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Phospholipase C Mediated Modulation of TRPV1 Channels

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Abstract The transient receptor potential vanilloid type 1 (TRPV1) channels are involved in both thermosensation and nociception. They are activated by heat, protons, and capsaicin and modulated by a plethora of other agents. This review will focus on the consequences of phospholipase C (PLC) activation, with special emphasis on the effects of phosphatidylinositol 4,5-bisphosphate (PIP_2) on these channels. Two opposing effects of PIP_2 have been reported on TRPV1. PIP_2 has been proposed to inhibit TRPV1, and relief from this inhibition was suggested to be involved in sensitization of these channels by pro-inflammatory agents. In excised patches, however, PIP_2 was shown to activate TRPV1. Calcium flowing through TRPV1 activates PLC and the resulting depletion of PIP_2 was proposed to play a role in capsaicin-induced desensitization of these channels. We will describe the data indicating involvement of PLC and PIP_2 in sensitization and desensitization of TRPV1 and will also discuss other pathways potentially contributing to these two phenomena. We attempt to resolve the seemingly contradictory data by proposing that PIP_2 can both activate and inhibit TRPV1 depending on the experimental conditions, more specifically on the level of stimulation of these channels. Finally, we also discuss data in the literature indicating that other TRP channels, TRPA1 and some members of the TRPC subfamily, may also be under a similar dual control by PIP_2.

Keyword TRPV1 · Capsaicin · Phospholipase C · PLC · PIP_2 · Phosphatidylinositol · Desensitization

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Introduction

Transient receptor potential vanilloid type 1 (TRPV1) was the first member of the mammalian transient receptor potential (TRP) ion channel family that was reported to be regulated by phosphatidylinositol 4,5-bisphosphate (PIP_2) [1]. The reported effect of PIP_2 on TRPV1 was inhibition. This paper was followed by a stream of articles on other members of the TRP channel family, all reporting the opposite effect, activation by PIP_2 [2–8]. Soon, inhibition of TRPV1 by PIP_2 became the odd man out on this field, especially considering the essential lack of confirmatory articles from other laboratories. Thus, the recent report by Stein et al. showing that PIP_2 activates TRPV1 channels in excised patches [9] was not very surprising, but it created some confusion in the field [10, 11]. Can PIP_2 both activate and inhibit TRPV1? An article published soon after Stein et al. confirmed the activating effect of PIP_2 in excised patches but also presented data supporting the idea that under certain circumstances, PIP_2 may also inhibit the same channels [12]. This short review will discuss the data on the PIP_2 regulation of TRPV1 and present some ideas how these two opposing effects of the lipid could be integrated.

Phospholipase C and PIP_2 Metabolism

The membrane phospholipid PIP_2 is the substrate for phospholipase C (PLC), which catalyzes the formation of the two classical second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Beyond its precursor function, PIP_2 has been shown to serve as a membrane anchor for a wide variety of cytoplasmic proteins. Recently, it was also found that PIP_2 regulates many different of ion channels, including several members

of the TRP channel family. The topic of phosphoinositide regulation of ion channels [13–16], including TRP channels [17–20], has been extensively reviewed recently.

When discussing PLC, our first thought is usually its activation by G-protein coupled receptors (GPCRs). Agonist binding to the heptahelical GPCRs leads to the activation of members of the PLCβ subfamily (PLCβ1–4) through direct activation by either the Gqα or Gβγ subunits of heterotrimeric G-proteins [21]. A large number of extracellular messenger molecules, such as bradykinin, angiotensin-2, acetycholine, and vasopressin, bind to receptors coupled to Gq and activate of PLCβ isoforms.

Stimulation of receptor tyrosine kinases (RTK) by growth factors such as nerve growth factor (NGF), on the other hand, leads to the activation of PLCγ isoforms (PLCγ1–2). The mechanism of activation of PLCγs is very different from that of PLCβs. The single transmembrane domain RTK dimerizes upon ligand binding then phosphorylates itself, which leads to docking of various effector molecules to the receptor, including PLCγ. Finally, the tyrosine kinase domain of the receptor also phosphorylates PLCγ which, in addition to docking, is also important for activation of this enzyme [21].

The third classical group of PLCs are PLCδs (PLCδ1, 3, and 4) [21]. PLCδ2 is a bovine homologue of the human/mouse PLCδ4 [22]. These enzymes are considered more ancient, even though certain lower animals such as drosophila lack these isoforms (www.flybase.org). Their regulation is less well understood than that of the other two classical groups. The activator most likely to be of physiological relevance is increased cytoplasmic Ca^{2+}. Thus, these enzymes are thought to serve as signal amplifiers after PLCβ or PLCγ activation. All PLC isoforms require some Ca^{2+} for activity and can be activated in vitro by high Ca^{2+}, but PLCδs are the most sensitive to changes of Ca^{2+} in the physiological Ca^{2+} range among the classical isoforms [23]. Thus, it is likely that these isoforms are activated in physiological conditions by increased cytoplasmic Ca^{2+}, especially since they do not have any obvious other activating mechanism, unlike the members of the other two classical groups. These three classical PLC subfamilies have been extensively reviewed [21, 24].

In the genomic era, several novel PLC isoforms have been cloned [25]. Briefly, PLCε (epsilon) was shown to be activated by the small G-protein ras [26]. PLCζ (zeta) is injected from the sperm into the oocyte (sperm factor) where it induces Ca^{2+} oscillations [27]. PLCη1 (eta) and PLCη2 are the most recently cloned PLC isoforms. Their regulation is not well understood, but they may be even more sensitive to Ca^{2+} than the PLCδ isoforms [25, 28]. Two PLC homologues with no apparent PLC activity (PLCL1 and PLCL2) have also been identified [29].

TRPV1 Channels

TRPV1 channels are members of the TRP ion channel family [30, 31]. They are nonselective cation channels that are permeable to calcium and are mainly expressed in sensory neurons [32, 33]. The cell bodies of these neurons are found in dorsal root and trigeminal ganglia (DRG, TG) and various other sensory ganglia such as the nodose and jugular ganglia of the vagus nerve [34]. TRPV1 channels are also expressed in various cell types other than sensory neurons, but discussion of this topic is beyond the scope of this review. The three best known activators of TRPV1 are heat, low extracellular pH, and vanilloids, such as capsaicin [35]. Several endogenous analogues of capsaicin (endovanilloids) such as anandamide have also been described [36], and there are countless other compounds and pathways that modulate these channels [37]. The activation of TRPV1 channels in sensory neurons evokes a painful burning sensation, which is well known to all who ever tasted hot chili peppers. Even though TRPV1 is clearly activated by heat with a threshold of ~42 C, its role in thermosensation is not well defined [38]. Deletion of the TRPV1 gene in mice, for example, resulted in only a moderate [39] or no change [40] in sensitivity to noxious heat. On the other hand, $TRPV1^{-/-}$ mice showed no behavioral responses to local capsaicin or resiniferatoxin injection [39] confirming that these compounds exert their effects exclusively through TRPV1. Various aspects of the regulation and pharmacology of TRPV1 have been extensively reviewed [32, 37, 41, 42].

