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Phosphoinositide regulation of non-canonical Transient Receptor Potential channels

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Abstract

Transient Receptor Potential (TRP) channels are involved in a wide range of physiological processes, and characterized by diverse activation mechanisms. Phosphoinositides, especially phosphatidylinositol 4,5-bisphosphate [PIP₂, or PtdIns(4,5)P₂] recently emerged as regulators of many TRP channels. Several TRP channels require PIP₂ for activity, and depletion of the lipid inhibits them. For some TRP channels, however, phosphoinositide regulation seems more complex, both activating and inhibitory effects have been reported. This review will discuss phosphoinositide regulation of members of the TRPM (Melastatin), TRPV (Vanilloid), TRPA (Ankyrin) and TRPP (Polycystin) families. Lipid regulation of TRPC (Canonical) channels is discussed elsewhere in this volume.

1. Introduction

Transient Receptor Potential (TRP) channels are distant relatives of the voltage gated ion channel superfamily [1]. Just like voltage gated ion channels, they have six transmembrane domains per subunit and four subunits form the functional channel [2]. Most TRP channels are non-selective, Ca²⁺ permeable cation channels, and many of them display weak voltage dependence. Based on sequence homology, mammalian TRP channels are subdivided into six groups: TRPC (Classical or Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin) and TRPA (Ankyrin).

TRP channels play roles in a wide variety of physiological processes. They are activated by a diverse set of factors, including temperature, mechanical stimuli, pH, and various chemical ligands. TRPC channels are activated downstream of phospholipase C (PLC) leading to Ca²⁺ influx and presumably depolarization in many different cell types [3]. Many TRP channels are involved in sensory functions [4]. TRPV1-4 are heat activated channels with various thresholds, TRPM8 and potentially TRPA1 are activated by cold. Temperature sensitivity was also reported for TRPM2, 4 and 5 [5]. TRPV1 and TRPA1 are involved in nociception, TRPM5 in taste, and TRPV4 in mechanosensation.

Several TRP channels play roles in mineral homeostasis at the cellular or organism level; TRPM6 and potentially TRPM7 function as Mg²⁺ transporters, mutation of TRPM6 leads to hypomagnesemia in humans [6]. TRPV5 and TRPV6 transport Ca²⁺ in renal and intestinal epithelial cells, respectively [7]. There are various other functions associated with TRP channels that do not fall into these categories, recent reviews discuss these in detail [2;8].

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Despite the high diversity of activation mechanisms and physiological functions, most, if not all TRP channels are regulated by phosphoinositides [9;10]. Phosphoinositides are membrane phospholipids, the phosphorylated derivatives of phosphatidylinositol (PtdIns). They undergo complex metabolism, as shown in Figure 1. Phosphatidylinositol 4,5-bisphosphate, [PtdIns (4,5)P₂ or PIP₂] is the most thoroughly studied phosphoinositide with respect to regulation of ion channels. It is generated via two sequential phosphorylation steps from PtdIns, by phosphoinositide 4 kinases (PI4K) and phosphatidylinositol 4-phosphate 5-kinases (PIP5K). Phosphoinositide 3-kinases (PI3K) generate PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ which play roles in signaling by various growth factors.

PIP₂ has various biological functions. It is the substrate for phospholipase C (PLC), thus the precursor of the two classical second messengers inositol 1,4,5 trisphosphate (IP₃) and diacylglycerol (DAG). It is also a substrate for PI3K to generate PtdIns(3,4,5)P₃. In addition to its precursor role, PIP₂ also plays a role on its own right in a variety of biological processes, such as membrane trafficking and cytoskeletal function. In many cases it serves as a membrane anchor for cytoplasmic proteins which bind to PIP₂ via various lipid binding domains, such as PH domains [11]. PIP₂ also regulates a wide variety of ion channels [12], including inwardly rectifying (Kir) and voltage gated K⁺ and Ca²⁺ channels, epithelial Na⁺ channels and as will be discussed here, TRP channels.

This review will focus on phosphoinositide regulation of mammalian TRP channels that are not in the TRPC family i.e., the “non-canonical” ones. Lipid regulation of mammalian TRPC-s and drosophila homologues of TRPC-s are discussed in detail in other articles in this volume.

2. General Features of Ion Channel Regulation by PIP₂

Most PIP₂ sensitive ion channels are activated by the lipid, probably through direct interactions. PIP₂ dependence of ion channels and transporters was discovered while studying the phenomenon of “run-down” i.e. loss of activity, in excised patches [13]. In ATP free medium, PIP₂ is gradually lost in this experimental setting leading to diminished channel activity. PIP₂ dependent ion channels can be reactivated by applying the lipid to the cytoplasmic face of the membrane patch. In intact cells, depletion of PIP₂ by PLC activation inhibits PIP₂ sensitive ion channels (Fig. 2A). Dependence of channel activity on PIP₂ is a common feature of many ion channels, including TRP channels. For several TRP channels, however, both positive and negative effects of the lipid have been reported, as discussed later. Before discussing data on TRP channels, I will briefly summarize general features of PIP₂ regulation of ion channels, emphasizing issues that may explain some of the complexities and problems specifically encountered with TRP channels.

2.1 Relationship between PIP₂ and other channel regulators

Many PIP₂ sensitive ion channels are constitutively active and they are open as long as PIP₂ is present. Examples several Kir channels, such as Kir2.1, or from the TRP channel family, TRPV5 and TRPV6, see later.

Most PIP₂ sensitive ion channels, however, require additional stimuli to open (Fig. 2A). For example, G-protein gated Inwardly Rectifying K⁺ (GIRK or Kir3.x) channels are opened by the βγ subunit of G-proteins (Gβγ); some GIRK channels are also activated by increased intracellular Na⁺. The ability of Gβγ and Na⁺ to open these channels depends on PIP₂. In excised patches none of these stimuli can activate the channel if PIP₂ is lost, but their ability to open the channel is restored if PIP₂ is applied. In the TRP channel field, TRPM8 is a good example of such regulation. TRPM8 is opened by menthol and cold, but the ability of these stimuli to activate the channel depends on the presence of PIP₂ [14]. As discussed in the next section, these other stimuli may also modify the effects of PIP₂.

2.2 The role of the apparent affinity

Under resting conditions the inner leaflet of the plasma membrane contains significant amounts of PIP₂. Whether this is enough to keep a particular PIP₂ sensitive channel maximally open, depends on the apparent affinity of the channel for the lipid (Fig. 2B). Channels with high affinity for PIP₂ cannot be further activated by excess PIP₂ because resting PIP₂ levels are saturating them. On the other hand, channels with lower PIP₂ affinity can be theoretically further activated by increased PIP₂ levels. Conversely, and more importantly, channels with lower PIP₂ affinity can be easily inhibited by depletion of PIP₂, whereas channels with high PIP₂ affinity are not inhibited significantly by moderate (physiological) PIP₂ depletions (Fig. 2B). Even high affinity channels can be inhibited by complete depletion of PIP₂, such as applying a PIP₂ chelator in excised patches.

It is important to note that the apparent affinity for PIP₂ is not necessarily static. For GIRK channels it was proposed that both Gβγ and Na⁺ open the channels by stabilizing its interaction with PIP₂ [15;16]. This would manifest as a left shift in the PIP₂ dose-response, and increased channel activity at a constant PIP₂ concentration (Fig. 2B). Increased affinity for PIP₂ also leads to reduced sensitivity to inhibition by PIP₂ depletion (Fig. 2B). For Na⁺, a compelling mechanistic model was proposed recently to explain how it increases PIP₂ sensitivity of GIRK channels: Na⁺ binds to an Asp and His that triggers a structural switch that frees a crucial Arg enabling it to interact with PIP₂ [17]. Among TRP channels, TRPM8 is a good example of this type of regulation. It was shown that both cold and menthol shifts the PIP₂ dose-response to the left, i.e. sensitizes the channel to PIP₂. This was accompanied by reduced sensitivity to inhibition by depletion of PIP₂ [14].

Mutation of PIP₂ interacting residues may convert a high affinity channel to a low affinity one, shifting its PIP₂ dose-response to the right and rendering it more sensitive to inhibition by PIP₂ depletion. This phenomenon is utilized in mutation studies aiming at locating putative PIP₂ interacting residues [18].

2.3 How does PIP₂ interact with ion channels?

The generally accepted view is that the negatively charged head group of PIP₂ interacts with positively charged amino acid residues in the cytoplasmic domains of ion channels (Fig. 2A). The most thorough studies locating these residues have been performed on Kir channels. Most positively charged residues, mutation of which altered PIP₂ interactions, were located in the C-terminus, but some were in the N-terminus [18]. A homology model based on partial crystal structures of various Kir channels has been proposed to depict PIP₂ interacting residues in Kir channels [19].

Even though the model just described is quite widely accepted, several points need to be made that make this seemingly simple picture more complicated. First, crystal structures of known phosphoinositide interacting soluble proteins show that the interactions with the lipid always involve 2-7 positively charged residues. It is also clear from these studies however, that non-charged residues also invariably contribute to phosphoinositide binding [11]. Some of the non-charged residues commonly contributing to phosphoinositide binding are Tyr, Asn and Ser [11]. Virtually no effort has been made so far to identify non-charged residues that interact with phosphoinositides in ion channels.

Second, even when a positively charged residue is identified, mutation of which alters channel activation by PIP₂, it is very difficult to tell with certainty, based on mutagenesis data whether this residue interacts directly with PIP₂, or its mutation alters PIP₂ interactions indirectly. This statement is also true for most mutagenesis based studies aiming to identify ligand binding sites. This problem is discussed in detail by Colquhoun [20]. Even when the crystal structure

of the channel is solved without the interacting lipid, it is not trivial to dock PIP₂ and tell which residue it is in contact with. Up to now, no ion channel has been co-crystallized with phosphoinositide ligands. Third, it is not well understood how phosphoinositide binding leads to channel opening.

When the partial crystal structures of various Kir channels were published, most of the putative PIP₂ interacting residues identified earlier by mutagenesis [18] lined up on the interface of the channel with the membrane, compatible with the idea that they are part of the PIP₂ binding site. This shows that the mutagenesis approach is useful in finding putative PIP₂ interacting residues. Some residues, however, were in locations incompatible with being part of the PIP₂ binding site, showing that this approach may also produce false positives, as implied in the previous paragraph.

