) An examination of the secretion-like coupling model for the activation of the Ca^{2+} release-activated Ca^{2+} current ICRAC in RBL-1 cells. J Physiol 532:55–71

Beech DJ (2005) Emerging functions of 10 types of TRP cationic channel in vascular smooth muscle. Clin Exp Pharmacol Physiol 32:597–603

Beech DJ, Muraki K, Flemming R (2004) Non-selective cationic channels of smooth muscle and the mammalian homologues of Drosophila TRP. J Physiol 559:685–706

Berridge MJ (2001) The versatility and complexity of calcium signalling. Novartis Found Symp 239:52–159

Berridge MJ (2002) The endoplasmic reticulum: a multifunctional signaling organelle. Cell Calcium 32:235–249

Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 4:517–529

Boulay G, Brown DM, Qin N, Jiang M, Dietrich A, Zhu MX, Chen Z, Birnbaumer M, Mikoshiba K, Birnbaumer L (1999) Modulation of Ca^{2+} entry by polypeptides of the inositol 1,4,5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca^{2+} entry. Proc Natl Acad Sci U S A 96:14955–14960

Broad LM, Braun FJ, Lievremont JP, Bird GS, Kurosaki T, Putney JW Jr (2001) Role of the phospholipase C-inositol 1,4,5-trisphosphate pathway in calcium release-activated calcium current and capacitative calcium entry. J Biol Chem 276:15945–15952

Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517–524

Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung EL, Derfler BH, Duggan A, Geleoc GS, Gray PA, Hoffman MP, Rehm HL, Tamasauskas D, Zhang DS (2004) TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432:723–730

Dietrich A, Mederos YSM, Gollasch M, Gross V, Storch U, Dubrovska G, Obst M, Yildirim E, Salanova B, Kalwa H, Essin K, Pinkenburg O, Luft FC, Gudermann T, Birnbaumer L (2005) Increased vascular smooth muscle contractility in TRPC6$^{-/-}$ mice. Mol Cell Biol 25:6980–6989

Gill DL, Patterson RL (2004) Toward a consensus on the operation of receptor-induced calcium entry signals. Sci STKE 2004:pe39

He LP, Hewavitharana T, Soboloff J, Spassova MA, Gill DL (2005) A functional link between store-operated and TRPC channels revealed by the 3,5-bis(trifluoromethyl)pyrazole derivative, BTP2. J Biol Chem 280:10997–11006

Hisatsune C, Kuroda Y, Nakamura K, Inoue T, Nakamura T, Michikawa T, Mizutani A, Mikoshiba K (2004) Regulation of TRPC6 channel activity by tyrosine phosphorylation. J Biol Chem 279:18887–18894

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

Inoue R, Okada T, Onoue H, Hara Y, Shimizu S, Naitoh S, Ito Y, Mori Y (2001) The transient receptor potential protein homologue TRP6 is the essential component of vascular alpha(1)-adrenoceptor-activated Ca^{2+}-permeable cation channel. Circ Res 88:325–332

Ishikawa J, Ohga K, Yoshino T, Takezawa R, Ichikawa A, Kubota H, Yamada T (2003) A pyrazole derivative, YM-58483, potently inhibits store-operated sustained Ca^{2+} influx and IL-2 production in T lymphocytes. J Immunol 170:4441–4449

Jung S, Strotmann R, Schultz G, Plant TD (2002) TRPC6 is a candidate channel involved in receptor-stimulated cation currents in A7r5 smooth muscle cells. Am J Physiol Cell Physiol 282:C347–359

Jung S, Muhle A, Schaefer M, Strotmann R, Schultz G, Plant TD (2003) Lanthanides potentiate TRPC5 currents by an action at extracellular sites close to the pore mouth. J Biol Chem 278:3562–3571

Kahr H, Schindl R, Fritsch R, Heinze B, Hofbauer M, Hack ME, Mortelmaier MA, Groschner K, Peng JB, Takanaga H, Hediger MA, Romanin C (2004) CaT1 knock-down strategies fail to affect CRAC channels in mucosal-type mast cells. J Physiol 557:121–132