Sensitization of TRPV1 and the Inhibitory Effect of PIP_2

Tissue damage induces inflammation, which is characterized by hypersensitivity to noxious (hyperalgesia) or even innocuous stimuli (allodynia). A number of substances are released upon tissue damage and the following inflammation that sensitize TRPV1 to activating stimuli [43]. These substances include bradykinin, NGF, ATP, chemokines, and prostaglandins. As $TRPV1^{-/-}$ mice show markedly decreased thermal hyperalgesia [39, 40], the sensitization of these channels is likely to be involved in this phenomenon.

Many of the modulators that are released in inflammation and tissue damage activate PLC, leading to the hydrolysis of PIP_2. It is well established that activation of PLC by cell surface receptors sensitizes TRPV1 channels to their three major activators: capsaicin, protons, and heat. For bradykinin [44], extracellular ATP [45], and the chemokine CCL3 [46], protein kinase C (PKC) was shown to be involved in sensitization of TRPV1. Sensitization has usually been studied at low stimulation strength, such as low concentrations of capsaicin (10–100 nM), a moderate drop of pH or moderate increase in temperature. Consistent

with this notion, both extracellular ATP [45] and PKC activation [47] shift the capsaicin dose-response curve to the left and the H^+ sensitivity to higher pH values. In contrast, several laboratories have shown that NGF increases the number of TRPV1 channels in the plasma membrane in a PI3K dependent manner [9, 48].

As mentioned earlier, it is quite well established that the activation of PKC plays a role in sensitization of TRPV1 by GPCR activation. An interesting alternative mechanism was proposed by David Julius' laboratory. In this paradigm, PIP_2 tonically inhibits TRPV1 channels. Upon activation of PLC, this tonic inhibition is relieved by PIP_2 hydrolysis, in effect leading to channel sensitization [1]. This model was very attractive, because members of one major subfamily of TRP channels (TRPCs) are activated downstream of PLC. As the regulation of TRPC channels is not well understood, activation by relief from PIP_2 inhibition was postulated to be a more general phenomenon among TRP channels, see also later. A subsequent study showed that the region responsible for PIP_2 inhibition of TRPV1 is in the very distal C terminus, a region that does not show homology to similar regions of other TRP channels [49].

The idea that PIP_2 inhibits TRPV1 channels was based mainly on indirect evidence; the effects of phosphoinositides were not tested in excised patches. When phosphoinositides were tested later in excised patches, they consistently activated TRPV1 channels [9, 12], seemingly contradicting the inhibitory role of PIP_2 [11]. However, two additional pieces of evidence were obtained recently supporting the idea of a partial inhibition by PIP_2 [12]. Depleting PIP_2 with a chemically inducible 5-phosphatase [50] potentiated TRPV1 currents at low but not high capsaicin concentrations [12]. The same intervention also potentiated currents evoked by moderate increases in temperature [12]. Overproducing PIP_2 by co-expressing a phosphatidylinositol 4-phosphate 5-kinase inhibited TRPV1 currents at low but not high capsaicin concentrations [12]. These latter data are consistent with a partial inhibition of TRPV1 by PIP_2 at low stimulation strength, where sensitization usually occurs. The inhibitory effect of PIP_2, however, is likely to be indirect as it was not detectable in excised patches regardless of the capsaicin concentration used [12].

Desensitization of TRPV1 and the Activating Effect of PIP_2

Activation of TRPV1 by high concentrations of capsaicin (≥1 μM) in the presence of extracellular calcium evokes a transient current. The decay of current during prolonged application of capsaicin is termed desensitization. The literature also uses the term tachyphylaxis that refers to the decrease in capsaicin-induced currents after repetitive applications of this compound. Tachyphylaxis was usually induced by repetitive very short (5–10 s) pulses of capsaicin. The boundaries between these two phenomena are often blurred however, because several studies used repetitive but longer (≥1 min) pulses of capsaicin. The following paragraphs will discuss the literature on the mechanism of desensitization. Most of the data described here refers to experiments with 1 min or longer application (s) of capsaicin.

First, we describe the evidence indicating the role of PIP_2 in the desensitization of TRPV1 then we will discuss alternative mechanisms that were proposed. Figure 1 shows a model explaining the potential role of PIP_2 depletion in the desensitization of TRPV1. According to this model, calcium flowing through TRPV1 activates a Ca^{2+} sensitive PLC, leading to depletion of PIP_2, which leads to diminished channel activity. This model is based on the following three key experimental findings: (1) as mentioned earlier, TRPV1 channels require PIP_2 for activity. Two laboratories have shown that TRPV1 currents are inhibited in excised inside–out patches by scavenging endogenous phosphoinositides with polylysine, and the channels could be reactivated by the application of PIP_2 or other phosphoinositides [9, 12]. (2) Several laboratories have shown using various techniques that TRPV1 activation in the presence of extracellular Ca^{2+} leads to depletion of PIP_2 [12, 51–53]. The likely mechanism is the activation of PLC, as capsaicin also increased the formation of IP_3, which is only expected if PIP_2 depletion is caused by activation of PLC but not if the mechanism is dephosphorylation by phosphatases [12]. Consistent with the role of PLC, capsaicin-induced PIP_2 depletion was prevented by a PLC inhibitor [12]. (3) Prevention of PIP_2 depletion by either inhibition of PLC or inclusion of PIP_2 in the whole-cell patch pipette inhibited desensitization [12, 54]. Both of

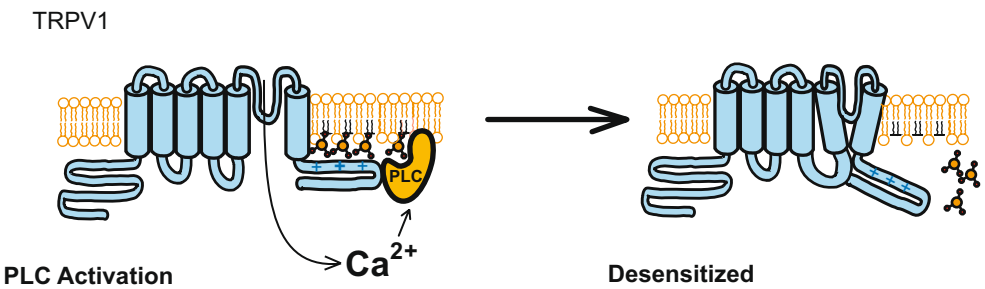

Fig. 1 Model of desensitization of TRPV1. Calcium entry through TRPV1 activates a calcium sensitive PLC isoform, and the ensuing depletion of PIP_2 inhibits TRPV1 activity. This figure does not include the potential roles of calmodulin and calcineurin in desensitization. See text for further details and references

these studies used water soluble diC_8 PIP_2 at 30 and 100 μM, respectively.