2.4 Phosphoinositide specificity

The majority of research has focused on the role of PtdIns(4,5)P₂, the isomer generally referred to as PIP₂. Other phosphoinositides, however, may also activate ion channels in excised patches with variable efficiency [21]. When studying the effects of other phosphoinositides (Fig. 1) we have to consider their potency/efficacy and their concentration in the plasma membrane relative to PtdIns(4,5)P₂. Even though the local concentration of a given phosphoinositide in the vicinity of a given ion channel is difficult to determine, the following numbers give a rough guideline on the relative abundance of these lipids in the plasma membrane [22;23]. PtdIns(4,5)P₂ constitutes up to 1% of the phospholipids in the plasma membrane. PtdIns(4)P (PIP), is thought to be in comparable quantities. The concentrations of PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ are generally assumed to be much lower than that of PtdIns(4,5)P₂ even in stimulated cells. PtdIns, the precursor of all phosphoinositides is relatively abundant; it is thought to constitute ~10% of all phospholipids in the cell and in the plasma membrane, but it is usually not a very efficient activator of ion channels. PtdIns(4,5)P₂ is likely to be the major regulator of most phosphoinositide sensitive ion channels, because it is more efficient and/or because its concentration is higher relative to other phosphoinositides. There are examples, however, where other phosphoinositides may also play a physiological role in addition to PtdIns(4,5)P₂, for example PtdIns(4)P for TRPV1, see later.

There are additional phosphoinositides, not depicted in Figure 1 that also exist in various cellular membranes; these are PtdIns(3)P, PtdIns(5)P and PtdIns(3,5)P₂. They are much less abundant than PtdIns(4,5)P₂, and virtually nothing is known about their role in ion channel regulation, thus they will not be further discussed here.

2.5 Possible indirect effects of PIP₂

The model in Figure 2 assumes direct activation of ion channels by PIP₂. As explained in reference [9] it is not trivial to prove that a given channel is activated by PIP₂ directly. Nevertheless, it is quite likely that most PIP₂ sensitive channels are activated by the lipid through direct interactions. Direct activation by the lipid is usually assumed if PIP₂ activates a channel reproducibly in excised patches, binds to it, and mutating (positively charged) residues in the channel protein affects PIP₂ activation and/or binding. A more definitive proof for direct effects of the lipid is the demonstration of phosphoinositide sensitivity on a purified reconstituted ion channel [24;25].

A much less explored possibility is that PIP₂ affects channel function indirectly. Given the complexity of the regulation of some TRP channels by PIP₂, we will discuss some possible scenarios for indirect effects of PIP₂, some of which are depicted in Figure 3. Indirect effects of PIP₂ may eventually explain some of the confusing findings on the TRP channel field, and resolve discrepancies between excised patch and intact cell data; see later.

Calmodulin, the ubiquitous Ca^{2+} binding protein can bind to other proteins through a large diversity of motifs [26] (<http://calcium.uhnres.utoronto.ca/ctdb/ctdb>). Some of these motifs contain several positively charged residues. This gives a structural basis for competition of PIP_2 and calmodulin for the same, or overlapping binding site(s). Competition between calmodulin and phosphoinositides was indeed demonstrated for several TRP and other ion channels [27] and soluble calmodulin binding proteins, such as MARCKS [28]. Figure 3A depicts this scenario; when cytoplasmic Ca^{2+} concentrations rise, Ca^{2+} binds to calmodulin and Ca^{2+} -calmodulin inhibits the channel via competing with PIP_2 for its activating site. A similar model was first proposed for TRPC6 where the inhibitory Ca^{2+} -calmodulin and the activating $\text{PtdIns}(3,4,5)\text{P}_3$ (PIP_3) compete for an overlapping binding site, resulting in complex regulation [27]. Biochemical competition between calmodulin and phosphoinositides was shown for the C-terminus of several other TRP channels including TRPV1 and other TRPCs, but no functional data were presented for those channels. There are several TRP and other ion channels where both calmodulin and PIP_2 regulation have been described, thus this theme may turn out to be a general mechanism of (TRP) channel regulation. This model still assumes a direct effect of PIP_2 on the channel, but it is altered by another factor, i.e. calmodulin. A modified version of this model is where PIP_2 activates the channel by displacing the inhibitory calmodulin, but once calmodulin (or some other soluble inhibitory factor) is missing, the channel would not require PIP_2 for activity. In this case, we would not expect a dependence on PIP_2 in excised patches, once this inhibitory factor is lost.

Conversely, PIP_2 may inhibit a channel by displacing a cytoplasmic factor that is needed for the activity of the channels (Fig. 3B). Alternatively, PIP_2 may have an effect on the channel through an intermediary soluble PIP_2 binding protein, which can either activate (Fig. 3C), or inhibit (Fig. 3D) the channel. These three models (Fig. 3B-D) are purely hypothetical, but in these cases we would expect a discrepancy between findings in intact cells and in excised patches, where these soluble factors would be lost. Such discrepancies indeed do exist for some TRPC channels [29] and for TRPV1, see later.

It is also possible that a channel is modulated by a PIP_2 sensitive membrane protein. PIP_2 was proposed to modulate TRPV1 through Pirt (Phosphoinositide interacting regulator of TRP), a two transmembrane domain protein that binds to PIP_2 [30] (Fig. 3E). Pirt, or another membrane resident PIP_2 interacting protein would not be lost in excised patches, thus it is unlikely to account for discrepancies with intact cell measurements. The presence or absence of such protein, however, could explain discordant findings in different cell types. Finally, phosphoinositides may also modify trafficking of ion channels or transporters [31].

2.6 Tools to study phosphoinositide regulation of ion channels

Perhaps the most direct experiment is to study the effects of PIP_2 in excised patches. Various PIP_2 analogues are available for these experiments; PIP_2 from natural sources has mainly arachidonyl-stearyl side chains, while synthetic PIP_2 usually contains two palmitoyl side chains. These long acyl chain lipids accumulate in the patch membrane, thus it is difficult to control their effective concentration. After activation with these PIP_2 analogues most ion channels run down very slowly upon cessation of the application of the lipid, making repeated application of these analogues impractical [32]. Increasingly popular are the short acyl chain (DiC_8) analogues of PIP_2 ; the activation of most ion channels by these lipids is quickly reversibly, presumably because they diffuse out the membrane easily upon washout [33]. Their effective concentration in the membrane is easier to control, but they are less “natural” than the long acyl chain analogues. It is important to keep in mind that the patch membrane contains significant amount of PIP_2 in the cell attached configuration. After excision, PIP_2 concentration in the patch decreases, probably because lipid phosphatases associated with the patch dephosphorylate PIP_2 . MgATP usually reverses run-down, presumably because it serves as a

substrate for lipid kinases associated with the patch. Mg^{2+} applied to the intracellular surface of the patch accelerates run down by providing a cofactor for lipid phosphatases [15]. Mg^{2+} also inhibits PIP_2 sensitive channels by screening the negative charges of the lipid [34]. The velocity of the rundown of the activity of a given channel generally correlates with its apparent affinity for PIP_2 ; channels with higher PIP_2 affinity run down slower than channels with lower affinity. One can also apply PIP_2 chelating agents, such as PIP_2 antibody [15] or poly-Lysine [18] to excised patches to accelerate run-down. Poly-Lys is less selective than the antibody, but it works more reliably.

A large variety of techniques have been used to measure direct biochemical binding of phosphoinositides to ion channels [15;35;36], including TRP channels [27]. Most of these studies were performed with purified cytoplasmic segments of ion channels. The advantage of this approach is that it clearly measures direct association of phosphoinositides with ion channels. The disadvantage is that isolated segments of the channel protein are studied, thus it is possible that the measured binding does not correspond to the biologically important interactions. In several cases mutations that affected PIP_2 binding were reintroduced into the full length channel and functional effect were shown on phosphoinositide sensitivity [15]. This is a strong argument for direct activation of a channel by PIP_2 . A perhaps even stronger evidence for direct activation of a channel by PIP_2 is the demonstration of the effect of the lipid on a purified reconstituted channel. It was shown that PIP_2 inhibits the purified bacterial KirBac channel reconstituted in lipid vesicles [24]. We have recently demonstrated that the activity of the purified TRPM8 depends on the presence of PIP_2 in lipid bilayer experiments [25], providing a strong evidence for direct activation of the channel by PIP_2 .

In intact cells PIP_2 levels can be modified using a variety of tools. PIP_2 levels can be decreased by activating PLC via G-protein coupled receptors ($PLC\beta$), receptor tyrosine kinases ($PLC\gamma$) or Ca^{2+} influx (probably $PLC\delta$). The pleiotropic effects of PLC activation (IP_3 , DAG, Ca^{2+}) make interpretation of these experiments difficult. An alternative approach to modify PIP_2 levels is over-expression of various lipid kinases and phosphatases. In these experiments the long term changes in phosphoinositide levels may have unanticipated effects, again complicating data interpretation. Only limited pharmacological tools are available to inhibit various enzymes involved in PIP_2 metabolism, and they are not very specific. At relatively low concentrations, wortmannin (10 – 100 nM) and LY294002 (10 μ M) are widely used as PI3K inhibitors. At higher concentrations (>5 μ M for wortmannin and 100 μ M for LY294002) they also inhibit PI4K isoforms, thus slowly depleting PIP_2 . PLC can be inhibited by U73122 and edelfosine, but these drugs have a number of side effects. PLC can also be activated pharmacologically by m-3M3FBS [37]. A novel approach is chemically, or electrically inducible PIP_2 phosphatases, both are based on the translocation of a PIP_2 phosphatase enzyme to the plasma membrane. One approach is based on the rapamycin-induced heterodimerization of mTOR and FKBP12 [38;39]. In this system, the plasma membrane anchored mTOR is dimerized upon the application of rapamycin with the FKBP12- PIP_2 5-phosphatase fusion protein. This leads to a rapamycin-inducible translocation of the 5-phosphatase from the cytoplasm to the plasma membrane and results in the depletion of $PtdIns(4,5)P_2$ converting it to $PtdIns(4)P$. This approach was successfully used to inhibit KCNQ [38] and TRPM8 channels [39]. The voltage sensitive PIP_2 5-phosphatase from *Ciona intestinalis* (Ci-VSP) is an alternative approach; its phosphatase domain moves closer to the plasma membrane upon depolarization, depletes PIP_2 , and inhibits PIP_2 sensitive Kir and KCNQ channels [40].

