Regulation of transient receptor potential (TRP) channels by phosphoinositides

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Abstract This review summarizes the modulation of transient receptor potential (TRP) channels, by phosphoinositides. TRP channels are characterized by polymodal activation and a surprising complexity of regulation mechanisms. Possibly, most if not all TRP channels are modulated by phosphoinositides. Modulation by phosphatidylinositol 4,5-bisphosphate (PIP₂) has been shown in detail for TRP vanilloid (TRPV) 1, TRPV5, TRP melastatin (TRPM) 4, TRPM5, TRPM7, TRPM8, TRP polycystin 2, and the *Drosophila* TRP-like (TRPL) channels. This review describes mechanisms of modulation of TRP channels mainly by PIP₂ and discusses some future challenges of this fascinating topic.

Keywords TRP · PIP₂ · PtdIns(4, 5)P₂ · TRPV · TRPM · TRPC · TRPA

Introduction

Transient receptor potential (TRP) channels are the most recently discovered major ion channel superfamily. They share similar topology with voltage-gated ion channels; they likely have six transmembrane segments per subunit and

probably form homo- or heterotetramers. Indeed, most TRP channels are regulated by voltage, although their voltage dependence is less steep than that of classical voltage-gated channels [67]. The regulation of TRP channels is polymodal. Regulatory factors include physical factors such as temperature, voltage, and mechanical stimuli, chemical factors such as pH and ions, mainly Ca²⁺ and Mg²⁺, and various signaling pathways including phospholipase C and various kinases. Many aspects of TRP channel regulation have been extensively reviewed [15, 60, 66, 70, 71], including their regulation by lipids [27, 28, 82]. In a recent review, one of the authors discussed phosphoinositide regulation of TRP channels, in the context of general aspects of ion channel regulation by phosphatidylinositol 4,5-bisphosphate (PIP₂) [82]. This review will place PIP₂ regulation of specific TRP channels into the context of their regulation by other factors as well as their biological function.

TRP channels have fascinated many scientists, ever since their discovery. We all encounter TRP channels in our daily lives on a regular basis. When we brush our teeth in the morning with a menthol-containing toothpaste, the resulting cool feeling is caused by the activation of TRP melastatin (TRPM) 8. Then, throughout the day, changes in ambient temperature are mainly sensed by channels in the TRPM and TRP vanilloid (TRPV) families [18]. At lunch or dinner, spices such as chili peppers [11], oregano [109], wasabi, horseradish, or mustard [37] all activate various TRP channels that lead to the familiar sensations we all know very well. A large number of other biological functions also involve TRP channels as key players. Calcium and magnesium absorption from the kidneys and intestines [32], Ca²⁺ signaling by hormones, and neurotransmitters that activate PLC, cell death, and vision in invertebrates [29] all involve TRP channels. There are also a number of diseases that are caused by mutations in TRP channels, the most common of

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them is polycystic kidney disease (PKD), for detailed reviews, see [38, 66, 70].

Seemingly, there is nothing in common that would hold this variety of biological functions together. There is an emerging attention to the role of phosphoinositides, especially PIP₂, in the regulation of TRP channels. It is hard to tell if PIP₂ is a common regulator of TRP channel, but even if it is not, it is important for many of them. Of course, the TRP channels are not unique in this respect ([31, 95], see also all the other reviews in this issue).

When studying phosphoinositide regulation of any biological process, including ion channels, there are several factors that need to be kept in mind. First of all, the lipids in the plasma membrane are essentially in two dimensions where “concentration” cannot be interpreted in the classical sense. Second, several phosphoinositides especially PIP₂ and phosphatidylinositol 4-phosphate (PIP) are found in substantial levels in resting cells, and their concentrations may decrease upon the activation of phospholipase C (PLC) but are unlikely to reach zero under physiological conditions. This makes the apparent affinity of a given ion channel for phosphoinositides a very important determinant of their regulation; that is, a channel with high affinity for PIP₂ may not be inhibited by a moderate PIP₂ depletion, whereas a channel with low affinity is inhibited. Third, phosphoinositides are local signaling molecules that are not distributed homogeneously in the plasma membrane; rather, it is likely that specific PIP₂ “microdomains” or “pools” exist [57]. These questions were discussed in detail recently in the context of PIP₂ regulation of TRP channels [82].

This review will discuss the available experimental tools, including recently developed ones, to study phosphoinositide regulation of ion channels, before discussing the specific data on TRP channels.