Furthermore, studies on the recovery from desensitization are also compatible with this model. If the mechanism of desensitization is the depletion of PIP_2, recovery from desensitization should require resynthesis of the lipid. Synthesis of PIP_2 requires ATP, and it can be prevented by inhibiting the kinase enzymes responsible for the synthesis of this lipid. Recovery of TRPV1 currents from desensitization in whole-cell patch clamp experiments required ATP in the pipette solution [52]. The same study showed that preventing resynthesis of PIP_2 by inhibiting phosphatidylinositol 4-kinases with phenylarsine oxide, or high concentrations of wortmannin delayed recovery from desensitization [52]. These data together quite convincingly demonstrate the role of PIP_2 depletion in the desensitization of TRPV1 currents (Fig. 1). This mechanism is similar to that proposed earlier for the menthol-induced desensitization of TRPM8 channels [6] and recently for Ca^{2+}-induced inactivation of TRPV6 [55].

It is likely, however, that PIP_2 depletion is not solely responsible for desensitization. In our hands, neither PLC inhibitors nor inclusion of PIP_2 in the patch pipette prevented desensitization completely [12]. The calcium sensor calmodulin has also been implicated in desensitization both directly and indirectly by activating the protein phosphatase calcineurin. Two different laboratories have shown that desensitization is reduced if calcineurin is inhibited by dialyzing cells with the cyclosporine cyclophilin complex [56, 57].

The direct role of calmodulin is somewhat controversial. It was shown that calmodulin interacts with the C terminus of TRPV1 channels, and removal of this part of the protein interfered with desensitization [58]. However, the same study found that the calmodulin blocker calmidazolium did not inhibit desensitization and neither did co-expression of a calcium insensitive mutant of calmodulin. Calcium calmodulin was shown to inhibit TRPV1 in excised patches; however, this inhibition was slow and moderate, only reaching ~50% [59], in marked contrast to the fast and almost complete disappearance of the current during desensitization. A recent article found that calmodulin interacts with the N-terminal ankyrin domains and inhibits TRPV1 currents [54]. In this article, calmidazolium inhibited capsaicin-induced desensitization of TRPV1.

ATP has been shown to bind to the C-terminal "walker motif" of TRPV1 and enhance currents induced by 1 μM capsaicin in excised patches [60]. The effect of ATP was independent of protein or lipid phosphorylation, as nonhydrolyzable analogues and ATP in the absence of Mg^{2+} had similar effects. Another study found that ATP binds to the N-terminal ankyrin-repeat domains and competes with calmodulin [54]. In this study, ATP included in whole-cell patch pipettes inhibited capsaicin-induced desensitization similar to nonhydrolyzable ATP analogues [54]. The authors argued that calmodulin inhibits TRPV1 by displacing ATP from this N-terminal binding site. The interplay between PIP_2, calmodulin, and ATP is probably quite complicated. Calmodulin and ATP seem to have multiple binding sites involving both the C- and N-terminal cytoplasmic domains [54, 58, 60, 61]. PIP_2, on the other hand, was shown to bind to the C-terminal cytoplasmic domain and compete with calmodulin [61]. Determining the exact role of PIP_2 depletion and the other potential mechanisms discussed here in desensitization will require further studies.

Paradoxically, capsaicin has long been used as a topical analgesic; after an initial burning pain, it induces pain relief. The rationale for its use is generally thought to be the desensitization we just described. It is worth mentioning that the over-the-counter preparations of capsaicin available in many pharmacies contain relatively low concentrations of capsaicin. As the human skin poses a significant barrier for capsaicin, it is unlikely that the drug reaches the nerve endings in high enough concentrations to induce desensitization [41]. Newer preparations, however, with much higher concentrations of capsaicin are under clinical trials for various painful disorders [62]. The pain relief induced by these preparations may last for several days or even weeks. Similarly, capsaicin-induced unresponsiveness in vivo can be quite long lasting. The patch clamp experiments we described in the previous paragraphs studied desensitization of TRPV1 currents on a much shorter time scale, minutes to tens of minutes. Recovery from desensitization in patch clamp experiments also happens within several minutes [52]. Whether the acute desensitization observed in electrophysiological experiments is underlying the long lasting unresponsiveness induced by capsaicin in vivo remains to be established.

Dual Regulation by PIP_2

How can we reconcile the opposing effects of PIP_2? PIP_2 clearly activates TRPV1 channels in excised patches [9, 12]; thus, it is likely that this effect is mediated by direct binding of the lipid to the channel. PIP_2 is thought to interact with ion channels via positively charged cytoplasmic residues [63–65]. Most TRP channels are also activated by PIP_2, but there is no consensus as to the binding site for PIP_2. For TRPM8 mutation of positively charged residues in the highly conserved proximal C-terminal, TRP domain significantly altered PIP_2 sensitivity, which is consistent with these residues being part of the PIP_2 binding site [6]. Similar results were obtained with TRPM5 and TRPV5 [6]. On the other hand, mutations of these residues in TRPM4 had no significant effect on PIP_2 activation [5]. Interesting-

ly, chimeras between TRPV1 and the cold sensitive TRPM8 are functional, and data obtained with them suggest that residues in the TRP domain are part of the PIP_2 binding site of TRPV1 [66]. The R701Q mutation in the same region markedly reduced activation of TRPV1 by lidocaine [67].

The distal C terminus has been suggested to be involved in the inhibitory effect of PIP_2 on TRPV1 [49]. This region has no sequence homology to any other TRP channel; its deletion eliminated sensitization by PLC coupled agonists [49] and reduced but not eliminated inhibition by over-producing PIP_2 [12]. Interestingly, the same region is also implicated in binding to calmodulin [58]. Calmodulin has been shown to bind to the C terminus of several other TRP channels, including TRPV1 [61], and phosphoinositides were shown to compete with calmodulin for binding. It was proposed that phosphoinositides activate TRPC channels by displacing the inhibitory calmodulin [61]. The competition between PIP_2 and calmodulin is also well documented for other proteins [68].

The inhibitory effect of PIP_2 is not detectable in excised patches; thus, it is likely to be indirect, i.e., requires a cytoplasmic factor that is lost upon patch excision. Figure 2a depicts a hypothetical model in which a cytosolic protein competes with PIP_2 for binding to this distal C-terminal region. In this model, binding of this protein to TRPV1 sensitizes the channel to its activating stimuli, e.g., low concentrations of capsaicin. Under resting conditions, binding of PIP_2 would displace this protein and inhibit the channel, i.e., reduce its sensitivity for capsaicin. In this scenario, activation of PLC would remove PIP_2 from this region. Thus, this hypothetical protein could bind to this region of the channel and sensitize it. Calmodulin, which as we saw can compete with PIP_2 for binding to various proteins, is unlikely to be this hypothetical protein, as it inhibits TRPV1 in excised patches [59].