In conclusion, there is a plethora of reagents to study involvement of PIP_2 in ion channel regulation, and it is necessary to use multiple of them to obtain conclusive results. This topic has been also discussed in more detail in two recent reviews: [12;41].

2.7 Controversies and open questions

The two best characterized ion channel families in terms of phosphoinositide regulation are probably Kir and KCNQ channels. In both cases, it is demonstrated beyond reasonable doubt that all members of the respective families require PIP₂ for activity. It is clear that depletion of the lipid in intact cells inhibits these channels, the question is just how much PIP₂ depletion they need, and as discussed earlier, this mainly depends on PIP₂ affinity. What is less clear in each case is to what extent the depletion of the lipid is responsible for the inhibition of channel activity upon PLC activation, especially in native cells. For both channel families various other mechanisms downstream of PLC, such as protein kinase C (PKC), calmodulin, or Ca²⁺ were also proposed, and likely to contribute to inhibition under certain circumstances. It is likely that the contribution of PIP₂ depletion vs. other mechanisms is receptor specific and depends on the cell type and agonist.

Many TRP channels are affected by PIP₂. In some cases the effect of the lipid is clear and reproducible activation of the channel; TRPM8 is a good example. In these cases the questions are similar to those described in the previous paragraph. In some other cases, however, (TRPA and some TRPC-s) it is not even clear if PIP₂ activates or inhibits the channel. Whether these controversies will be explained in a coherent way, is still an open question. This review will discuss the experimental findings on PIP₂ regulation of TRPM, TRPV, TRPA and TRPP channels.

3. Trpm Channels

TRPMs are the functionally most diverse group in the TRP channel superfamily with eight mammalian members. Most TRPMs are non-selective Ca²⁺ permeable cation channels, similar to most other TRP-s. Exceptions are TRPM4 and TRPM5, which are Ca²⁺-activated, non-selective cation channels that are not permeable to Ca²⁺. PIP₂ regulation has been reported for 4 members of this group, in all cases PIP₂ activated the respective channel (Table 1) and thus PIP₂ dependence is probably a common feature of TRPM channels.

3.1 TRPM7

TRPM7 has been proposed to play roles in a variety of functions, including anoxic cell death, cellular Mg²⁺ and trace metal ion homeostasis, neurotransmitter release, cell adhesion, and development of the immune system [42-46]. Consistent with its pleiotropic role, its genetic deletion in mice is embryonic lethal [46]. TRPM7, similar to TRPM6, has an atypical kinase domain at its C-terminal end, the functional role of which not yet clear.

TRPM7 was the first TRP channel that was reported to require PIP₂ for activity [47]. The channel runs down in excised patches; it can be reactivated by PIP₂ and inhibited by a PIP₂ antibody [47]. Run down is prevented by MgATP, presumably because it serves as a substrate for lipid kinases resynthesizing PIP₂ [47]. Mg²⁺ itself on the other hand inhibits the channel via screening of the charges of phospholipids, probably PIP₂ [48]. In native myocardial cells, PIP₂ was also shown to be required for the activity of a cardiac “Mg²⁺-inhibited cation channel”, which is probably TRPM7 [49]. TRPM7 channels conduct Mg²⁺, in addition to Ca²⁺ and monovalent cations. TRPM7 may function as the major cellular Mg²⁺ uptake mechanism [50], even though this notion was challenged recently [46]. Intracellular Mg²⁺ inhibits TRPM7, serving as a negative feedback mechanism to inhibit Mg²⁺ uptake. Under conditions of low intracellular Mg²⁺, relief from this inhibition may open the channel to restore Mg²⁺ levels. Mg²⁺ was proposed to inhibit these channels via screening of the charges in phospholipids, probably PIP₂ [48].

TRPM7 was proposed to be inhibited by PLC mediated PIP₂ hydrolysis [47]. Runnels et al reported that activation of muscarinic receptors lead to inhibition of TRPM7 activity. Channel

activity after washout of the stimulus recovered faster if PIP₂ was dialyzed through the patch pipette, and slower is resynthesis of PIP₂ was inhibited with wortmannin [47]. The role of PIP₂ hydrolysis in TRPM7 regulation was challenged later by two articles. Takezawa et al proposed that cyclic adenosine monophosphate and protein kinase A are the major regulators of TRPM7 and Gq-coupled receptors played only a minor role [51]. Another study reported that bradykinin acting through a PLC-coupled receptor activates TRPM7 in the perforated patch configuration, but inhibits it in the whole-cell configuration, if Mg²⁺ is not provided in the pipette solution [52]. Providing Mg²⁺ 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. In summary, TRPM7 requires PIP₂ for activity, but under what circumstances PIP₂ depletion regulates it and to what extent, needs further clarification.

3.2 TRPM8

TRPM8 is an ion channel activated by cold temperatures and cooling agents such as menthol or icilin in sensory neurons [53;54]. Knockout studies convincingly demonstrated the involvement of this channel in sensing moderately cold temperatures [55-57]. TRPM8 is also likely to be involved in mediating the analgesic effects of moderate cold and menthol [58].

TRPM8 clearly requires PIP₂ for activity. Its activity runs down in excised patches, and application of PIP₂ reactivates the channel [14;59]. The activating effect is isomer specific; PtdIns(4,5)P₂ is more effective than PtdIns(3,4)P₂, PtdIns(3,4,5)P₃ or PtdIns(4)P [14]. PIP₂ chelating agents, such as PIP₂ antibody, or poly-Lysine also inhibit TRPM8 in excised patches [14;59]. The activity of the purified TRPM8 reconstituted into lipid bilayers depends on the presence of PIP₂, providing a strong evidence for direct activation of the channel by PIP₂ [25]. Activation of PLC via cell surface receptors [14;59], by Ca²⁺ influx through TRPM8 [14;37] or pharmacologically with m-3M3FBS [37] inhibits TRPM8. PLC independent depletion of PIP₂ using a rapamycin-inducible phosphatase [37;39;60] or high concentrations of wortmannin [14;59] inhibits TRPM8.

In addition to being important for channel activity, PIP₂ is also likely to be involved in desensitization of TRPM8. TRPM8 currents activated by menthol [14;37;53], cold [37;61] and icilin [14;53] gradually diminish in the presence of extracellular Ca²⁺, a process called desensitization or adaptation. This effect has been reported both in expression systems [14; 53] and in native sensory neurons [61]. Similarly, physiological responses to cold [62] and menthol [63] have been shown to desensitize. We have proposed that the mechanism of desensitization is the Ca²⁺-induced activation of PLC and the ensuing depletion of PIP₂ [14] (Fig. 4A). This idea is based on the following findings. As mentioned earlier PIP₂ activates TRPM8 in excised patches and depletion of the lipid inhibits the channel in intact cells. Ca²⁺ influx through TRPM8 leads to activation of PLC and the depletion of PIP₂, [14;37]. Desensitization is slowed down by co-expressing PIP5K that synthesizes PIP₂, and accelerated by co-expressing the highly Ca²⁺ sensitive PLC isoform PLCδ1 [14].

Another potential mechanism which may contribute to Ca²⁺-induced desensitization is PKC. It was shown that PKC activators inhibit TRPM8 [64;65]. As menthol activates PLC, it is likely that it also activates PKC. The effects of PKC inhibitors on the menthol-induced, Ca²⁺ dependent desensitization of TRPM8, however, have not been examined yet.

How does PIP₂ activate TRPM8? Two questions will be discussed here: what is the relationship of PIP₂ to other regulators of TRPM8, and where are the PIP₂ interacting residues. TRPM8 is activated by cold and cooling agents, such as menthol. Cooling agents shift the activation threshold of the channel to warmer temperatures [53]. TRPM8 is also voltage dependent. The gating charge of TRPM8 was estimated to be 0.89 [66], which is an order of magnitude lower than that of classical voltage gated channels. This results in weak voltage dependence (outward

rectification). It was proposed that menthol and cold activate TRPM8 by shifting its voltage dependence to more positive potentials [67]. Subsequent articles found that while cold does shift the normalized voltage dependence to the left, it also increases maximum open probability, thus challenging the previous model [68;69]. Voltage also influences cold and menthol sensitivity, the channel is easier to open both by cold and menthol at positive voltages [67]. It is likely that cold, cooling agents and voltage regulate TRPM8 in a complex allosteric manner, which is not completely understood yet.

When PIP₂ is also considered in this equation, the picture becomes even more complicated. Both cold and menthol increase sensitivity of TRPM8 to PIP₂, i.e. shift PIP₂ dose-response curves to the left. Concurrently, the channel becomes less sensitive to PIP₂ depletion in the presence of menthol [14]. PIP₂ also shifts the voltage dependence of the channel towards negative voltages. Conversely, the effect of PIP₂ also depends on membrane voltage. At positive voltages the channel displays higher apparent affinity for the lipid than at negative voltages. Concurrently the channel is more sensitive to inhibition by PIP₂ depletion at negative than at positive voltages [14]. Conflicting data have been published on whether or not PIP₂ changes menthol sensitivity of TRPM8. In one report the dose-response to menthol was shifted to the left in the presence of PIP₂ in excised patches compared to no added PIP₂ [59]. Another study found that depletion of PIP₂ using the rapamycin-inducible phosphatase inhibited TRPM8 currents, but did not change the menthol or cold sensitivity [37]. The major effect of PIP₂ depletion was to shift the voltage dependence of TRPM8 currents to more positive potentials. In other words, more inhibition was observed at negative than at positive voltages [37], agreeing with earlier studies [14]. In conclusion the regulation of TRPM8 is a complex interplay between temperature, cooling agents, voltage and PIP₂. No comprehensive model has been proposed so far to incorporate all these variables. Given the number of variables, such model is not trivial to build.