Tools to study phosphoinositide regulation of ion channels

Perhaps the simplest and most direct evidence for PIP₂ regulation of an ion channel is to test the effect of the lipid in excised patches [34, 65, 84, 86, 87, 97]. Keep in mind, however, that the patch membrane already contains endogenous PIP₂, which in the case of a channel with high PIP₂ affinity may already produce saturated activity. The effect of exogenous PIP₂ in this case can only be determined if endogenous PIP₂ levels are substantially decreased. This may happen spontaneously, by letting the patch stand in an adenosine triphosphate (ATP)-free solution in the inside-out configuration, thus allowing endogenous lipid phosphatases to dephosphorylate PIP₂. This phenomenon causes the well known “rundown” of current activity for many PIP₂-sensitive channels [30, 34, 97]. The putative lipid phosphatases

can be inhibited by a cocktail containing fluoride, vanadate, and pyrophosphate (FVPP) [34, 84]. The activity of the phosphatases on the other hand can be enhanced by adding millimolar concentrations of Mg²⁺ [34]. Similarly, micromolar concentrations of Ca²⁺ can activate PLC in the excised patches, also leading to a decrease in PIP₂ levels [34, 65]. Note that although both phosphatases and PLC reduce PIP₂ and probably PIP levels, their end products are different; phosphatases remove only the phosphates, producing PI and PIP, whereas PLC removes the whole head group, thus producing diacylglycerol (DAG) and inositol triphosphate (IP₃).

Endogenous PIP₂ levels can also be reduced using various PIP₂-chelating agents, such as poly-lysine [84, 86], PIP₂ antibody [30, 34, 84], and neomycin [34]. Unfortunately, there is not a single PIP₂ scavenger that is ideal to use. Poly-lysine and neomycin are not specific; they probably also screen the charges of other negatively charged lipids, such as phosphatidylinositol and phosphatidylserine. The PIP₂ antibody on the other hand is supposed to be specific to PIP₂ over other negatively charged lipids, but it works much less reliably than poly-Lys [84]. Although there are numerous older [34, 97] and more recent reports [48] on the effectiveness of the PIP₂ antibody, there are also reports on the lack of its effects on ion channels that are shown to be PIP₂ sensitive using other methods [22, 50]. Considering the reluctance of many investigators to include negative data in publications, the incidence of the lack of effect of the PIP₂ antibody is probably higher.

Once ready to apply phosphoinositides to the patch, we have several choices. The first reports used either PIP₂ [30, 97] or mixed phosphoinositides [23] purified from biological sources, usually bovine brain. These contain a mixture of acyl chains, the most common combination being arachidonyl–stearyl (AASt). Synthetic dipalmitoyl (DiP) PIP₂ is also available from many vendors; it is supposedly more stable than the natural form and has similar effects to AASt PIP₂ in most cases. Although, be cautioned that on Kir3.1/3.4 (GIRK) channels, DiP PIP₂ was substantially less effective than AASt [83].

Another choice, with increasing popularity, is the synthetic dioctanoyl (diC₈) PIP₂ [65, 83, 86]. This lipid is water soluble, and it activates ion channels in a quickly reversible fashion, as opposed to DiP or AASt PIP₂, which activate most ion channels essentially irreversibly [84]. An important advantage of diC₈ PIP₂ is that dose–response measurements can be performed and specificity profiles of phosphoinositide effects can easily be evaluated. Apparent affinities obtained with diC₈ PIP₂ dose–response measurements show excellent correlation with PIP₂ affinities obtained with other methods such as PIP₂ antibody or poly-Lys inhibition kinetics, inhibition by PIP₂ depletion,

and direct binding [19, 51, 110]. Specificity profiles obtained by diC₈ phosphoinositide analogues also show excellent correlation with specificity profiles using natural or long acyl-chain synthetic phosphoinositides [41, 83, 85].

A more recently described alternative is the PLC resistant analogue of PIP₂ that was shown to activate TRPM4 [111]. Again, just as with PIP₂ scavengers, there is not a single ideal phosphoinositide to use in excised patches. The caveat of the long acyl chain phosphoinositides is that usually they exert their effects slowly and they accumulate in the patch membrane eventually reaching concentrations that are probably much higher than those that occur naturally. The diC₈ phosphoinositide analogues obviously have different physicochemical characteristics from those of the natural lipid, and rigorous comparison of the effects of DiC₈ phosphoinositides and the natural lipids have not yet been performed for most channels. There is only one report so far on the effect of PLC-resistant PIP₂ on ion channels, and it showed PLC-resistant PIP₂ analogues [111] activated TRPM4 in excised patches.

PIP₂ levels can also be modified indirectly in excised patches, by applying either enzymes that modify their levels or substrates for such enzymes. Providing Mg-ATP for endogenous lipid kinases prevents rundown or reactivates many PIP₂-sensitive ion channels [34, 97], including TRP channels [48, 65]. Purified phosphatidylinositol-specific PLC has also been used by several laboratories to deplete PIP₂ from the intracellular surface of excised patches [22, 30].

Phosphoinositides can also be directly applied in whole-cell patch clamp experiments by dialyzing them through the patch pipette. This approach has been successfully used to demonstrate PIP₂ sensitivity of several ion channels, including TRP channels [87]. Another way of directly applying phosphoinositides in intact cells is to use various shuttle molecules such as neomycin [72]. The caveat of this approach is that the shuttle molecules themselves bind phosphoinositides and thus may also alter endogenous lipid levels.