An alternative scenario is shown in Fig. 2b, where a cytosolic protein binds to PIP_2 and inhibits TRPV1 (reduces its sensitivity to capsaicin). In this model, this hypothetical cytoplasmic protein would have to bind both to PIP_2 and TRPV1 to inhibit the channel. Depletion of PIP_2 would release this protein from the plasma membrane; thus, it could not interact with TRPV1, leading to the relief from inhibition and sensitization.

Both these models are hypothetical, but they may help visualize possible scenarios for the requirement of cytoplasmic factors in the inhibitory effects of PIP_2. Other themes, such as requirement for cytoskeletal element, could also explain the indirect inhibitory effect of PIP_2, as was proposed for TRPC4α channels [69]. In addition, both models in Fig. 2 assume that PLC activation removes only the inhibitory but not the activating PIP_2 molecules. This is highly unlikely to happen. The following paragraph describes a possible model explaining how partial removal

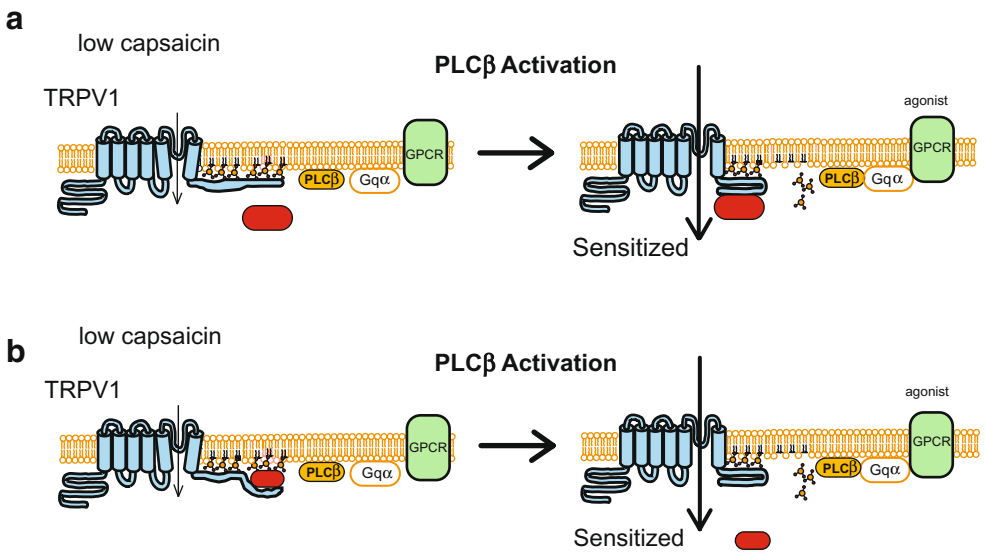

Fig. 2 Two alternative schemes to explain an indirect inhibitory effect of PIP_2 on TRPV1 channels. **a** The distal C terminus of TRPV1 binds to PIP_2, which prevents binding of the same region to a hypothetical cytoplasmic protein (*red*). Upon activation of PLC, the C terminus ceases to bind PIP_2, and the hypothetical protein can bind to the C terminus and sensitize the channel. **b** A hypothetical cytoplasmic protein (*red*) binds to PIP_2 that anchors it to the plasma membrane. This protein, when bound to PIP_2, also binds to the distal C terminus of TRPV1 and inhibits the channel, i.e., the TRPV1 is less sensitive to its activating stimuli. Upon activation of PLC, the protein falls off from the plasma membrane and does not bind to the C terminus of TRPV1; the channel is relieved from inhibition and becomes sensitized. The *larger arrows* in the *right panels* symbolize larger currents in the sensitized state. For simplicity, this figure assumes that PLC activation only removes the inhibitory PIP_2, see legend of Fig. 3 and main text for further details. The model does not include the potential role of PKC in sensitization

of both the inhibitory and activating PIP_2 molecules can result in activation (sensitization) of the channel.

On the descriptive level, the product of an inhibitory and activating effect could be a bell-shaped dependence on PIP_2 (Fig. 3a, dashed line). If resting PIP_2 levels are on the right side of this bell-shaped curve, a moderate depletion of PIP_2 could result in activation and a more severe depletion could result in inhibition. According to our data [12], this model could be valid at low capsaicin concentrations. At high capsaicin concentrations, there is likely to be a simple unidirectional dependence on PIP_2 (Fig. 3b). During desensitization, a severe depletion of PIP_2 may shut down these channels. Again, this model is obviously an oversimplification; it only serves as a guide to imagine the results of changing PIP_2 concentrations in the context of the opposing effects of the lipid.

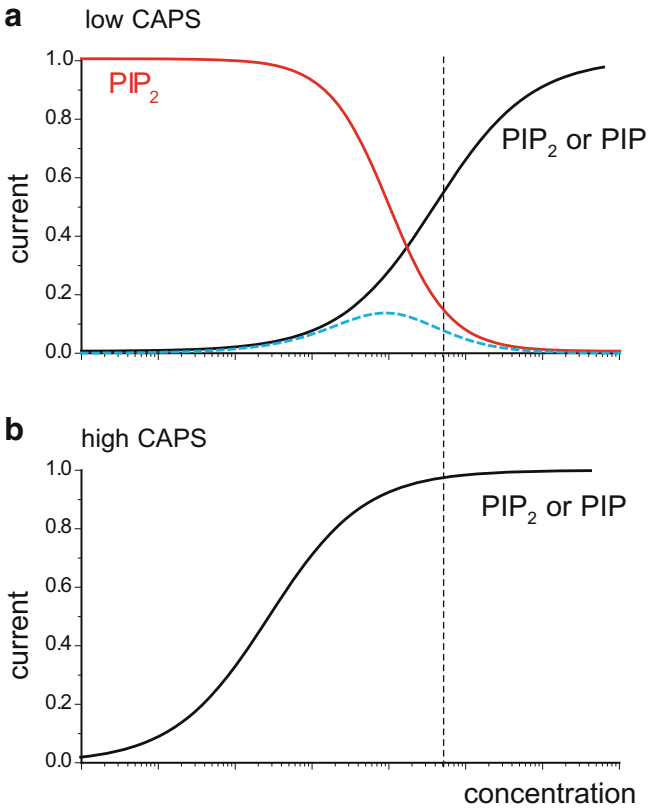

Fig. 3 Model to explain the inhibitory and activating effects of PIP_2 on TRPV1 based on the data in reference [12]. PIP_2 (and PIP) activates TRPV1 channels, presumably directly (*black curve*). In addition, there is an inhibitory effect (*red curve*), which is probably indirect and specific to PIP_2. The sum of these two effects results in a *bell-shaped* dose response to phosphoinositides (*dashed line*), in intact cells. The *vertical dotted line* shows possible resting cellular PIP_2 levels. For simplicity, we labeled the figure with low capsaicin (CAPS; *top*) and high CAPS (*bottom*), but these may also apply to moderate and large drops in pH. The result of the changes in phosphoinositide affinities is a *bell-shaped* dose responses at low capsaicin, and a simple activating *curve* for high capsaicin, respectively. The model also implies that moderate depletion of PIP_2 may activate TRPV1 at low capsaicin but not at high capsaicin. For intermediary capsaicin concentrations, these *curves* may lie between the two scenarios depicted here. The regulation of TRPV1 is obviously more complex than described here; our model serves only to illustrate the possible effect of the coexistence of inhibitory and activating effects of phosphoinositides and their changes at various stimulation strengths