Where are the PIP₂ interacting residues in TRP channels? Much less is done in this respect on TRP channels than on Kir channels. No systematic mutation analysis has been performed so far. We have found that mutation of positively charged residues in the highly conserved TRP domain of TRPM8 substantially decreased the apparent affinity of the channel for PIP₂ [14]. The same mutations rendered the channel more sensitive to inhibition by depletion of PIP₂. This is compatible with the idea that these residues are part of a PIP₂ binding site. However, the R1008Q mutation that had the most dramatic effect on PIP₂ sensitivity also affected menthol, cold sensitivity. PIP₂ sensitivity of this mutant was however still much less than that of the wild-type channel when examined at lower temperatures and higher menthol concentrations arguing for a primary effect on PIP₂ sensitivity. Nevertheless, as discussed earlier, it cannot be excluded that these mutations affect PIP₂ sensitivity indirectly. Two of the three TRP domain mutants only moderately affected PIP₂ sensitivity, thus it is unlikely that this domain is solely responsible for PIP₂ sensitivity. It is likely that other parts of the channel also contribute to PIP₂ binding, especially that mutation of equivalent TRP domain residues did not affect PIP₂ sensitivity of TRPM4 [70] and TRPV6 (T.R. unpublished observation).

In summary, there is a general agreement that TRPM8 requires PIP₂ for activity. Several important questions remain. What is the relationship of PIP₂ to the other regulators of TRPM8, i.e. cold, cooling agents and voltage? Where are the (other) PIP₂ interacting residues? What is the contribution of PIP₂ depletion and other potential mechanisms (PKC) to desensitization / adaptation.

3.3 TRPM4 and TRPM5

TRPM5 is a Ca²⁺-activated non-selective cation channel that is not permeable to Ca²⁺ [71]. This channel plays a role in taste perception; its genetic deletion in mice leads to defects in sensation of sweet, bitter, and amino acid tastes [72;73]. TRPM5 is also activated by moderate

heat, which was proposed to be responsible for the thermal sensitivity of sweet taste [74]. TRPM4, a close homologue of TRPM5, is also a Ca^{2+} -activated non-selective cation channel [75]. Its physiological function is different; TRPM4 is expressed in many different cell types, including immune cells, where it regulates Ca^{2+} oscillations [76]. Genetic deletion of TRPM4 in mice leads to increased IgE-dependent mast cell activation and anaphylactic responses [77].

TRPM5 was the second TRP channel that was reported to require PIP_2 for activity [78]. In excised patches, the sensitivity of the channel to Ca^{2+} activation gradually diminishes, but it can be restored with the application of PIP_2 . TRPM4 also becomes gradually desensitized to Ca^{2+} in excised patches and its Ca^{2+} sensitivity can be restored by application of PIP_2 or by MgATP [70;79]. Desensitization to Ca^{2+} could be inhibited by U73122 in excised patches, indicating that the patches contain Ca^{2+} -inducible PLC activity [70] and suggesting a similar mechanism for desensitization to that discussed for TRPM8 (Fig. 4C). Interestingly, mutation of positively charged residues in the TRP domain of TRPM4 did not affect PIP_2 activation, in contrast to mutation of positive charges in a more distant, “PH-domain-like” motif [70].

With some minor quantitative differences, TRPM4 and TRPM5 behave very similarly, with respect to Ca^{2+} and PIP_2 activation. Both TRPM4 and TRPM5 are expressed in the preBötzinger complex. The neurons of this area synchronously discharge bursts of action potentials during the inspiratory phase of respiratory network activity. Consistent with the role of PIP_2 in keeping these channels open, excess PIP_2 increased the inspiratory drive potential and decreasing PIP_2 reduced it [80].

4. TRPV Channels

The TRPV family has 6 mammalian members. They can be separated into 2 groups. TRPV1-4 are sensory channels, all are activated by heat with various thresholds. Most of these channels are expressed in sensory neurons, or keratinocytes in the skin. TRPV4, in addition to being activated by heat, is also a mechanosensitive channel. TRPV5 and 6 on the other hand are epithelial Ca^{2+} channels; they share high homology to each other, but much less to the other members of the TRPV family. Unlike all other TRP channels, TRPV5 and 6 show inward rectification, and are selective for calcium, and other divalent cations [7]. PIP_2 regulation was reported for three members of this family: TRPV1, TRPV5 and TRPV6 (Table 1). All three of these channels are activated by PIP_2 in excised patches, but for TRPV1 an additional indirect inhibitory effect of the lipid in intact cells may complicate the picture.

4.1 TRPV1

TRPV1 was the first non-canonical TRP channel to be cloned [81]. Its major activators are heat, capsaicin (the pungent compound in hot peppers), and tissue acidosis. This channel is involved in nociception and there are many other factors that activate or regulate it [82]. Phosphoinositide regulation of TRPV1 is probably quite complex, and somewhat controversial. This topic has been recently discussed in more detail, in the context of two important phenomena, sensitization and desensitization of TRPV1 [83].

The least controversial part of phosphoinositide regulation of TRPV1 currently is the activation of the channel by PIP_2 . It was shown unequivocally, three different laboratories reaching the same conclusion, that PIP_2 activates TRPV1 in excised patches [84-87]. Agents that chelate PIP_2 such as poly-Lysine inhibit TRPV1 in excised patches, thus supporting the activating effect of the lipid [84;85]. Other phosphoinositides also activate TRPV1 in excised patches. $\text{PtdIns}(4)\text{P}$ is somewhat less potent than $\text{PtdIns}(4,5)\text{P}_2$, but the effects of the two lipids at maximal concentrations are similar. The effect of $\text{PtdIns}(3,4,5)\text{P}_3$ is comparable to that of $\text{PtdIns}(4,5)\text{P}_2$ [85;86].

What is functional role of the activating effect of PIP₂? It is likely that depletion of the lipid plays a role in the Ca²⁺ dependent desensitization of TRPV1, similarly to several other TRP channels, such as TRPM8 [14], TRPM4 [70] and TRPV6 [88]. The model is simple, when Ca²⁺ enters a cell through TRPV1, it activates a Ca²⁺ sensitive PLC, and the resulting depletion leads/contributes to decreased channel activity (Fig. 4A). This model is based on the following data. 1. As already mentioned, TRPV1 requires PIP₂ for activity in excised patches. 2. Application of capsaicin in the presence of extracellular Ca²⁺ leads to depletion of PIP₂ [85; 89-91]. 3. Recovery from desensitization depends on the ability of the cell to resynthesize PIP₂ [89]. 4. PLC inhibitors reduce desensitization [85;92]. 5. Supplying excess PIP₂ or PtdIns(4)P through the patch pipette in whole-cell patch clamp experiments reduces desensitization [85;92]. As mentioned earlier, PtdIns(4)P also activates TRPV1 in excised patches, and it is also depleted upon PLC activation [85]. As the concentration of PtdIns(4)P is thought to be comparable to that of PIP₂, it may also play a role, together with PIP₂, in keeping TRPV1 open.

The picture seems clear and reassuring. However, PIP₂ depletion is unlikely to be the mechanism solely responsible for desensitization of TRPV1, as both PLC inhibition and supplying excess PIP₂ only partially inhibited desensitization. Also in one study supplying PIP₂ through the patch pipette in whole-cell experiments only moderately reduced capsaicin-induced desensitization [90]. The ubiquitous Ca²⁺ sensor calmodulin has also been proposed to play a role in desensitization, both acting on the channel directly [92-94], and by activating calcineurin [95;96], and thus inducing dephosphorylation of the channel. Again, as with most other PIP₂ sensitive ion channels, the question remaining is to what extent PIP₂ depletion contributes to channel desensitization under various conditions, relative to other mechanisms.

Similarly to TRPM8, the activating effect of PIP₂ is modulated by other regulators of TRPV1. The channels display higher apparent affinity for PIP₂ at positive than at negative voltages [85]. Capsaicin also shifts the PIP₂ dose-response to the left [85]. Consistent with this, when the capsaicin-activated channels (1 μM) are inhibited by depletion of PIP₂, the inhibition can be relieved by further increasing the concentration of capsaicin to 10-100 μM [91]. Similar relationships have been described for other regulators of TRPV1. Both capsaicin and heat shifts the voltage dependence of TRPV1 to the left [67]. Low pH sensitizes the channel to activation by both heat and capsaicin [97].

PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ activate TRPV1 in excised patches, with a similar efficiency to PIP₂ [85;86]. As discussed earlier, the concentration of these lipids is much lower than that of PIP₂ in the plasma membrane, thus the effect of PIP₂ probably overrides their effects. PtdIns(3,4,5)P₃ as well as PtdIns(3,4)P₂ are the products of PI3K. It was shown by several laboratories that PI3K is involved in sensitization of TRPV1 by NGF [84;98;99]. The major effect of NGF was the increased number of TRPV1 channels in the plasma membrane, thus PtdIns(3,4,5)P₃ and/or PtdIns(3,4)P₂ may play a role of trafficking of TRPV1.

Although by now there seems to be a general agreement on the role of PIP₂ in activating TRPV1, the picture was not always so clear. In fact, originally it was proposed that PIP₂ tonically inhibits TRPV1, and depletion of the lipid contributes to sensitization of the channel by proinflammatory mediators activating PLC [100]. The idea was based on indirect evidence, and the effects of the lipid were not tested in excised patches. It is important to note that sensitization upon PLC activation occurs when the channel is moderately stimulated, for example at low capsaicin concentrations. As we discussed, in excised patches, PIP₂ clearly activates TRPV1, seemingly refuting the inhibitory effect of PIP₂. Is it possible that in intact cells, there is an additional, probably indirect effect of PIP₂ leading to partial channel inhibition? Here the picture is less clear. Our laboratory found that depletion of the lipid with the rapamycin-inducible PIP₂ phosphatase system [39] leads to further activation when the channel is only moderately stimulated by capsaicin or heat [85]. This finding suggests a partial

inhibition by PIP₂ in intact cells, in addition to its activating effect. Importantly, potentiation by PIP₂ depletion was only seen when the channel was stimulated by low concentration of capsaicin, or moderate heating, conditions where PLC mediated sensitization also occurs. When the channel was maximally stimulated by high capsaicin concentrations, we observed neither activation, nor inhibition by the inducible phosphatase [85]. We explained the lack of inhibition at high capsaicin concentrations with PtdIns(4)P keeping the channel open under such conditions. PtdIns(4)P is not depleted by the phosphatase, indeed it is expected that its level increases when PIP₂ is converted to PtdIns(4)P. Conversely, when we over-expressed the PIP5K enzyme, generating excess PIP₂, TRPV1 activity was inhibited at low, but not at high capsaicin concentrations [85]. This finding is also compatible with a partial inhibitory effect of PIP₂ at moderate stimulation levels. This inhibitory effect, however, is likely to be indirect, because it is not detectable in excised patches. We have presented some ideas in Figure 3 to explain such indirect effects.