In intact cells, we can activate PLC to decrease PIP₂ levels by stimulating endogenous or overexpressed cell surface receptors [86, 87]. Activating PLC, however, also generates IP₃ and DAG, thus leading to a Ca²⁺ release and activation of protein kinase C (PKC). The effects of the downstream molecules are sometimes difficult to differentiate from that of decreased PIP₂ levels. Lipid phosphatases and kinases do not have such obvious activators; thus, their effects can either be studied by overexpressing them [12, 92] or inhibiting them using pharmacological tools [62]. Overexpression of kinases or phosphatases can be achieved using a constitutive promoter [86, 92] or a tetracycline-inducible system [12, 65]. Unfortunately, the available inhibitors of phosphatidylinositol 4-kinases (PI4K), wortmannin, and phenylarsine oxide are not very specific [3].

Pharmacological tools to inhibit lipid phosphatases and phosphatidylinositol 4-phosphate 5-kinase (PIP5K) are even more limited. When overexpressing lipid phosphatases and kinases, we have to keep in mind that long-term changes in phosphoinositides may induce nonspecific changes in the cellular environment that may also affect channel activity.

Finally and more recently, a novel approach was introduced to the study of phosphoinositide signaling. It is based on the rapamycin-induced heterodimerization of the rapamycin binding domain of mTOR and FKBP12, which was successfully used earlier to manipulate small guanosine-triphosphate-binding proteins at the plasma membrane [36]. This approach was utilized concurrently by two laboratories to rapidly and selectively modify PIP₂ levels in the plasma membrane, without activating other signaling pathways and study the regulation of ion channels [56]. Rapamycin-induced translocation of the phosphatase domain of type-IV 5-phosphatase inhibited the activity of TRPM8, showing that the activity of these channels depends on the presence of PIP₂ [104]. A similar approach was also utilized concurrently to study the regulation of KCNQ channels [96].

In summary, a variety of tools is available to study phosphoinositide regulation of ion channels; keeping their advantages and caveats in mind, their combined use is necessary to establish regulatory roles for these lipids on ion channels.

TRPC channels

Canonical transient receptor potential (TRPC) channels are the closest mammalian homologues of the *Drosophila* TRP channel (Table 1). TRPCs, just like most other TRP channels, are nonselective cation channels that also conduct Ca²⁺. Although they are generally assumed to mediate Ca²⁺ influx, they have substantial conductance of Na⁺ ions; thus, they also depolarize the cells, which should also be considered [21] when discussing their physiological roles. Both mammalian TRPCs and the *Drosophila* TRP/TRP-like (TRPL) complex are activated by cell surface receptors that activate PLC. PLC hydrolyzes PIP₂ to form IP₃ and DAG. IP₃ releases Ca²⁺ from internal stores, and Ca²⁺ activates a wide variety of biological processes. Given the finite capacity of the internal stores, a secondary Ca²⁺ influx from the extracellular space is necessary for sustained Ca²⁺ signaling [80]. The search for the mechanism of this enhanced Ca²⁺ influx undoubtedly accelerated the early progress in the cloning and study of mammalian TRP channels [115]. TRPC channels are activated by PLC-coupled agonists, but the exact mechanism that couples PLC activation to channel opening is not really clear even now.

Table 1 Some properties of the TRPC family members

	Selectivity P_{Ca}/P_{Na}	Proposed activation mechanisms	Effect of phosphoinositides (PIPs)
TRPC1, ENSG00000144935	Nonselective	PLC, 1-oleoyl-2-acetyl- <i>sn</i> -glycerol (OAG), mechanical (stretch), phosphoinositide-3 kinase (PI3K), calmodulin, receptor tyrosine-kinases (RTK)-PLC γ . upregulated by PKC, NO?, store depletion?	?
TRPC2, ENSMUSG00000058020	2.7	PLC, DAG, store depletion?	?
TRPC3, ENSG00000138741	1.6	PLC, DAG, OAG, src TK, IP ₃ , intracellular Ca ²⁺ , store depletion	?
TRPC4, ENSG00000100991	1.1–7.7	PLC, GTP γ S, micromolar La ³⁺ , store depletion?	?
TRPC5, ENSG00000072315	1.8–9.0	PLC, GTP γ S, receptor operated, lysophosphatidylcholine (LPC), micromolar La ³⁺ or Gd ³⁺ , store depletion?, PIP5K, Rho GTPases (e.g., Rac1), PI3K, myosin light chain kinase (MLCK), NO nitrosylation of pore cysteines	PIPs play a role in traffic to the plasma membrane [5].
TRPC6, ENSG00000137672	5	PLC, DAG, OAG, Src, 20-hydroxyeicosatetraenoic acid (20-HETE), tyrosine kinase Fyn, Ca ²⁺ -Calmodulin kinase II (CamK-II), mechano-activated, <i>Alf</i> ₄ ⁻ , PtdIns(3,4,5)P ₃ activates [102]	C-terminus binds phosphoinositides competing with calmodulin [42]
TRPC7, ENSG00000069018	2	PLC, DAG, OAG, 20-HETE, store depletion	?

For more information see <http://www.ensembl.org/>, <http://clapham.tch.harvard.edu/>, Special Issues “TRP channels: facts, fiction, challenges,” Cell Calcium 33, 2003, and “Functional role of TRP channels” Pflügers Arch 451, 2005; for detailed reviews see also references [15, 25, 63, 66, 69, 73, 108]

One widely used experimental paradigm to study this question is the *Drosophila* eye. There, DAG or some of its metabolites are clearly involved in opening the channel. As the *Drosophila* TRPL channel is inhibited by PIP₂ in excised patches [22], a simultaneous depletion of PIP₂ may also contribute to the full activation of the TRP/TRPL complex [28]. The involvement of various lipids in signal transduction in invertebrate vision has been extensively reviewed [27, 28].