We also need to consider that the PLC isoforms that are involved in sensitization and desensitization are likely to be different. GPCRs that induce sensitization of TRPV1 activate PLCβs, whereas RTKs such as NGF activate PLCγs. During desensitization, the most likely isoform that is activated is a PLCδ isoform [12]. It is feasible to think that different isoforms may deplete PIP_2 to different extents. Compatible with this notion, it was shown that PLCδ has much higher activity in vitro than PLCβ or PLCγ [21].

Another factor that can affect the level of PIP_2 depletion is the localization and relative abundance of the effector molecules. Ca^{2+} entering through TRPV1 presumably activates PLC at the right place to remove PIP_2 from the channel. GPCRs in the nerve terminal may activate PLCβ that is not in the exact vicinity of TRPV1; thus, the local depletion of PIP_2 maybe more moderate. The relative abundance of the various PLC isoforms and receptors may also affect the extent of PIP_2 depletion, but very little is known about this topic, especially in native neurons.

Another hypothetical possibility for differential regulation by various PLC isoforms is the following. TRPV1 is also activated by the precursor of PIP_2, phosphatidylinositol 4-phosphate (PIP), but the inhibitory effect is likely to be specific for PIP_2 [12]. The concentration of PIP in the plasma membrane is thought to be comparable to that of PIP_2. PIP is also the substrate for PLC; thus, it is also depleted during PLC activation. In case different PLC isoforms deplete PIP and PIP_2 to different extents, they could have distinct effects on TRPV1 channel activity. No comprehensive study has been performed to our knowledge on PLC isoforms concerning their relative efficiencies to hydrolyze PIP_2 versus PIP.

The inhibitory effect of PIP_2 is likely to be indirect, and the activating effect is likely to be direct [12]; thus, it not impossible that PIP_2 in different compartments differentially affects these two phenomena. Phosphoinositides are long thought to be local, compartmentalized signaling molecules, and it is possible that the different PLC isoforms deplete PIP_2 in different compartments/pools. There is a large and often contradictory literature on the compartmentalization of phosphoinositides and local signaling. Two excellent reviews have recently discussed this topic [68, 70].

Finally, a note on experimental protocols, most studies examined desensitization using saturating capsaicin concentrations in the presence of extracellular calcium. Sensitization, on the other, hand was usually studied using low concentrations of capsaicin, in the absence of extracellular calcium, presumably to avoid desensitization. Desensitiza-

tion, however, may also happen at lower concentrations of capsaicin, as long as extracellular calcium is present [71]. Thus, in the presence of extracellular calcium, sensitization and desensitization may happen simultaneously, further adding to the complexity of the picture. In addition, we generally discussed activation of PLC by GPCRs and RTKs in the context of sensitization. It is worth noting, however, that there are reports that GPCR activation by carbachol [72] and RTK activation by NGF [52] may inhibit TRPV1 currents induced by high capsaicin concentrations.

A very recent article identified a novel protein phosphoinositide interacting regulator of TRP (Pirt) that associates with TRPV1 and regulates its function [73]. Pirt is specifically expressed in DRG neurons and has two transmembrane domains. Heat and capsaicin-induced currents in DRG neurons were significantly attenuated in *Pirt*$^{-/-}$ mice. Heterologous expression of Pirt enhanced TRPV1 currents in HEK293 cells but only at high capsaicin concentrations. This effect is different from what is observed during sensitization, where marked enhancement of currents is seen at low but not high capsaicin concentrations. Dialysis of 10 μM diC_8 PIP_2 through the whole-cell patch pipette potentiated currents induced by 5 μM capsaicin in wild type but not *Pirt*$^{-/-}$ DRG neurons. The authors concluded that Pirt is required for the stimulatory effect of PIP_2 on TRPV1. PIP_2, however, activates TRPV1 in excised patches in expression systems [12], where Pirt is unlikely to be present. The authors argued that high concentrations of PIP_2 used in expression systems may override the requirement for Pirt. DiC_8 PIP, however, activated TRPV1 at concentrations as low as 0.5 μM in excised patches in Xenopus oocytes [12]. In our experience, this concentration of the lipid exerts a much lower activity on most ion channels than resting PIP_2 levels in the plasma membrane. Thus, Pirt is not strictly required for the stimulatory effect of phosphoinositides on TRPV1, unless it is also expressed in Xenopus oocytes, which has not been tested yet. Interestingly, genetic deletion of *Pirt* attenuated but not eliminated sensitization of capsaicin-induced currents by bradykinin. As Pirt is a transmembrane protein, it is unlikely to be the hypothetic factor that is lost in excised patches and presumably responsible for the inhibitory effect of PIP_2 (Fig. 2). More experiments are needed to clarify the contribution of Pirt to phosphoinositide regulation of TRPV1.

Other Examples of Potential Dual Effects of PIP_2 on TRP Channels

TRPA1

There are many parallels between the functions of TRPV1 and TRPA1. Both channels are involved in nociception and have chemical agonists that evoke a painful sensation. TRPA1 was first described as a noxious cold sensor [74], but this notion is controversial, and it has not been resolved even by studies with deletion of this gene in mice [75, 76]. Another interesting proposal was that TRPA1 serves as the mechanosensitive auditory channel in the inner ear. Genetic deletion of this channel clearly refuted this idea, TRPA1$^{-/-}$ mice have normal auditory function [75, 76]. In contrast to these contradictory studies, the literature is very consistent on the notion that this channel is involved in generating the pain that is evoked by many noxious chemicals such as formaldehyde and acrolein. TRPA1 is also clearly responsible for the pungency of mustard oil and related compounds, which are responsible for the characteristic feeling evoked by eating horseradish or wasabi [75–78].