Another article, on the other hand found that the rapamycin-inducible PIP₂ phosphatase inhibited TRPV1 both high and low concentrations of capsaicin [86]. This is compatible with the activating effect of PIP₂ in excised patches, and argues against an inhibitory effect of the lipid. It is hard to tell what causes the discrepancies between the two studies [85;86]. There are a number of differences in the experimental conditions including, the cell-type, the rapamycin analogue, the concentrations of capsaicin used, and the origin of the rapamycin-phosphatase system (ref [38] vs. ref [39]). Some of these differences may explain the opposing findings of the two studies. It is worth noting however, that the same two articles reached very similar conclusions on the effects of the phosphoinositides PtdIns(4,5)P₂, PtdIns(4)P and PtdIns(3,4,5)P₃ in excised patches, despite several differences in experimental conditions [85;86].

A recent addition to the complexity of phosphoinositide regulation of TRPV1 is the discovery of Pirt [30]. Pirt is a two transmembrane domain protein, specifically expressed in DRG neurons and it interacts both with TRPV1 and phosphoinositides. Heat and capsaicin-induced currents in dorsal root ganglion (DRG) neurons were significantly attenuated in *Pirt*^{-/-} mice compared to wild-type. 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. When 10 μM diC₈ PIP₂ was dialyzed through the whole-cell patch pipette, it 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 PIP₂ on TRPV1.

PIP₂ however activates TRPV1 in excised patches in expression systems [85], where Pirt is unlikely to be present. The authors argued that high concentrations of PIP₂ used in expression systems may override the requirement for Pirt. DiC₈ PIP, however, activated TRPV1 at concentrations as low as 0.5 μM in excised patches in *Xenopus* oocytes [85]. In our experience, these concentrations of the lipid exert a much lower activity on most ion channels than resting PIP₂ levels in the plasma membrane. We found that ~25 μM DiC₈ PIP₂ exerts a roughly equivalent effect of the endogenous PIP₂ in oocyte membranes on Kir channels (unpublished observation). This is in very good agreement with data published on KCNQ channels expressed in mammalian cells [101], where ~23 μM diC₈ PIP₂ was reported to exert similar effect to that of endogenous PIP₂. Thus, Pirt is probably not strictly required for the stimulatory effect of phosphoinositides on TRPV1, unless it is expressed in oocytes. Pirt, however, is unlikely to be expressed in *Xenopus* oocytes, as in mice it is only expressed in DRG, and we have not found *Xenopus laevis* or *tropicalis* homologues of Pirt in genomic databases. Interestingly the EC₅₀ for PIP₂ activation was lower in neuronal F-11 cells [86] than in *Xenopus* oocytes [85]. Whether this is caused by Pirt expression in the neuronal cell line needs to be tested.

Genetic deletion of *Pirt* attenuated but did not eliminate sensitization of capsaicin-induced currents by bradykinin. As *Pirt* is a transmembrane protein, it is unlikely to be the hypothetical factor that is lost in excised patches and presumably responsible for the inhibitory effect of PIP₂. Also we found evidence for the indirect inhibitory effect of PIP₂ in *Xenopus* oocytes, where a *Pirt* homologue is unlikely to be present [85]. In conclusion, *Pirt* is unlikely to be strictly responsible for either the activating or the inhibitory effects of PIP₂ on TRPV1. Nevertheless, this phosphoinositide binding protein is present in the native environment of TRPV1; it interacts with the channel and modulates its function. Thus it is likely that *Pirt* is an important modulator of native TRPV1 channels, but clarifying its exact role in phosphoinositide regulation of these channels will require further experimental work.

In conclusion, TRPV1 clearly requires phosphoinositides for activity; PIP₂ reproducibly activates the channel in excised patches. There also seems to be an agreement that depletion of the lipid contributes to Ca²⁺-induced desensitization. If there is a partial inhibition by PIP₂ in intact cells, it is likely to depend on a factor lost upon patch excision (indirect effect) because we and others found no evidence of it in excised patches using a variety of tools [85; 86]. We have recently reviewed PIP₂ regulation of TRPV1 and discussed ideas to integrate the activating and the possible inhibitory effects of PIP₂ in the PLC mediated regulation of TRPV1 [83].

4.2 TRPV5 and TRPV6

TRPV5 and TRPV6 are Ca²⁺ selective channels, located on the apical membrane of epithelial cells that are responsible for active transcellular Ca²⁺ transport [7]. Calcium entering the cell on the apical side through these channels is pumped out on the basolateral side by either a Ca²⁺-ATP-ase or a Na⁺/Ca²⁺ exchanger. TRPV5 is expressed in the kidney, in the late distal convoluted and the connecting tubules, whereas TRPV6 is mainly expressed in the duodenum. TRPV6 is regulated at the transcriptional level by active vitamin D3 (calcitriol). Genetic deletion of either of these channels results in disturbances in calcium homeostasis in mice [102;103]. The rate of TRPV6 protein evolution was shown to be accelerated in the human lineage [104]. The ancestral variant of TRPV6 was shown to be overactive and associated with increased prevalence of kidney stones in humans, presumably by increased intestinal Ca²⁺ absorption and compensatory hypercalciuria [105]. Both TRPV5 and TRPV6 undergo Ca²⁺-induced inactivation, which presumably protects the cells from toxic Ca²⁺ levels and limits epithelial Ca²⁺ transport.

Both TRPV5 and TRPV6 require PIP₂ for activity; their activity runs down in excised patches, which is accelerated by poly-Lysine [14] and they are reactivated by application of PIP₂ [88; 106]. We have proposed that Ca²⁺-induced inactivation of TRPV6 proceeds through PLC activation and the resulting depletion of PIP₂ [88;107], similarly to TRPM8 and TRPV1 (Fig. 4B). This model is based on the following findings. TRPV6 is activated in excised patches by PIP₂ but not PIP. Ca²⁺-induced inactivation is inhibited by dialyzing PIP₂, but not PIP through the patch pipette in whole-cell patch clamp experiments. Ca²⁺ influx through TRPV6 leads to depletion of PIP₂ and formation of IP₃, indicating activation of PLC. PLC independent depletion of PIP₂ with the rapamycin-inducible PIP₂ phosphatase, or high concentrations of wortmannin inhibited TRPV6 [88]. Both PIP₂ depletion and Ca²⁺-induced inactivation of TRPV6 were inhibited by PLC inhibitors [107].

The calcium sensor calmodulin has also been proposed to play a role in Ca²⁺-induced inactivation of TRPV6 [108;109]. Again, just like in other cases, it is possible that both mechanisms contribute to Ca²⁺-induced inactivation. Competition of CaM with PIP₂, as described earlier is a feasible mechanism that would integrate CaM and PIP₂ regulation, but it has not been experimentally tested.

5. TRPA1

TRPA1 is the single member of the mammalian TRPA family. This channel is involved in nociception; it mediates the painful sensation evoked by many noxious environmental chemicals including mustard oil, formaldehyde, acrolein and allicin. It is noteworthy that many of these chemicals activate TRPA1 through covalent modification. TRPA1 is also expressed in sensory neurons of the airways, where it plays a role in chemosensory airway reflexes [110]. Two additional functions were proposed for TRPA1, sensation of noxious cold [111], and mechanosensation in the inner ear (hearing) [112]. The latter was clearly refuted by knockout studies, while the role of TRPA1 in sensation of noxious cold is still controversial [113]. While probably receiving less attention than its role in cold sensation, regulation of TRPA1 by phosphoinositides is also highly controversial, as discussed below.

The first connection of TRPA1 with phosphoinositides was the observation that in the inner ear mechanotransduction and adaptation requires PIP₂ [114]. This observation together with the proposed role of TRPA1 as the mechanotransductive channel [112] raised the possibility that TRPA1 is a PIP₂ sensitive channel. TRPA1 turned out to be dispensable for hearing [115;116]; nevertheless, it may still be a PIP₂ sensitive channel.

The first actual evidence for the role of PIP₂ in the regulation TRPA1 came from a study that examined the mechanism of homologous and heterologous desensitization of TRPA by mustard oil and capsaicin, respectively [90]. The authors found that application of capsaicin lead to depletion of PIP₂ in both trigeminal ganglion neurons and in heterologous systems expressing TRPV1. In both systems, dialyzing PIP₂ through the patch pipette prevented heterologous desensitization of TRPA1 by capsaicin [90]. These results imply a positive role for PIP₂ in the regulation of TRPA1, but the direct effects of PIP₂ were not tested in excised patches.

Essentially at the same time, studying sensitization of TRPA1 currents by the PLC coupled proteinase activated (PAR2) receptors, Dai et al proposed an inhibitory role for PIP₂ [117]. They found that that activation of PLC either pharmacologically (m-3M3FBS) or by activating PAR2 receptors, potentiated the effects of TRPA1 agonists (sensitization). Sequestering PIP₂ with an antibody mimicked the effect of PLC activation, whereas dialyzing PIP₂ through the whole-cell patch pipettes inhibited it. Again, the effects of PIP₂ in excised patches were not tested.

Subsequently the laboratory of Donghee Kim reported that TRPA1 runs down in excised patches, a common property of PIP₂ activated channels. However, the cofactor lost there was not PIP₂, but inorganic polyphosphate (PPPi) [118]. In their study, PIP₂ was not able to reactivate the channels. This work was followed by another study from the same laboratory, showing that PIP₂ actually inhibits TRPA1 in excised patches, when they are preactivated with mustard oil and PPPi [87]. In agreement with this idea, they also showed that poly-Lysine and an antibody against PIP₂ activates TRPA1 in the presence of PPPi.