Mammalian TRPC3, TRPC6, and TRPC7 are activated by DAG. This mechanism provides a rational basis for their activation. TRPC4 and TRPC5 are not activated by DAG. PIP₂ depletion could serve as a mechanism for their activation, but an inhibitory effect of PIP₂ has not yet been reported on these channels. TRPC5 was reported to be activated by the epidermal growth factor (EGF) via vesicular translocation to the plasma membrane [5]. Both phosphoinositide 3-kinase and PIP5K were required for this phenomenon, showing the involvement of phosphoinositides. As phosphoinositides are important for membrane trafficking, similar mechanisms may also regulate the activity of other ion channels. These indirect effects of phosphoinositides should also be considered in addition to the presumably direct effects of the lipids on ion channels.

A recent report showed that in TRPC6, a C-terminal calmodulin-binding domain also binds phosphoinositides [42]. The lipids compete with calmodulin for binding, PtdIns(3,4,5)P₃ being the most effective. It was proposed

that the competition between phosphoinositides and calmodulin plays a role in the regulation of TRPC6 channels [42]. Extracellular application of PtdIns(3,4,5)P₃ was also shown to induce TRPC6 activity [102]. Biochemical evidence for competition between calmodulin and phosphoinositides was also provided for other channels including TRPV1, voltage-gated Ca²⁺ channels, and KCNQ K⁺ channels [42]. Competition of phosphoinositides with calmodulin has also been extensively discussed for cytoplasmic proteins [57] and was also shown for cyclic-nucleotide-gated channels [7]. Thus, it is possible that this paradigm is a general phenomenon for ion channels and should be considered as a possible regulatory mechanism.

Initially, the prevailing view was that the event that initiates TRPC channel activation is the depletion of internal Ca²⁺ stores (capacitative or store-operated mechanism) [80, 115]. This was clearly disproved for the activation of the *Drosophila* TRP channel complex [27], but for mammalian TRPCs, it could be the mechanism of activation in certain cases [81]. A recent development in this field is the cloning of STIM1 [112], which functions as a Ca²⁺ sensor in the endoplasmic reticulum, and Orai1 or CRACM1 [24, 105], which probably constitutes the pore-forming subunit of the long-sought I_{CRAC}. All three members of the Orai family have four putative transmembrane regions and show no homology to TRP channels or to any other known ion channels. Interestingly, STIM also interacts with and activates certain TRPC channels [35, 52].

I_{CRAC} has been shown to be inhibited by depletion of phosphoinositides [9]. Inspection of the sequences of the three human Orai proteins shows a highly conserved cluster of positively charged residues just before the first putative transmembrane domain, which could serve as phosphoinositide interaction sites, if indeed these channels need phosphoinositides for activity. Interestingly, however, depletion of PIP_2 with rapamycin-inducible PIP_2 5-phosphatase did not inhibit thapsigargin-induced Ca^{2+} entry [104]. In summary, relatively little is known about phosphoinositide regulation of TRPC channels or the recently cloned I_{CRAC} channels, but we can expect progress on this field in the near future [64].

TRPV channels

Mammalian TRPV channels can be separated into two functionally distinct groups (Table 2). TRPV1–4 channels are all activated by warm temperatures with various thresholds, while some of them are also activated by mechanical stimuli. These four channels are outwardly

rectifying, nonselective cation channels, similar to most other TRP channels. They are mainly expressed in sensory neurons. TRPV5 and 6 on the other hand are inwardly rectifying, Ca^{2+} -selective channels. They are expressed mainly in epithelial tissues, such as the kidneys and intestines. Their physiological function is Ca^{2+} absorption [32]. There are reports of PIP_2 regulation of members of both groups.

TRPV1 was the first non-TRPC mammalian TRP channel that was cloned [11]. A quick PubMed search reveals that there are more articles about TRPV1 than any other individual TRP channel. If we extend the search to capsaicin, its specific activator, there are more articles than about all other TRP channels combined. What drives this fascination? There are several factors; clearly, one of them is its involvement in pain sensation [10] and other disease states [70], but its exceedingly complex regulation is certainly another important factor. This complexity also applies to its regulation by phosphoinositides. TRPV1 is activated by warm temperatures, protons, endogenous cannabinoids, depolarization, various lipids, and capsaicin (the pungent