As to the PLC mediated regulation, there are also similarities between TRPA1 and TRPV1. As with TRPV1, both stimulatory and inhibitory effects of PIP_2 have been described or implied for TRPA1. These channels, just as TRPV1, undergo sensitization upon activation of PLC by cell surface receptors such as the proteinase activated receptor 2 [79, 80]. Unlike for TRPV1 [79], PKC does not seem to be involved in this phenomenon [80]. On the other hand, dialysis of PIP_2 through the patch pipette inhibited the sensitization of TRPA1 by bradykinin [80]. This assumes that PIP_2 inhibits TRPA1, and bradykinin sensitizes these channels by depleting PIP_2 and relieving this inhibition.

As with TRPV1, these channels undergo desensitization upon prolonged application of mustard oil. Interestingly, in neurons expressing both TRPV1 and TRPA1, prolonged application of capsaicin also diminishes subsequent currents evoked by mustard oil (heterologous desensitization). The mechanism of the heterologous and homologous desensitization was proposed to be different. For the homologous desensitization of TRPA1, internalization of the channel was suggested to be the main mechanism [51]. Capsaicin, unlike mustard oil, was shown to deplete PIP_2 in TG neurons and dialysis of PIP_2 through the patch pipette inhibited heterologous desensitization, assuming an activating effect of PIP_2 on TRPA1 [51]. Interestingly, the effect of PIP_2 in excised patches was not tested in any of these two articles [51, 80]. One study found that TRPA1 shows fast rundown in excised patches and PIP_2 did not reactivate the channels [81]. A very recent article, on the other hand, showed that PIP_2 reactivates TRPA1 in excised patches after rundown [82]. The same study also found that dialysis of PIP_2 through the whole-cell patch pipette inhibited homologous desensitization of TRPA1.

Interestingly, in one study, application of bradykinin activated TRPA1 currents, but this activation was transient, followed by an actual inhibition [83]. Even though this finding can have multiple interpretations, it is compatible

with the idea of dual effects of PIP_2 on these channels. Further studies are required to elucidate the roles of PIP_2 in the regulation of TRPA1 channels.

TRPC Channels

All TRPC channels are activated by PLC coupled agonists and mediate Ca^{2+} influx and probably depolarization in the sustained phase of stimulation. The mechanism of activation of these channels originally was thought be coupled to the emptying of intracellular stores. Even though it may be the mechanism in certain cases [84], it is controversial and clearly not the general mechanism of TRPC channel activation [85]. DAG, the breakdown product of PIP_2, consistently activates TRPC3, TRPC6, and TRPC7 [86, 88] independently of the activation of PKC. The activation mechanism of TRPC4 and TRPC5 is less clear. An attractive paradigm would be if PIP_2 inhibited these channels, and depletion of PIP_2 upon PLC activation would relieve this inhibition leading to channel activation. Indeed, it was shown for the drosophila TRPL, a homolog of mammalian TRPCs, that PIP_2 inhibits these channels in excised patches [87]. This study was performed in an expression system. In the drosophila eye relief from inhibition by PIP_2, depletion does not seem to be the mechanism of activation of TRP by light, even though it may play some auxiliary role [88]. Recently, TRPC4α but not TRPC4β was shown to be inhibited by dialysis of PIP_2 through the whole-cell patch pipette [69]. In this study, disruption of the actin filaments with cytochalasin D eliminated the inhibitory effect of PIP_2, showing the requirement for cytoskeletal elements. The effects of PIP_2 in excised patches were not tested in this study.

Interestingly, two recent papers showed an activating effect of PIP_2 on various TRPCs. Lemonnier et al. showed that in excised patches, PIP_2 activates TRPC3, 6, and 7 channels [89]. Jardin et al. showed that in platelets, extracellular application of PIP_2 enhanced Ca^{2+} entry through TRPC6 [90]. Thus, it seems that PIP_2 can be both inhibitory and activating on certain TRPC channels. It remains to be seen whether these opposing effects can be observed on the same channel isoform.

Conclusions and Future Questions

PIP_2 regulates an ever increasing number of ion channels and transporters [13–16]. In most cases, the described effect was a simple activation or requirement for PIP_2 for channel activity. Indeed, one wonders sometimes whether there is any mammalian ion channel that does not require PIP_2 for activity. On the other hand, it seems that on some channels, the effects of PIP_2 are more complex than just a simple activation. Apart from the ones we discussed, it was shown for voltage dependent Ca^{2+} channels that PIP_2 has two opposing effects, requirement for channel activity and shift of voltage dependence to the right, making it more difficult to open the channels [91]. On the Na/Ca exchanger, it was shown that, in addition to direct effects of PIP_2 on the transporter, there are complex effects of PIP_2 on trafficking [92].

On the role of PIP_2 in the regulation of TRPV1, there are several open questions. Even though the picture on the involvement of PIP_2 depletion in desensitization seems relatively well supported, most of the studies we described were performed in various expression systems. The only exception is that the capsaicin was shown to deplete PIP_2 in TG neurons too [51]. Interestingly, in the same study, PIP_2 dialysis only moderately inhibited capsaicin-induced desensitization of TRPV1 in TG neurons. Clearly, more experiments are needed in native neurons to resolve the contribution of PIP_2 depletion to TRPV1 desensitization. Even experiments in DRG or TG neuron preparations, which are mainly the cell bodies, do not necessarily represent events in the nerve terminals. Experiments on more physiological preparations such as skin-nerve preparations [93] will be important to establish the role of PIP_2 depletion in desensitization of capsaicin responses.

As to the partial inhibition of TRPV1 by PIP_2 at low stimulation strength, there are two outstanding questions. What is the relative contribution of PIP_2 depletion (if any) and PKC to the sensitization of these channels by PLC activation. This question can be answered by relatively straightforward experiments, but it may be different for different agonist. Second, what is the mechanism of the (indirect) inhibition of the channel by PIP_2? The most likely mechanism is through a cytosolic PIP_2 binding protein or other factor that becomes lost in excised patches (Fig. 2). Given the large number of the potential candidates, this hypothetical factor will not be easy to identify.

Finally, the recent discovery of Pirt adds to the complexity of the picture. This protein is expressed DRG neurons but presumably not in expression systems, where many of the experiments addressing the role of PIP_2 were performed. Interestingly, the absence of Pirt in expression systems is only an assumption at this point; it needs to be tested experimentally. This is a key experiment in deciding whether phosphoinositides regulate TRPV1 through binding to this protein, or Pirt has only a modulatory role. Even if Pirt is a modulator, its presence in the native environment of the channel makes it an important player in the phosphoinositide regulation of TRPV1 channels.