To make the story more complicated, a paper from Bernd Nilius' laboratory showed that PIP₂ activates TRPA1 channels in excised patches, and it reduces Ca²⁺-induced desensitization if dialyzed through the patch pipette [119]. This suggests a similar model for desensitization to that proposed for TRPM8 and TRPV1 (Fig. 4A). They also applied an array of commonly used tools to study the effects of PIP₂. High concentrations of wortmannin inhibited TRPA1 currents and mustard oil-induced Ca²⁺ signals in sensory neurons, presumably through depleting PIP₂. Neomycin, often used as a PIP₂ scavenger inhibited TRPA1 in excised patches. MgATP activated TRPA1 in excised patches, a common property of PIP₂ dependent ion channels. In contrast to these supporting data, poly-Lysine, a commonly used PIP₂ scavenger activated TRPA1 in excised patches, a finding at odds with the activating effect of PIP₂, but

in agreement with the previously discussed paper reporting inhibition by PIP₂ [87]. The authors also confirmed the positive effect of PPPi, but the effects of PIP₂ were not tested in the presence of PPPi in excised patches.

Very recently, it was shown that the rapamycin-inducible PIP₂ phosphatase, which inhibited TRPM8, neither activated nor inhibited TRPA1 [60]. In conclusion, at this point three seemingly incompatible views exist on TRPA1, PIP₂ inhibits, PIP₂ activates or it has no effect on the channel, all three possibilities supported by data from more than one laboratory (Table 1). Clearly, more work is needed to clarify the role of phosphoinositides in the regulation of TRPA1.

6. TRPP2

TRPP2 (PKD2, Polycystin 2) is a member of the TRPP family [120]. It co-assembles with PKD1, and loss of function mutation of either proteins leads to autosomal dominant polycystic kidney disease [121]. Despite their well established role in the pathophysiology of this quite common genetic disorder, their physiological roles and regulation are not very well understood. TRPP2 is activated by epidermal growth factor (EGF). It was proposed that PIP₂ inhibits TRPP2 and EGF activates it by depleting PIP₂ thus relieving the channel from inhibition [122].

7. Conclusions

Phosphoinositides regulate many, if not all TRP channels. The first two published articles on TRP channels reported inhibition of the drosophila TRPL channel [123] and the mammalian TRPV1 by PIP₂ [100]. For a while, it seemed that PIP₂ may be a general inhibitor of TRP channels. By now it seems that many more TRP channels are activated by this lipid. Activation by PIP₂, or dependence on PIP₂ has been demonstrated convincingly for several TRP channels: TRPM4,5,7,8, TRPV1, 5 and 6. Further studies are needed to see what extent the depletion of PIP₂ is involved in regulation of these channels, and what interplay exists with other signaling pathways. Nevertheless, PIP₂ dependence is probably a common feature of most TRP channels.

Inhibition by PIP₂, even though it started out as the dogma, seems rather the exception than the rule by now. It is also more controversial than the activating effect; on most TRP channels where inhibitory effects were reported, there are also opposing results showing that PIP₂ activates the same channel i.e. TRPV1 and TRPA1. There are also conflicting results on activating vs. inhibitory effects of PIP₂ on several members of the TRPC family (see other reviews in this volume). Can the same lipid both activate and inhibit the same channel? It may be possible, but finding out how clearly requires further studies.

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References

1. Yu FH, Catterall WA. The VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci STKE* 2004;2004:re15. [PubMed: 15467096]
2. Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. *Annu Rev Physiol* 2006;68:619–647. [PubMed: 16460286]
3. Putney JW Jr. The enigmatic TRPCs: multifunctional cation channels. *Trends Cell Biol* 2004;14:282–286. [PubMed: 15183184]

4. Voets T, Talavera K, Owsianik G, Nilius B. Sensing with TRP channels. *Nat Chem Biol* 2005;1:85–92. [PubMed: 16408004]
5. Dhaka A, Viswanath V, Patapoutian A. TRP Ion Channels and Temperature Sensation. *Annu Rev Neurosci.* 2006
6. Hoenderop JG, Bindels RJ. Epithelial Ca^{2+} and Mg^{2+} channels in health and disease. *J Am Soc Nephrol* 2005;16:15–26. [PubMed: 15574510]
7. Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. *Physiol Rev* 2005;85:373–422. [PubMed: 15618484]
8. Nilius B, Owsianik G, Voets T, Peters JA. Transient Receptor Potential Channels in Disease. *Physiol Rev* 2007;87:165–217. [PubMed: 17237345]
9. Rohacs T. Regulation of TRP channels by PIP_2 . *Pflugers Arch* 2007;453:753–762. [PubMed: 17031667]
10. Qin F. Regulation of TRP ion channels by phosphatidylinositol-4,5-bisphosphate. *Handb Exp Pharmacol* 2007;509–525. [PubMed: 17217076]
11. Rosenhouse-Dantsker A, Logothetis DE. Molecular characteristics of phosphoinositide binding. *Pflugers Arch* 2007;455:45–53. [PubMed: 17588168]
12. Suh BC, Hille B. PIP_2 is a necessary cofactor for ion channel function: how and why? *Annu Rev Biophys* 2008;37:175–195. [PubMed: 18573078]
13. Hilgemann DW, Ball R. Regulation of cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchange and KATP potassium channels by PIP_2 . *Science* 1996;273:956–959. [PubMed: 8688080]
14. Rohacs T, Lopes CMB, Michailidis I, Logothetis DE. $\text{PI}(4,5)_2$ regulates the activation and desensitization of TRPM8 channels through the TRP domain. *Nat Neurosci* 2005;8:626–634. [PubMed: 15852009]
15. Huang CL, Feng S, Hilgemann DW. Direct activation of inward rectifier potassium channels by PIP_2 and its stabilization by $\text{G}\beta\gamma$. *Nature* 1998;391:803–806. [PubMed: 9486652]
16. Zhang H, He C, Yan X, Mirshahi T, Logothetis DE. Activation of inwardly rectifying K^+ channels by distinct $\text{PtdIns}(4,5)\text{P}_2$ interactions. *Nature Cell Biology* 1999;1:183–188.
17. Rosenhouse-Dantsker A, Sui JL, Zhao Q, et al. A sodium-mediated structural switch that controls the sensitivity of Kir channels to $\text{PtdIns}(4,5)\text{P}_2$. *Nat Chem Biol* 2008;4:624–631. [PubMed: 18794864]
18. Lopes CMB, Zhang H, Rohacs T, Jin T, Logothetis DE. Alterations in Conserved Kir Channel- PIP_2 Interactions Underlie Channelopathies. *Neuron* 2002;34:933–944. [PubMed: 12086641]
19. Logothetis DE, Lupyán D, Rosenhouse-Dantsker A. Diverse Kir modulators act in close proximity to residues implicated in phosphoinositide binding. *J Physiol* 2007;582:953–965. [PubMed: 17495041]
20. Colquhoun D. Binding, gating, affinity and efficacy: the interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. *Br J Pharmacol* 1998;125:924–947. [PubMed: 9846630]
21. Rohacs T, Lopes CM, Jin T, Ramdya PP, Molnar Z, Logothetis DE. Specificity of activation by phosphoinositides determines lipid regulation of Kir channels. *Proc Natl Acad Sci U S A* 2003;100:745–750. [PubMed: 12525701]
22. Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. *Annu Rev Biochem* 1998;67:481–507. [PubMed: 9759495]
23. Downes, CP.; Hawkins, PT.; Stephens, L. Identification of the stimulated reaction in intact cells, its substrate supply and the metabolism of inositol phosphates. In: Michell, RH.; Drummond, AH.; Downes, CP., editors. *Inositol lipids in cell signalling*. London: Academic Press; 1989. p. 1-38.
24. Enkvetchakul D, Jeliaskova I, Nichols CG. Direct modulation of Kir channel gating by membrane phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 2005;280:35785–35788. [PubMed: 16144841]
25. Zakharian E, Rohacs T. Inorganic polyphosphate modulates TRPM8 channels. *Biophys J* 2009;96:265a.
26. Yap KL, Kim J, Truong K, Sherman M, Yuan T, Ikura M. Calmodulin target database. *J Struct Funct Genomics* 2000;1:8–14. [PubMed: 12836676]
27. Kwon Y, Hofmann T, Montell C. Integration of phosphoinositide- and calmodulin-mediated regulation of TRPC6. *Mol Cell* 2007;25:491–503. [PubMed: 17317623]