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

Leypold BG, Yu CR, Leinders-Zufall T, Kim MM, Zufall F, Axel R (2002) Altered sexual and social behaviors in trp2 mutant mice. Proc Natl Acad Sci U S A 99:6376–6381

Li HS, Xu XZ, Montell C (1999) Activation of a TRPC3-dependent cation current through the neurotrophin BDNF. Neuron 24:261–273

Liman ER, Innan H (2003) Relaxed selective pressure on an essential component of pheromone transduction in primate evolution. Proc Natl Acad Sci U S A 100:3328–3332

Ma HT, Patterson RL, van Rossum DB, Birnbaumer L, Mikoshiba K, Gill DL (2000) Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. Science 287:1647–1651

Ma HT, Venkatachalam K, Li HS, Montell C, Kurosaki T, Patterson RL, Gill DL (2001) Assessment of the role of the inositol 1,4,5-trisphosphate receptor in the activation of transient receptor potential channels and store-operated Ca^{2+} entry channels. J Biol Chem 276:18888–18896

Ma HT, Venkatachalam K, Parys JB, Gill DL (2002) Modification of store-operated channel coupling and inositol trisphosphate receptor function by 2-aminoethoxydiphenyl borate in DT40 lymphocytes. J Biol Chem 277:6915–6922

Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP (2005) TRPC1 forms the stretch-activated cation channel in vertebrate cells. Nat Cell Biol 7:179–185

Montell C (1999) Visual transduction in Drosophila. Annu Rev Cell Dev Biol 15:231–268

Montell C (2001) Physiology, phylogeny, and functions of the TRP superfamily of cation channels. Sci STKE 2001:RE1

Montell C, Birnbaumer L, Flockerzi V (2002a) The TRP channels, a remarkably functional family. Cell 108:595–598

Montell C, Birnbaumer L, Flockerzi V, Bindels RJ, Bruford EA, Caterina MJ, Clapham DE, Harteneck C, Heller S, Julius D, Kojima I, Mori Y, Penner R, Prawitt D, Scharenberg AM, Schultz G, Shimizu N, Zhu MX (2002b) A unified nomenclature for the superfamily of TRP cation channels. Mol Cell 9:229–231

Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, Mori Y (1999) Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+}-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J Biol Chem 274:27359–27370

Parekh AB, Penner R (1997) Store depletion and calcium influx. Physiol Rev 77:901–930

Parekh AB, Putney JW Jr (2005) Store-operated calcium channels. Physiol Rev 85:757–810

Patterson RL, van Rossum DB, Ford DL, Hurt KJ, Bae SS, Suh PG, Kurosaki T, Snyder SH, Gill DL (2002) Phospholipase C-gamma is required for agonist-induced Ca^{2+} entry. Cell 111:529–541

Prakriya M, Lewis RS (2001) Potentiation and inhibition of Ca^{2+} release-activated Ca^{2+} channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP3 receptors. J Physiol 536:3–19

Putney JW Jr (2001) Pharmacology of capacitative calcium entry. Mol Interv 1:84–94

Putney JW Jr (2004) The enigmatic TRPCs: multifunctional cation channels. Trends Cell Biol 14:282–286

Putney JW Jr, Broad LM, Braun FJ, Lievremont JP, Bird GS (2001) Mechanisms of capacitative calcium entry. J Cell Sci 114:2223–2229

Reading SA, Earley S, Waldron BJ, Welsh DG, Brayden JE (2005) TRPC3 mediates pyrimidine receptor-induced depolarization of cerebral arteries. Am J Physiol Heart Circ Physiol 288:H2055–2061

Schaefer ML, Wong ST, Wozniak DF, Muglia LM, Liauw JA, Zhuo M, Nardi A, Hartman RE, Vogt SK, Luedke CE, Storm DR, Muglia LJ (2000) Altered stress-induced anxiety in adenylyl cyclase type VIII-deficient mice. J Neurosci 20:4809–4820

Schindl R, Kahr H, Graz I, Groschner K, Romanin C (2002) Store depletion-activated CaT1 currents in rat basophilic leukemia mast cells are inhibited by 2-aminoethoxydiphenyl borate. Evidence for a regulatory component that controls activation of both CaT1 and CRAC (Ca^{2+} release-activated Ca^{2+} channel) channels. J Biol Chem 277:26950–26958