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References

1. Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P_2 mediated inhibition. Nature 411:957–962
2. Lee J, Cha SK, Sun TJ, Huang C-L (2005) PIP_2 activates TRPV5 and releases its inhibition by intracellular Mg^{2+}. J Gen Physiol 126:439–451
3. Liu B, Qin F (2005) Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. J Neurosci 25:1674–1681
4. Liu D, Liman ER (2003) Intracellular Ca^{2+} and the phospholipid PIP_2 regulate the taste transduction ion channel TRPM5. Proc Natl Acad Sci U S A 100:15160–15165
5. Nilius B, Mahieu F, Prenen J, Janssens A, Owsianik G, Vennekens R, Voets T (2006) The Ca^{2+}-activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-biphosphate. EMBO J 25:467–478
6. Rohacs T, Lopes CMB, Michailidis I, Logothetis DE (2005) PI(4,5)P_2 regulates the activation and desensitization of TRPM8 channels through the TRP domain. Nat Neurosci 8:626–634
7. Runnels LW, Yue L, Clapham DE (2002) The TRPM7 channel is inactivated by PIP_2 hydrolysis. Nat Cell Biol 4:329–336
8. Zhang Z, Okawa H, Wang Y, Liman ER (2005) Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. J Biol Chem 280:39185–39192
9. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE (2006) Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol 128:509–522
10. Nilius B, Mahieu F (2006) A road map for TR(I)Ps. Mol Cell 22:297–307
11. Zhang X, McNaughton PA (2006) Why pain gets worse: the mechanism of heat hyperalgesia. J Gen Physiol 128:491–493
12. Lukacs V, Thyagarajan B, Balla A, Varnai P, Balla T, Rohacs T (2007) Dual regulation of TRPV1 by phosphoinositides. J Neurosci 27:7070–7080
13. Gamper N, Shapiro MS (2007) Regulation of ion transport proteins by membrane phosphoinositides. Nat Rev Neurosci 8:921–934
14. Hilgemann DW, Feng S, Nasuhoglu C (2001) The complex and intriguing lives of PIP_2 with ion channels and transporters. Sci STKE 2001:RE19
15. Logothetis DE, Nilius B (2007) Dynamic changes in phosphoinositide levels control ion channel activity. Pflugers Arch 455:1–3
16. Suh BC, Hille B (2005) Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. Curr Opin Neurobiol 15:370–378
17. Hardie RC (2007) TRP channels and lipids: from Drosophila to mammalian physiology. J Physiol 578:9–24
18. Qin F (2007) Regulation of TRP ion channels by phosphatidylinositol-4,5-bisphosphate. Handb Exp Pharmacol 179:509–525
19. Rohacs T (2007) Regulation of TRP channels by PIP_2. Pflugers Arch 453:753–762
20. Rohacs T, Nilius B (2007) Regulation of transient receptor potential (TRP) channels by phosphoinositides. Pflugers Arch 455:157–168
21. Rebecchi MJ, Pentyala SN (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. Physiol Rev 80:1291–1335
22. Irino Y, Cho H, Nakamura Y, Nakahara M, Furutani M, Suh PG, Takenawa T, Fukami K (2004) Phospholipase C delta-type consists of three isozymes: bovine PLCdelta2 is a homologue of human/mouse PLCdelta4. Biochem Biophys Res Commun 320:537–543
23. Allen V, Swigart P, Cheung R, Cockcroft S, Katan M (1997) Regulation of inositol lipid-specific phospholipase cdelta by changes in Ca^{2+} ion concentrations. Biochem J 327(Pt 2):545–552
24. Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. Annu Rev Biochem 70:281–312
25. Cockcroft S (2006) The latest phospholipase C, PLCeta, is implicated in neuronal function. Trends Biochem Sci 31:4–7
26. Bunney TD, Katan M (2006) Phospholipase C epsilon: linking second messengers and small GTPases. Trends Cell Biol 16:640–648
27. Swann K, Saunders CM, Rogers NT, Lai FA (2006) PLCzeta (zeta): a sperm protein that triggers Ca^{2+} oscillations and egg activation in mammals. Semin Cell Dev Biol 17:264–273
28. Stewart AJ, Morgan K, Farquharson C, Millar RP (2007) Phospholipase C-eta enzymes as putative protein kinase C and Ca^{2+} signalling components in neuronal and neuroendocrine tissues. Neuroendocrinology 86:243–248
29. Takenaka K, Fukami K, Otsuki M, Nakamura Y, Kataoka Y, Wada M, Tsuji K, Nishikawa S, Yoshida N, Takenawa T (2003) Role of phospholipase C-L2, a novel phospholipase C-like protein that lacks lipase activity, in B-cell receptor signaling. Mol Cell Biol 23:7329–7338
30. Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517–524
31. Montell C (2005) The TRP superfamily of cation channels. Sci STKE 2005:re3
32. Caterina MJ, Julius D (2001) The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 24:487–517
33. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
34. Kollarik M, Undem BJ (2006) Sensory transduction in cough-associated nerves. Respir Physiol Neurobiol 152:243–254
35. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543
36. Starowicz K, Nigam S, Di MV (2007) Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 114:13–33
37. Pingle SC, Matta JA, Ahern GP (2007) Capsaicin receptor: TRPV1 a promiscuous TRP channel. Handb Exp Pharmacol 179:155–171
38. Dhaka A, Viswanath V, Patapoutian A (2006) TRP ion channels and temperature sensation. Annu Rev Neurosci 29:135–161
39. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313
40. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183–187
41. Szallasi A, Blumberg PM (1999) Vanilloid (capsaicin) receptors and mechanisms. Pharmacol Rev 51:159–212
42. Szallasi A, Cruz F, Geppetti P (2006) TRPV1: a therapeutic target for novel analgesic drugs? Trends Mol Med 12(11):545–554
43. Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. Nature 413:203–210
44. Premkumar LS, Raisinghani M, Pingle SC, Long C, Pimentel F (2005) Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation. J Neurosci 25:11322–11329
45. Tominaga M, Wada M, Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci U S A 98:6951–6956