Table 2 Some important properties of the TRPV family members

	Selectivity P_{Ca}/P_{Na}	Proposed activation mechanisms	Effect of phosphoinositides (PIPs)
TRPV1, ENSG00000043316	~10 (capsaicin activated current), ~4 (heat activated current)	Depolarization, heat ($\geq 43^\circ\text{C}$), low pH (≤ 5.9), vanilloids, endovanilloids, PKC, CamK-II, PI3K, anandamide, nerve growth factor (NGF), glia-derived growth factor (GDGF), neurotrophin 3 (NT3), reducing agents like dithiothreitol (DTT), sensitization by ethanol, jelly fish toxin, venoms of the Indian tarantulas contain inhibitor cysteine knot peptides (ICK), 12/15-hydroperoxyeicosatetraenoic acid (12-(S)-HPETE, 15-(S)-HPETE), 5-(S)-HETE, leukotriene B ₄ , N-arachidonoyl dopamine (NADA), protease-activated receptor 2 (PAR2), spermine, 2-aminoethoxydiphenyl borate (2-APB), oleylethanolamide (OEA), protein kinase A (PKA), PGE ₂ and PGI ₂ via their receptor EP ₁ or IP	PIP_2 inhibits in intact cells [14, 53, 78]; PIP_2 activates excised patches [53, 93]
TRPV2, ENSG00000154039	1–3	Noxious heat ($>53^\circ\text{C}$), mechanical (stretch, swelling), PKC, PI3K, growth factors, insulin-like growth factor 1 (IGF-1), head activator (HA), 2-APB	?
TRPV3, ENSG00000167723	2.6	Heat ($23\text{--}29^\circ\text{C}$), camphor, 2-APB, voltage dependent	?
TRPV4, ENSG00000111199	6–10	Moderate heat ($>24^\circ\text{C}$), cell swelling, shear stress, phospholipase A ₂ (PLA ₂) activation, PKC, PAR ₂ , anandamide, epoxyeicosatrienoic acid (EETs), 4 α -phorbol 12,13-didecanoate (4 α -PDD) and other phorbols, bisandrographolide A (BAA) from a Chinese herb	?
TRPV5, ENSG00000127412	>100	Low $[Ca^{2+}]_i$, hyperpolarization, voltage dependent block by Mg^{2+} , serum- and glucocorticoid-dependent kinase 1 (SGK1), serine/threonine kinase WNK4	PIP_2 activates [47, 86]
TRPV6, ENSG00000165125	>100	Low $[Ca^{2+}]_i$, hyperpolarization, voltage dependent block by Mg^{2+}	?

For references for channel names, numbering, and additional information, see legend to Table 1

compound of chili peppers). All the latter compounds induce a dramatic shift of the TRPV1 activation curve towards negative potentials [67, 106, 108]. TRPV1 is involved in nociception, the sensation of pain. A drop in pH that often accompanies tissue injury is one factor that contributes to activation of TRPV1 in these circumstances [100].

Another event that is involved in the activation of TRPV1 by painful stimuli is the release of proinflammatory substances upon tissue damage. These factors include bradykinin, extracellular ATP, and chemokines. Many of these factors activate PLC. We all know that an inflamed area feels warmer than normal and even moderately warm temperatures or other innocuous stimuli can feel painful. This phenomenon, thermal hyperalgesia, at least in part, is caused by the increased sensitivity of TRPV1. The sensitization of TRPV1 has been shown to involve PKC [6], and it was also proposed that PIP₂ inhibits these channels and relief from this inhibition leads to the sensitized state of these channels [14].

The idea that PIP₂ inhibits TRPV1 was based on indirect measurements; the effects of the lipid were not tested in excised patches. Note that sensitization mainly occurs at moderate stimuli; the manifestation of sensitization is generally a left shift in the dose response to capsaicin and pH [14, 101]. In other words, if there is an inhibitory effect of PIP₂, it is mainly prevalent at low stimulation strength, such as low capsaicin concentration. The region responsible for inhibition by PIP₂ was proposed to be in the very distal C terminus of TRPV1, which shares no homology to the same region of any other TRP channel [78]. Consistent with the role of the distal C terminus in PIP₂ inhibition, it was found that when the C terminus of TRPM8 was replaced with the C terminus of TRPV1, the chimeric channel was inhibited by PIP₂ dialyzed through the patch pipette [8]. As all other mammalian TRP channels that were tested so far are activated by PIP₂, the inhibitory effect of PIP₂ seems unique to TRPV1. Indeed, it was recently reported that PIP₂ activates TRPV1 in excised patches [53, 93]. How can we put these data together? It is likely that TRPV1 needs PIP₂ to be open, just like most other TRP channels. Under certain circumstances, such as low capsaicin concentrations, however, PIP₂ may partially inhibit TRPV1, leading to a bell-shaped dependence on PIP₂ concentration [53].

What is the relevance of the activating effect of PIP₂ on TRPV1? TRPV1 channels undergo desensitization in response to high concentrations of capsaicin in the presence of extracellular Ca²⁺. This phenomenon is likely to play an important role in the paradoxical use of capsaicin as a local analgesic [89]. This phenomenon was shown to involve the calcium-sensitive protein phosphatase calcineurin [58]. Inhibition of desensitization by calcineurin inhibitors was partial [58]. It was shown earlier that resynthesis of PIP₂ is required for recovery from desensitization [49], which was

difficult to put together with the originally published inhibition of TRPV1 by PIP₂. In view of the recent reports showing activation of TRPV1 by PIP₂ [53, 93], the role of PIP₂ depletion in desensitization seems a feasible mechanism. We have indeed found that interfering with depletion of PIP₂ either by inhibiting PLC with U73122 or dialyzing PIP₂ or PIP through the patch pipette in whole-cell experiments inhibited desensitization of TRPV1 [53]. Note that desensitization of TRPV1 is usually studied at high capsaicin concentrations, where the potential inhibitory effect of PIP₂ is likely not to be present.