Soboloff J, Spassova M, Xu W, He LP, Cuesta N, Gill DL (2005) Role of endogenous TRPC6 channels in Ca^{2+} signal generation in A7r5 smooth muscle cells. J Biol Chem 280:39786–39794

Spassova MA, Soboloff J, He LP, Hewavitharana T, Xu W, Venkatachalam K, van Rossum DB, Patterson RL, Gill DL (2004) Calcium entry mediated by SOCs and TRP channels: variations and enigma. Biochim Biophys Acta 1742:9–20
Stowers L, Holy TE, Meister M, Dulac C, Koentges G (2002) Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science 295:1493–1500
Sugawara H, Kurosaki M, Takata M, Kurosaki T (1997) Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. EMBO J 16:3078–3088
Suh PG, Hwang JI, Ryu SH, Donowitz M, Kim JH (2001) The roles of PDZ-containing proteins in PLC-beta-mediated signaling. Biochem Biophys Res Commun 288:1–7
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
Tang Y, Tang J, Chen Z, Trost C, Flockerzi V, Li M, Ramesh V, Zhu MX (2000) Association of mammalian trp4 and phospholipase C isozymes with a PDZ domain-containing protein, NHERF. J Biol Chem 275:37559–37564
van Rossum DB, Patterson RL, Sharma S, Barrow RK, Kornberg M, Gill DL, Snyder SH (2005) Phospholipase Cgamma1 controls surface expression of TRPC3 through an intermolecular PH domain. Nature 434:99–104
Vazquez G, Lievremont 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 U S A 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
Venkatachalam K, Ma HT, Ford DL, Gill DL (2001) Expression of functional receptor-coupled TRPC3 channels in DT40 triple receptor InsP3 knockout cells. J Biol Chem 276:33980–33985
Venkatachalam K, van Rossum DB, Patterson RL, Ma HT, Gill DL (2002) The cellular and molecular basis of store-operated calcium entry. Nat Cell Biol 4:E263–272
Venkatachalam K, Zheng F, Gill DL (2003) Regulation of canonical transient receptor potential (TRPC) channel function by diacylglycerol and protein kinase C. J Biol Chem 278:29031–29040
Venkatachalam K, Zheng F, Gill DL (2004) Control of TRPC and store-operated channels by protein kinase C. Novartis Found Symp 258:172–185
Voets T, Prenen J, Fleig A, Vennekens R, Watanabe H, Hoenderop JG, Bindels RJ, Droogmans G, Penner R, Nilius B (2001) CaT1 and the calcium release-activated calcium channel manifest distinct pore properties. J Biol Chem 276:47767–47770
Welsh DG, Morielli AD, Nelson MT, Brayden JE (2002) Transient receptor potential channels regulate myogenic tone of resistance arteries. Circ Res 90:248–250
Xu XZ, Li HS, Guggino WB, Montell C (1997) Coassembly of TRP and TRPL produces a distinct store-operated conductance. Cell 89:1155–1164
Yue L, Peng JB, Hediger MA, Clapham DE (2001) CaT1 manifests the pore properties of the calcium-release-activated calcium channel. Nature 410:705–709
Zagranichnaya TK, Wu X, Villereal ML (2005) Endogenous TRPC1, TRPC3, and TRPC7 proteins combine to form native store-operated channels in HEK-293 cells. J Biol Chem 280:29559–29569

Zhang L, Saffen D (2001) Muscarinic acetylcholine receptor regulation of TRP6 Ca^{2+} channel isoforms. Molecular structures and functional characterization. J Biol Chem 276:13331–13339
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 U S A 98:3168–3173
Zitt C, Halaszovich CR, Luckhoff A (2002) The TRP family of cation channels: probing and advancing the concepts on receptor-activated calcium entry. Prog Neurobiol 66:243–264
Zitt C, Strauss B, Schwarz EC, Spaeth N, Rast G, Hatzelmann A, Hoth M (2004) Potent inhibition of Ca^{2+} release-activated Ca^{2+} channels and T-lymphocyte activation by the pyrazole derivative BTP2. J Biol Chem 279:12427–12437