28. McLaughlin S, Murray D. Plasma membrane phosphoinositide organization by protein electrostatics. *Nature* 2005;438:605–611. [PubMed: 16319880]
29. Trebak M, Lemonnier L, Dehaven WI, Wedel BJ, Bird GS, Putney JW Jr. Complex functions of phosphatidylinositol 4,5-bisphosphate in regulation of TRPC5 cation channels. *Pflugers Arch*. 2008
30. Kim AY, Tang Z, Liu Q, et al. Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. *Cell* 2008;133:475–485. [PubMed: 18455988]
31. Yaradanakul A, Feng S, Shen C, et al. Dual control of cardiac Na^+ Ca^{2+} exchange by PIP_2 : electrophysiological analysis of direct and indirect mechanisms. *J Physiol* 2007;582:991–1010. [PubMed: 17540705]
32. Rohacs T, Lopes C, Mirshahi T, Jin T, Zhang H, Logothetis DE. Assaying phosphatidylinositol bisphosphate regulation of potassium channels. *Methods Enzymol* 2002;345:71–92. [PubMed: 11665643]
33. Rohacs T, Chen J, Prestwich GD, Logothetis DE. Distinct specificities of inwardly rectifying K^+ channels for phosphoinositides. *J Biol Chem* 1999;274:36065–36072. [PubMed: 10593888]
34. Suh BC, Hille B. Electrostatic interaction of internal Mg^{2+} with membrane PIP_2 Seen with KCNQ K^+ channels. *J Gen Physiol* 2007;130:241–256. [PubMed: 17724161]
35. Soom M, Schonherr R, Kubo Y, Kirsch C, Klinger R, Heinemann SH. Multiple PIP_2 binding sites in Kir2.1 inwardly rectifying potassium channels. *FEBS Lett* 2001;490:49–53. [PubMed: 11172809]
36. MacGregor GG, Dong K, Vanoye CG, Tang L, Giebisch G, Hebert SC. Nucleotides and phospholipids compete for binding to the C terminus of K_{ATP} channels. *Proc Natl Acad Sci U S A* 2002;99:2726–2731. [PubMed: 11880626]
37. Daniels RL, Takashima Y, McKemy DD. The activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositol-4,5-bisphosphate. *J Biol Chem*. 2008
38. Suh BC, Inoue T, Meyer T, Hille B. Rapid Chemically Induced Changes of $\text{PtdIns}(4,5)\text{P}_2$ Gate KCNQ Ion Channels. *Science* 2006;314:1454–1457. [PubMed: 16990515]
39. Varnai P, Thyagarajan B, Rohacs T, Balla T. Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact cells. *J Cell Biol* 2006;175:377–382. [PubMed: 17088424]
40. Murata Y, Okamura Y. Depolarization activates the phosphoinositide phosphatase Ci-VSP, as detected in *Xenopus* oocytes coexpressing sensors of PIP_2 . *J Physiol* 2007;583:875–889. [PubMed: 17615106]
41. Rohacs T, Nilius B. Regulation of transient receptor potential (TRP) channels by phosphoinositides. *Pflugers Arch* 2007;455:157–168. [PubMed: 17479281]
42. Aarts M, Iihara K, Wei WL, et al. A key role for TRPM7 channels in anoxic neuronal death. *Cell* 2003;115:863–877. [PubMed: 14697204]
43. Krapivinsky G, Mochida S, Krapivinsky L, Cibulsky SM, Clapham DE. The TRPM7 Ion Channel Functions in Cholinergic Synaptic Vesicles and Affects Transmitter Release. *Neuron* 2006;52:485–496. [PubMed: 17088214]
44. Su LT, Agapito MA, Li M, et al. TRPM7 regulates cell adhesion by controlling the calcium-dependent protease calpain. *J Biol Chem* 2006;281:11260–11270. [PubMed: 16436382]
45. Monteilh-Zoller MK, Hermosura MC, Nadler MJ, Scharenberg AM, Penner R, Fleig A. TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. *J Gen Physiol* 2003;121:49–60. [PubMed: 12508053]
46. Jin J, Desai BN, Navarro B, Donovan A, Andrews NC, Clapham DE. Deletion of *Trpm7* disrupts embryonic development and thymopoiesis without altering Mg^{2+} homeostasis. *Science* 2008;322:756–760. [PubMed: 18974357]
47. Runnels LW, Yue L, Clapham DE. The TRPM7 channel is inactivated by PIP_2 hydrolysis. *Nat Cell Biol* 2002;4:329–336. [PubMed: 11941371]
48. Kozak JA, Matsushita M, Nairn AC, Cahalan MD. Charge screening by internal pH and polyvalent cations as a mechanism for activation, inhibition, and rundown of TRPM7/MIC channels. *J Gen Physiol* 2005;126:499–514. [PubMed: 16260839]
49. Gwanyanya A, Sipido K, Vereecke J, Mubagwa K. ATP- and PIP_2 -dependence of the magnesium-inhibited, TRPM7-like cation channel in cardiac myocytes. *Am J Physiol Cell Physiol* 2006;291:C627–C635. [PubMed: 16707555]

50. Schmitz C, Perraud AL, Johnson CO, et al. Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. *Cell* 2003;114:191–200. [PubMed: 12887921]
51. Takezawa R, Schmitz C, Demeuse P, Scharenberg AM, Penner R, Fleig A. Receptor-mediated regulation of the TRPM7 channel through its endogenous protein kinase domain. *Proc Natl Acad Sci U S A* 2004;101:6009–6014. [PubMed: 15069188]
52. Langeslag M, Clark K, Moolenaar WH, van Leeuwen FN, Jalink K. Activation of TRPM7 channels by PLC-coupled receptor agonists. *J Biol Chem* 2007;282:232–239. [PubMed: 17095511]
53. McKemy DD, Neuhauss WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 2002;416:52–58. [PubMed: 11882888]
54. Peier AM, Moqrich A, Hergarden AC, et al. A TRP Channel that Senses Cold Stimuli and Menthol. *Cell* 2002;108:705–715. [PubMed: 11893340]
55. Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A. TRPM8 is required for cold sensation in mice. *Neuron* 2007;54:371–378. [PubMed: 17481391]
56. Colburn RW, Lubin ML, Stone DJ Jr, et al. Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 2007;54:379–386. [PubMed: 17481392]
57. Bautista DM, Siemens J, Glazer JM, et al. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* 2007;448:204–208. [PubMed: 17538622]
58. Proudfoot CJ, Garry EM, Cottrell DF, et al. Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. *Curr Biol* 2006;16:1591–1605. [PubMed: 16920620]
59. Liu B, Qin F. Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. *J Neurosci* 2005;25:1674–1681. [PubMed: 15716403]
60. Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER. The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J Biol Chem*. 2008
61. Reid G, Babes A, Pluteanu F. A cold- and menthol-activated current in rat dorsal root ganglion neurones: properties and role in cold transduction. *J Physiol* 2002;545:595–614. [PubMed: 12456837]
62. Darian-Smith I, Johnson KO, Dykes R. “Cold” fiber population innervating palmar and digital skin of the monkey: responses to cooling pulses. *J Neurophysiol* 1973;36:325–346. [PubMed: 4196271]
63. Eccles R. Menthol and related cooling compounds. *J Pharm Pharmacol* 1994;46:618–630. [PubMed: 7529306]
64. Premkumar LS, Raisinghani M, Pingle SC, Long C, Pimentel F. Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation. *J Neurosci* 2005;25:11322–11329. [PubMed: 16339027]
65. Abe J, Hosokawa H, Sawada Y, Matsumura K, Kobayashi S. Ca^{2+} -dependent PKC activation mediates menthol-induced desensitization of transient receptor potential M8. *Neurosci Lett* 2006;397:140–144. [PubMed: 16380208]
66. Voets T, Owsianik G, Janssens A, Talavera K, Nilius B. TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. *Nat Chem Biol* 2007;3:174–182. [PubMed: 17293875]
67. Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature* 2004;430:748–754. [PubMed: 15306801]
68. Brauchi S, Orio P, Latorre R. Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor TRPM8. *Proc Natl Acad Sci U S A* 2004;101:15494–15499. [PubMed: 15492228]
69. Matta JA, Ahern GP. Voltage is a partial activator of rat thermosensitive TRP channels. *J Physiol* 2007;585:469–482. [PubMed: 17932142]
70. Nilius B, Mahieu F, Prenen J, et al. The Ca^{2+} -activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-bisphosphate. *EMBO J* 2006;25:467–478. [PubMed: 16424899]
71. Prawitt D, Monteilh-Zoller MK, Brixel L, et al. TRPM5 is a transient Ca^{2+} -activated cation channel responding to rapid changes in $[Ca^{2+}]_i$. *Proc Natl Acad Sci U S A* 2003;100:15166–15171. [PubMed: 14634208]

72. Perez CA, Huang L, Rong M, et al. A transient receptor potential channel expressed in taste receptor cells. *Nat Neurosci* 2002;5:1169–1176. [PubMed: 12368808]
73. Zhang Y, Hoon MA, Chandrashekar J, et al. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* 2003;112:293–301. [PubMed: 12581520]
74. Talavera K, Yasumatsu K, Voets T, et al. Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. *Nature* 2005;438:1022–1025. [PubMed: 16355226]
75. Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP. TRPM4 is a Ca^{2+} -activated nonselective cation channel mediating cell membrane depolarization. *Cell* 2002;109:397–407. [PubMed: 12015988]
76. Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet JP. TRPM4 regulates calcium oscillations after T cell activation. *Science* 2004;306:1374–1377. [PubMed: 15550671]
77. Vennekens R, Olausson J, Meissner M, et al. Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4. *Nat Immunol* 2007;8:312–320. [PubMed: 17293867]
78. Liu D, Liman ER. Intracellular Ca^{2+} and the phospholipid PIP_2 regulate the taste transduction ion channel TRPM5. *Proc Natl Acad Sci U S A* 2003;100:15160–15165. [PubMed: 14657398]
79. Zhang Z, Okawa H, Wang Y, Liman ER. Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. *J Biol Chem* 2005;280:39185–39192. [PubMed: 16186107]
80. Crowder EA, Saha MS, Pace RW, Zhang H, Prestwich GD, Del Negro CA. Phosphatidylinositol 4,5-bisphosphate regulates inspiratory burst activity in the neonatal mouse preBotzinger complex. *J Physiol* 2007;582:1047–1058. [PubMed: 17599963]
81. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–824. [PubMed: 9349813]
82. Pingle SC, Matta JA, Ahern GP. Capsaicin receptor: TRPV1 a promiscuous TRP channel. *Handb Exp Pharmacol* 2007;155–171. [PubMed: 17217056]
83. Rohacs T, Thyagarajan B, Lukacs V. Phospholipase C mediated modulation of TRPV1 channels. *Mol Neurobiol* 2008;37:153–163. [PubMed: 18528787]
84. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE. Phosphoinositide 3-Kinase Binds to TRPV1 and Mediates NGF-stimulated TRPV1 Trafficking to the Plasma Membrane. *J Gen Physiol* 2006;128:509–522. [PubMed: 17074976]
85. Lukacs V, Thyagarajan B, Balla A, Varnai P, Balla T, Rohacs T. Dual regulation of TRPV1 by phosphoinositides. *J Neurosci* 2007;27:7070–7080. [PubMed: 17596456]
86. Klein RM, Ufret-Vincenty CA, Hua L, Gordon SE. Determinants of molecular specificity in phosphoinositide regulation: $\text{PI}(4,5)\text{P}_2$ is the endogenous lipid regulating TRPV1. *J Biol Chem* 2008;283:26208–26216. [PubMed: 18574245]
87. Kim D, Cavanaugh EJ, Simkin D. Inhibition of transient receptor potential A1 channel by phosphatidylinositol-4,5-bisphosphate. *Am J Physiol Cell Physiol* 2008;295:C92–C99. [PubMed: 18495815]
88. Thyagarajan B, Lukacs V, Rohacs T. Hydrolysis of phosphatidylinositol 4,5-bisphosphate mediates calcium induced inactivation of TRPV6 channels. *J Biol Chem* 2008;283:14980–14987. [PubMed: 18390907]
89. Liu B, Zhang C, Qin F. Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. *J Neurosci* 2005;25:4835–4843. [PubMed: 15888659]
90. Akopian AN, Ruparel NB, Jeske NA, Hargreaves KM. Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. *J Physiol* 2007;583:175–193. [PubMed: 17584831]
91. Yao J, Qin F. Interaction with phosphoinositides confers adaptation onto the TRPV1 pain receptor. *PLoS Biol* 2009;7:e46. [PubMed: 19243225]
92. Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R. The Ankyrin Repeats of TRPV1 Bind Multiple Ligands and Modulate Channel Sensitivity. *Neuron* 2007;54:905–918. [PubMed: 17582331]
93. Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H, Tominaga M. Structural determinant of TRPV1 desensitization interacts with calmodulin. *Proc Natl Acad Sci U S A* 2003;100:8002–8006. [PubMed: 12808128]