The only other TRPV channel that has been reported to be regulated by PIP₂ is TRPV5, which is activated by PIP₂ [47, 86]. It was reported that PIP₂ relieves the inhibition of TRPV5 by Mg²⁺. PIP₂ decreases the sensitivity of the channel to inhibition by Mg²⁺, which probably binds to a conserved aspartate (D542) residue in the selectivity filter, causing not only fast, voltage-dependent inhibition but also slow inhibition. The slow inhibition is abolished by PIP₂, but the activation of TRPV5 by PIP₂ was not dependent on Mg²⁺. For detailed description of the Mg²⁺ block of the highly homologous TRPV6 channel, see [107]. Interestingly, a somewhat similar mechanism was proposed for TRPM7 ([39], see later). As Mg²⁺ is found in the cytoplasm at a relatively high concentration and it can interfere with PIP₂ activation of ion channels, this ion should be considered when studying regulation of other ion channels by phosphoinositides. As representative members of the two groups of TRPVs that are activated by PIP₂, it is quite likely that PIP₂ is a general activator of TRPV channels, but we have to wait for further publications to confirm this.

TRPM channels

TRPM channels are the most recently cloned and most diverse members of the mammalian TRP family (Table 3). There are not one or two simple themes as for TRPCs and TRPVs that could describe their functions. There are four members of the TRPM family that have been reported to be activated by PIP₂; these are TRPM7 [26, 87], TRPM5 [50], TRPM8 [48, 86], and TRPM4 [65, 114]. We will provide some background on the functions of these four channels in the context of their phosphoinositide regulation. It is likely that PIP₂ is a general activator of TRPM channels, but again we have to wait for further data to confirm this.

TRPM7 is the first mammalian TRP channel that was reported to be activated by PIP₂ [87]. TRPM7 is an intriguing channel. It has an atypical kinase domain at the end of its C terminus, similarly to TRPM6. The exact function of this kinase domain and its role in channel regulation is not yet clear. It is hard to describe the functional role of TRPM7 in one sentence, as multiple

Table 3 Some important properties of the TRPM family members

	Selectivity P_{Ca}/P_{Na}	Proposed activation mechanisms	Effect of phosphoinositides (PIPs)
TRPM1, ENSG00000134160	n.d.	Translocation, microphthalmia-associated transcription factor (MITF) induced expression	?
TRPM2, ENSG00000142185	0.5–1.6	ADP-ribose, cyclic ADP-ribose at higher temperatures, nicotinamide adenine dinucleotide (NAD), heat, H_2O_2 and other reactive oxygen species (ROS), poly(ADP)ribose polymerase-1 (PARP-1), Ca^{2+} , NAD dependent deacetylases sirtuins	?
TRPM3, ENSG00000083067	1.6–2.0	Cell swelling, store depletion? D-erythrospingosine	?
TRPM4, ENSG00000130529	Selective for monovalent cations	Elevated $[Ca^{2+}]_i$, ATP, PKC, decavanadate, voltage dependent, heat, 3,5-bis(trifluoromethyl)pyrazole (BTP2)	PIP ₂ activates [65, 114]
TRPM5, ENSG00000070985	Selective for monovalent cations	Elevated $[Ca^{2+}]_i$, voltage dependent, heat	PIP ₂ activates [50]
TRPM6, ENSG00000119121	$P_{Mg}/P_{Na} \sim 6$	Decreased $[Mg^{2+}]_i$, 2-APB	?
TRPM7, ENSG00000092439	3	Decreased $[Mg^{2+}]_i$, Mg-ATP, CAMP, G proteins, shear stress, membrane translocation, PARP	PIP ₂ activates [26, 87] challenged [43, 98]; role in traffic to the plasma membrane [40]
TRPM8, ENSG000000144481	1–3	Depolarization, cold (8–28°C), menthol, icilin, Ca^{2+} , increased intracellular pH	PIP ₂ activates [48, 86]

For references for channel names, numbering, and additional information, see legend to Table 1. For details and references see legend Table 1.

and quite diverse functions have been attributed to it. Deletion of TRPM7 in mice is embryonically lethal (A. G. Ryazanov, personal communication), suggesting important functions in fundamental processes.

TRPM7 channels conduct not only Ca^{2+} and monovalent cations but also Mg^{2+} . It was proposed that TRPM7 functions as the major cellular Mg^{2+} uptake mechanism [91]. TRPM7 knockout cells have severely altered Mg^{2+} uptake mechanism; the residual Mg^{2+} transport is provided by the solute transporter SLC41A2 [88]. Intracellular Mg^{2+} inhibits TRPM7, which may serve as a negative feedback mechanism to inhibit Mg^{2+} uptake when the cell has enough Mg^{2+} . Under conditions of low intracellular Mg^{2+} , relief from this inhibition may open the channel to restore Mg^{2+} levels. It has been proposed that Mg^{2+} inhibits these channels via screening of the charges in PIP₂ [39]. TRPM7 has also been shown to be involved in cell death [1], neurotransmitter release [40], cell adhesion [94], and transport of trace metals [59].