46. Zhang N, Inan S, Cowan A, Sun R, Wang JM, Rogers TJ, Caterina M, Oppenheim JJ (2005) A proinflammatory chemokine, CCL3, sensitizes the heat- and capsaicin-gated ion channel TRPV1. Proc Natl Acad Sci U S A 102:4536–4541
47. Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001) Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534:813–825
48. Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24:4211–4223
49. Prescott ED, Julius D (2003) A modular PIP_2 binding site as a determinant of capsaicin receptor sensitivity. Science 300:1284–1288
50. Varnai P, Thyagarajan B, Rohacs T, Balla T (2006) Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact cells. J Cell Biol 175:377–382
51. Akopian AN, Ruparel NB, Jeske NA, Hargreaves KM (2007) Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. J Physiol 583:175–193
52. Liu B, Zhang C, Qin F (2005) Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. J Neurosci 25:4835–4843
53. Novakova-Tousova K, Vyklicky L, Susankova K, Benedikt J, Samad A, Teisinger J, Vlachova V (2007) Functional changes in the vanilloid receptor subtype 1 channel during and after acute desensitization. Neuroscience 149:144–154
54. Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R (2007) The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 54:905–918
55. Thyagarajan B, Lukacs V, Rohacs T (2008) Hydrolysis of phosphatidylinositol 4,5-bisphosphate mediates calcium induced inactivation of TRPV6 channels. J Biol Chem 283:14980–14987
56. Docherty RJ, Yeats JC, Bevan S, Boddeke HW (1996) Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. Pflugers Arch 431:828–837
57. Mohapatra DP, Nau C (2005) Regulation of Ca^{2+}-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. J Biol Chem 280:13424–s13432
58. Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H, Tominaga M (2003) Structural determinant of TRPV1 desensitization interacts with calmodulin. Proc Natl Acad Sci U S A 100:8002–8006
59. Rosenbaum T, Gordon-Shaag A, Munari M, Gordon SE (2004) Ca^{2+}/calmodulin modulates TRPV1 activation by capsaicin. J Gen Physiol 123:53–62
60. Kwak J, Wang MH, Hwang SW, Kim TY, Lee SY, Oh U (2000) Intracellular ATP increases capsaicin-activated channel activity by interacting with nucleotide-binding domains. J Neurosci 20:8298–8304
61. Kwon Y, Hofmann T, Montell C (2007) Integration of phosphoinositide- and calmodulin-mediated regulation of TRPC6. Mol Cell 25:491–503
62. Knotkova H, Pappagallo M, Szallasi A (2008) Capsaicin (TRPV1 agonist) therapy for pain relief: farewell or revival? Clin J Pain 24:142–154
63. Huang CL, Feng S, Hilgemann DW (1998) Direct activation of inward rectifier potassium channels by PIP_2 and its stabilization by $G\beta\gamma$. Nature 391:803–806
64. Lopes CMB, Zhang H, Rohacs T, Jin T, Logothetis DE (2002) Alterations in conserved Kir channel-PIP_2 interactions underlie channelopathies. Neuron 34:933–944
65. Rosenhouse-Dantsker A, Logothetis DE (2007) Molecular characteristics of phosphoinositide binding. Pflugers Arch 455:45–53
66. Brauchi S, Orta G, Mascayano C, Salazar M, Raddatz N, Urbina H, Rosenmann E, Gonzalez-Nilo F, Latorre R (2007) Dissection of the components for PIP_2 activation and thermosensation in TRP channels. Proc Natl Acad Sci U S A 104:10246–10251
67. Leffler A, Fischer MJ, Rehner D, Kienel S, Kistner K, Sauer SK, Gavva NR, Reeh PW, Nau C (2008) The vanilloid receptor TRPV1 is activated and sensitized by local anesthetics in rodent sensory neurons. J Clin Invest 118:763–776
68. McLaughlin S, Murray D (2005) Plasma membrane phosphoinositide organization by protein electrostatics. Nature 438:605–611
69. Otsuguro KI, Tang J, Tang Y, Xiao R, Freichel M, Tsvilovskyy V, Ito S, Flockerzi V, Zhu MX, Zholos AV (2008) Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate. J Biol Chem 283(15):10026–10036
70. Hilgemann DW (2007) Local PIP_2 signals: when, where, and how? Pflugers Arch 455:55–67
71. Koplas PA, Rosenberg RL, Oxford GS (1997) The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J Neurosci 17:3525–3537
72. Mohapatra DP, Wang SY, Wang GK, Nau C (2003) A tyrosine residue in TM6 of the vanilloid receptor TRPV1 involved in desensitization and calcium permeability of capsaicin-activated currents. Mol Cell Neurosci 23:314–324
73. Kim AY, Tang Z, Liu Q, Patel KN, Maag D, Geng Y, Dong X (2008) Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell 133:475–485
74. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112:819–829
75. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124:1269–1282
76. Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50:277–289
77. Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, Cravatt B, Corey DP, Patapoutian A (2007) An ion channel essential for sensing chemical damage. J Neurosci 27:11412–11415
78. McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM (2007) TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A 104:13525–13530
79. Dai Y, Moriyama T, Higashi T, Togashi K, Kobayashi K, Yamanaka H, Tominaga M, Noguchi K (2004) Proteinase-activated receptor 2-mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced inflammatory pain. J Neurosci 24:4293–4299
80. Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T, Kobayashi K, Obata K, Yamanaka H, Noguchi K (2007) Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. J Clin Invest 117:1979–1987
81. Kim D, Cavanaugh EJ (2007) Requirement of a soluble intracellular factor for activation of transient receptor potential A1 by pungent chemicals: role of inorganic polyphosphates. J Neurosci 27:6500–6509
82. Karashima Y, Prenen J, Meseguer V, Owsianik G, Voets T, Nilius B (2008) Modulation of the transient receptor potential channel TRPA1 by phosphatidylinositol 4,5-bisphosphate manipulators. Pflugers Arch (in press)

83. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849–857
84. Liu X, Cheng KT, Bandyopadhyay BC, Pani B, Dietrich A, Paria BC, Swaim WD, Beech D, Yildrim E, Singh BB, Birnbaumer L, Ambudkar IS (2007) Attenuation of store-operated Ca^{2+} current impairs salivary gland fluid secretion in $TRPC1^{-/-}$ mice. Proc Natl Acad Sci U S A 104(44):17542–17547
85. Putney JW Jr (2007) Recent breakthroughs in the molecular mechanism of capacitative calcium entry (with thoughts on how we got here). Cell Calcium 42:103–110
86. 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
87. Estacion M, Sinkins WG, Schilling WP (2001) Regulation of Drosophila transient receptor potential-like (TrpL) channels by phospholipase C-dependent mechanisms. J Physiol 530:1–19
88. Hardie RC (2003) Regulation of TRP channels via lipid second messengers. Annu Rev Physiol 65:735–759
89. Lemonnier L, Trebak M, Putney JW Jr (2008) Complex regulation of the TRPC3, 6 and 7 channel subfamily by diacylglycerol and phosphatidylinositol-4,5-bisphosphate. Cell Calcium 43:506–514
90. Jardin I, Redondo PC, Salido GM, Rosado JA (2007) Phosphatidylinositol 4,5-bisphosphate enhances store-operated calcium entry through hTRPC6 channel in human platelets. Biochim Biophys Acta 1783(1):84–97
91. Wu L, Bauer CS, Zhen XG, Xie C, Yang J (2002) Dual regulation of voltage-gated calcium channels by $PtdIns(4,5)P_2$. Nature 419:947–952
92. Yaradanakul A, Feng S, Shen C, Lariccia V, Lin MJ, Yang J, Kang TM, Dong P, Yin HL, Albanesi JP, Hilgemann DW (2007) Dual control of cardiac Na^+ Ca^{2+} exchange by PIP_2: electrophysiological analysis of direct and indirect mechanisms. J Physiol 582:991–1010
93. Reeh PW (1986) Sensory receptors in mammalian skin in an in vitro preparation. Neurosci Lett 66:141–146