TRPM7 has been proposed to be inhibited by PLC-mediated PIP₂ hydrolysis [87], but this was challenged later by proposing that cyclic adenosine monophosphate and protein kinase A were the major regulators of TRPM7 and Gq-coupled receptors played only a minor role [98]. This contradiction has been discussed in a previous review on this topic [82]; therefore, we will not discuss it in detail here. An interesting recent addition to this debate is the

finding that bradykinin acting through a PLC-coupled receptor activates TRPM7 in the perforated patch configuration [43]. The same report confirms previous findings that PLC activation inhibits TRPM7 in the whole-cell configuration, if Mg^{2+} is not provided in the pipette solution. Providing Mg^{2+} in the pipette solution reconstituted the activating effect of PLC. The mechanism of the activation of TRPM7 by bradykinin and the involvement of PIP₂ was not examined. It would be an interesting hypothesis to test if PIP₂ has different effects on TRPM7 depending on the Mg^{2+} concentration. Note that it was proposed that bradykinin receptor stimulation may not appreciably alter membrane PIP₂ levels in contrast to muscarinic stimulation, as it may also activate PI4 kinase that could offset the decrease in PIP₂ levels induced by PLC [17]. In native myocardial cells, PIP₂ is required for maintaining the activity of a cardiac channel (the Mg^{2+} -inhibited cation channel), which is probably TRPM7 [26].

Interestingly, TRPM7 is present in synaptic vesicles and forms a complex with synapsin I and synaptotagmin I and directly binds to snapin. PIP₂ seems to target these vesicles to the plasma membrane, and it may also be responsible for TRPM7 activation upon fusion of the synaptic vesicle (devoid of PIP₂) to the plasma membrane (contains PIP₂). Likely, TRPM7 is involved in acetylcholine release: The influx of cations into the vesicles via vesicle-attached TRPM7 may mediate an influx of counter-ions, which facilitate acetylcho-

line release from a negatively charged site in an ion exchange matrix, probably proteoglycans [40, 61].

TRPM4 and TRPM5 are nonselective cation channels that have negligible Ca^{2+} permeabilities [33, 45, 76]. Both are activated by an increase in intracellular $[\text{Ca}^{2+}]_i$. TRPM5 was first described in taste tissue [75]; TRPM5^{-/-} mice show markedly diminished [16] or no responses [113] to bitter, sweet, and umami tastes. Interestingly, TRPM5 is also inhibited by cold temperatures, and this effect is responsible for the thermal sensitivity of sweet taste [99]. The technical term “thermal sensitivity of sweet taste” is best explained by everyday experience: Warmer sugary solutions taste sweeter than cold ones.

In contrast to the well-defined role of TRPM5 in taste sensation, TRPM4 has more diverse roles. TRPM4 has been shown to play a role in Ca^{2+} oscillations in T cells [44], insulin secretion [13], and myogenic constriction of cerebral arteries [20], and it constitutes the Ca^{2+} -activated nonselective Ca^{2+} channel in cardiac cells [68].

Despite their different physiological roles, TRPM4 and TRPM5 share activation by PIP_2 . Both channels are activated by Ca^{2+} in excised patches, but their Ca^{2+} sensitivity decreases with time. This desensitization can be reversed by the application of PIP_2 [50, 65, 114]. PIP_2 activation of TRPM4 is much more prominent than that of TRPM5 [65]. The mechanism of activation by PIP_2 involves an increase in the Ca^{2+} sensitivity of TRPM4 and a dramatic shift of the voltage range for activation to negative potentials. Depletion of PIP_2 from the cell via receptor stimulation, incubation of cells with wortmannin

(an inhibitor of phosphatidylinositol 4-kinase, which delays PIP_2 replenishment) application of the PIP_2 -scavenging agent poly-L-lysine, and overexpression of a PIP_2 -depleting enzyme 5ptase IV lead to a reduction in TRPM4 activity. The most striking proof is obtained in inside-out patches. Desensitization of the channel can be prevented or reversed when PIP_2 is reapplied to the cytosolic side of excised patches. Recovery from desensitization can be achieved even in inside-out patches when Mg-ATP is added to the intracellular side during free perfusion, indicating that PIP_2 is probably replenished via activation of lipid kinases. Because all these modulator effects prevail in excised inside-out patches, it is intriguing to speculate that PIP_2 -modulating enzymes, such as lipid kinases (PIP4K and PIP5K), a Ca^{2+} -dependent PLC, or phosphatidylinositol phosphatases form structural complexes with TRPM4 [65, 68] and other PIP_2 -sensitive channels [34, 97].

The voltage sensor of the *Aeropyrum pernix* voltage-gated potassium (KvAP) requires negatively charged phospholipids, such as phosphatidylglycerol, to maintain its gating properties [46, 90]. It is likely that the two first arginines in the S4 region of the voltage sensor interact with the negatively charged phosphate head groups of the surrounding lipid. Interestingly, it has been shown for TRPM4 that the apparent gating charge of $\sim 0.7e$ under conditions of PIP_2 depletion is reduced in the presence of PIP_2 to ~ 0.34 [65]. A possible explanation could be the direct interaction between the voltage sensor and the negatively charged phosphate head group in PIP_2 similarly to KvAP [90].

Table 4 Some important properties of members of the TRPP and TRPML families, as well as of TRPA1

	Selectivity $P_{\text{Ca}}/P_{\text{Na}}$	Proposed activation mechanisms	Effect of phosphoinositides (PIPs)
TRPML1, ENSG00000090674	~ 1 , H^+ permeable	Increased $[\text{Ca}^{2+}]_i$, inhibited by proteolytic cleavage	?
TRPML2, ENSG00000153898	n.d.	n.d.	?
TRPML3, ENSG00000055732	n.d.	n.d.	?
TRPP2, ENSG00000118762	1–5	Mechanical stress, $[\text{Ca}^{2+}]_i$	PIP_2 inhibits [54]
TRPP3, ENSG00000107593	4	$[\text{Ca}^{2+}]_i$	?
TRPP5, ENSG00000078795	1–5	$[\text{Ca}^{2+}]_i$	?
TRPA1, ENSG00000104321	0.8–1.4	Isothiocyanates, allicin, Δ^9 -tetrahydrocannabinol (THC), cinnamaldehyde, prolonged noxious cold?, mechanical stress, voltage dependent?, $[\text{Ca}^{2+}]_i$ dependent, OAG and arachidonic acid downstream of receptor-mediated PLC activation, menthol?, thymol, carvacrol, acrolein, artemin (a neuronal survival factor), covalent modification of N-terminal cysteines activates	?

For references for channel names, numbering, and additional information, see legend to Table 1

TRPM8 is a cold-sensitive ion channel expressed in sensory neurons. It is also activated by menthol [55, 74], depolarization [106], and PIP₂ [48, 86]. These factors have a complex interplay in regulating this channel. Menthol sensitizes to cold [55], shifts the voltage dependence of the channel [106], and increases its apparent affinity for PIP₂ [86]. Depolarization also increases sensitivity to PIP₂ [86] and menthol [106]. Cold also shifts voltage dependence [106] and PIP₂ sensitivity [86]. The channel displays an almost exclusive dependence on PIP₂, chelating PIP₂ in excised patches blocks channel activity completely [86]. Breaking down PIP₂ in intact cells with rapamycin-inducible 5-phosphatase almost completely inhibits TRPM8 [104]. TRPM8, similar to TRPV1, undergoes desensitization in response to menthol and cold in the presence of extracellular Ca²⁺. It was proposed that the mechanism of this desensitization is the depletion of PIP₂ via Ca²⁺-mediated activation of PLC (presumably a PLC δ isoform) [86]. PKC has also been shown to inhibit TRPM8 [2, 77]. As PKC is also activated upon PLC activation, inhibition by PKC is also a feasible mechanism for desensitization. More experiments are needed to differentiate between these two alternative pathways. Menthol and the supercooling agent icilin have analgesic effects, which is mediated by the activation of TRPM8 [79]. A recent report showed that high concentrations of ethanol inhibit TRPM8 [4], and it was proposed that the mechanism of this inhibition is the interference with PIP₂ activation.

TRPM8 is also expressed in the prostate, where neither cold temperatures nor cooling agents could serve as their physiological regulators. It has recently been shown that lysophospholipids (LPLs) also activate TRPM8 [103]. Whether the effects of LPLs depend on phosphoinositides needs to be determined in future experiments.

TRPP2

Polycystins are distant homologues of TRP channels. They were discovered by positional cloning of the gene responsible for the autosomal dominant form of PKD. PKD is one of the most common genetic disorders and a leading cause of end-stage renal disease worldwide. It was shown that polycystin 2 (PKD2, TRPP2) is inhibited by PIP₂, and EGF activates PKD2 by relief from this inhibition via PIP₂ hydrolysis [54]. For properties of the members of the TRPP and TRPML families, as well as TRPA1, see Table 4.

Conclusions

From the data reviewed here, it is tempting to assume that maybe the majority, if not all TRP channels are regulated by phosphoinositides. Only a few examples, however, have

been studied in detail. Because we assume that this lipid regulation presents a general mechanism for modulation of TRP channel activity, some urgent questions have to be answered. First, a close correlation between PIP₂ levels in the plasma membrane and channel activity has to be monitored in simultaneous measurements. Elegant tools are now available for such studies [96, 104]. Second, PIP₂ probably interacts with clusters of positive charges in the TRP channel proteins. Possible binding motifs for TRP channel interaction with PIP₂ are pleckstrin homology domain-like sequences [65], which can be identified in all TRP proteins, or positively charged residues in the TRP domain [86]. However, the structure of a putative PIP₂-binding pocket is probably complex and still unknown. Furthermore, it has to be shown whether the effects of PIPs are really due to binding or due to charge compensation. Third, the functional impact of TRP channel modulation by PIP₂ has to be shown in native cells under conditions of physiological stimulation, which would cause changes in PIP₂ levels under in vivo conditions.

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