94. Rosenbaum T, Gordon-Shaag A, Munari M, Gordon SE. Ca^{2+} /calmodulin modulates TRPV1 activation by capsaicin. *J Gen Physiol* 2004;123:53–62. [PubMed: 14699077]
95. Docherty RJ, Yeats JC, Bevan S, Boddeke HW. Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. *Pflugers Arch* 1996;431:828–837. [PubMed: 8927498]
96. Mohapatra DP, Nau C. Regulation of Ca^{2+} -dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. *J Biol Chem* 2005;280:13424–13432. [PubMed: 15691846]
97. Tominaga M, Caterina MJ, Malmberg AB, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998;21:531–543. [PubMed: 9768840]
98. Zhang X, Huang J, McNaughton PA. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *EMBO J* 2005;24:4211–4223. [PubMed: 16319926]
99. Zhuang ZY, Xu H, Clapham DE, Ji RR. Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. *J Neurosci* 2004;24:8300–8309. [PubMed: 15385613]
100. Chuang HH, Prescott ED, Kong H, et al. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P₂ mediated inhibition. *Nature* 2001;411:957–962. [PubMed: 11418861]
101. Li Y, Gamper N, Hilgemann DW, Shapiro MS. Regulation of Kv7 (KCNQ) K⁺ channel open probability by phosphatidylinositol 4,5-bisphosphate. *J Neurosci* 2005;25:9825–9835. [PubMed: 16251430]
102. Bianco SD, Peng JB, Takanaga H, et al. Marked Disturbance of Calcium Homeostasis in Mice with Targeted Disruption of the Trpv6 Calcium Channel Gene. *J Bone Miner Res* 2006;22:274–285. [PubMed: 17129178]
103. Hoenderop JG, van Leeuwen JP, van der Eerden BC, et al. Renal Ca^{2+} wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. *J Clin Invest* 2003;112:1906–1914. [PubMed: 14679186]
104. Akey JM, Swanson WJ, Madeoy J, Eberle M, Shriver MD. TRPV6 exhibits unusual patterns of polymorphism and divergence in worldwide populations. *Hum Mol Genet* 2006;15:2106–2113. [PubMed: 16717058]
105. Suzuki Y, Pasch A, Bonny O, Mohaupt MG, Hediger MA, Frey FJ. Gain-of-function haplotype in the epithelial calcium channel TRPV6 is a risk factor for renal calcium stone formation. *Hum Mol Genet* 2008;17:1613–1618. [PubMed: 18276610]
106. Lee J, Cha SK, Sun TJ, Huang C-L. PIP₂ activates TRPV5 and releases its inhibition by intracellular Mg^{2+} . *J Gen Physiol* 2005;126:439–451. [PubMed: 16230466]
107. Thyagarajan B, Benn BS, Christakos S, Rohacs T. Phospholipase C mediated regulation of TRPV6 channels: implications in active intestinal Ca^{2+} transport. *Mol Pharmacol* 2009;75:608–616. [PubMed: 19073818]
108. Derler I, Hofbauer M, Kahr H, et al. Dynamic but not constitutive association of calmodulin with rat TRPV6 channels enables fine tuning of Ca^{2+} -dependent inactivation. *J Physiol* 2006;577:31–44. [PubMed: 16959851]
109. Niemeyer BA, Bergs C, Wissenbach U, Flockerzi V, Trost C. Competitive regulation of Ca^T-like-mediated Ca^{2+} entry by protein kinase C and calmodulin. *Proc Natl Acad Sci U S A* 2001;98:3600–3605. [PubMed: 11248124]
110. Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, Jordt SE. TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J Clin Invest* 2008;118:1899–1910. [PubMed: 18398506]
111. Story GM, Peier AM, Reeve AJ, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* 2003;112:819–829. [PubMed: 12654248]
112. Corey DP, Garcia-Anoveros J, Holt JR, et al. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* 2004;432:723–730. [PubMed: 15483558]
113. Latorre R. Perspectives on TRP channel structure and the TRPA1 puzzle. *J Gen Physiol* 2009;133:227–229. [PubMed: 19237586]
114. Hirano M, Denis CS, Richardson GP, Gillespie PG. Hair cells require phosphatidylinositol 4,5-bisphosphate for mechanical transduction and adaptation. *Neuron* 2004;44:309–320. [PubMed: 15473969]

115. Kwan KY, Allchorne AJ, Vollrath MA, et al. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* 2006;50:277–289. [PubMed: 16630838]
116. Bautista DM, Jordt SE, Nikai T, et al. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 2006;124:1269–1282. [PubMed: 16564016]
117. Dai Y, Wang S, Tominaga M, et al. Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J Clin Invest* 2007;117:1979–1987. [PubMed: 17571167]
118. Kim D, Cavanaugh EJ. Requirement of a soluble intracellular factor for activation of transient receptor potential A1 by pungent chemicals: role of inorganic polyphosphates. *J Neurosci* 2007;27:6500–6509. [PubMed: 17567811]
119. Karashima Y, Prenen J, Meseguer V, Owsianik G, Voets T, Nilius B. Modulation of the transient receptor potential channel TRPA1 by phosphatidylinositol 4,5-bisphosphate manipulators. *Pflugers Arch* 2008;457:77–89. [PubMed: 18461353]
120. Giamarchi A, Padilla F, Coste B, et al. The versatile nature of the calcium-permeable cation channel TRPP2. *EMBO Rep* 2006;7:787–793. [PubMed: 16880824]
121. Igarashi P, Somlo S. Genetics and pathogenesis of polycystic kidney disease. *J Am Soc Nephrol* 2002;13:2384–2398. [PubMed: 12191984]
122. Ma R, Li WP, Rundle D, Kong J, Akbarali HI, Tsiokas L. PKD2 functions as an epidermal growth factor-activated plasma membrane channel. *Mol Cell Biol* 2005;25:8285–8298. [PubMed: 16135816]
123. Estacion M, Sinkins WG, Schilling WP. Regulation of *Drosophila* transient receptor potential-like (TrpL) channels by phospholipase C-dependent mechanisms. *J Physiol* 2001;530:1–19. [PubMed: 11136854]
124. Prescott ED, Julius D. A modular PIP₂ binding site as a determinant of capsaicin receptor sensitivity. *Science* 2003;300:1284–1288. [PubMed: 12764195]

A

B

Figure 1.

Phosphoinositide metabolism. A: Chemical formula for PtdIns(4,5)P₂. B. Metabolism of phosphoinositides and the generation of phosphoinositide derived second messengers upon PLC activation.

Figure 2.

The effect of PIP₂ depletion on ion channels. *A. Left*, under resting conditions, PIP₂ cannot activate the channels. *Middle*, upon activation, the channel changes conformation and the negatively charged head group of PIP₂ interacts with positively charged residues in the cytoplasmic domain(s) of the channels, leading to channel opening. It is also possible that the channel interacts with PIP₂ in the absence of the stimulus, but the lipid cannot activate it. Both these possibilities manifest as an increased apparent affinity of the channel for PIP₂ upon activation. *Right*, upon PLC activation PIP₂ is depleted and as a consequence, the channel closes. See text for further details. *B*. The effect of the apparent affinity for PIP₂ on channel function (from reference [9]). Channels with high affinity for PIP₂ (left, solid line) are fully

active at resting PIP_2 concentrations. Physiological changes in PIP_2 levels, i.e. moderate depletion of PIP_2 and increased PIP_2 levels do not affect them significantly. Channels with low affinity for PIP_2 (right, dashed line) on the other hand can be regulated by physiological changes in PIP_2 levels. The apparent affinity for PIP_2 is not static, it can be changed by other factors acting on the channel, for example, menthol increases the apparent affinity for PIP_2 on TRPM8. This is represented in the figure by the bidirectional arrow. See text for further details.

Figure 3.

Different possibilities of indirect effects of PIP₂ on ion channels. A. Calmodulin (CaM) competes for the same or overlapping binding site with PIP₂ or other phosphoinositides. Upon increased cytoplasmic Ca^{2+} concentrations, Ca^{2+} - CaM displaces PIP₂ or other phosphoinositides from their activating site, thus closing the channel. B. PIP₂ inhibits the channel by competing with an activating factor for binding to the channel. Depletion of PIP₂ allows the activating factor to open the channel. C. A PIP₂ binding protein keeps the channel open. Upon depletion of PIP₂, the hypothetical PIP₂ binding protein falls off from the plasma membrane, leading to channel closing. D. The reverse of the previous scenario. A PIP₂ binding protein keeps the channel closed; depletion of PIP₂ leads to channel opening. E. A

phosphoinositide interacting transmembrane protein (Pirt) modifies the activity of a channel (TRPV1). Scenarios B, C and D are purely hypothetical, see text for further details.

Figure 4.

Calcium-induced activation of PLC leads to channel inactivation. A. Menthol or cold opens TRPM8, capsaicin or heat opens TRPV1. Calcium flowing through the channels activates a Ca^{2+} sensitive PLC, probably a PLC δ isoform. This leads to the depletion of PIP_2 (and PIP) and diminished channel activity (desensitization or adaptation). This model has been tested for menthol [14;37] and capsaicin [85;91], partially for cold [37], but not for heat. A similar model was proposed for TRPA1 [119], but it is quite controversial, see text for details. Also note that there are possibly other mechanisms contributing to diminished activity, calmodulin and calcineurin for TRPV1, and PKC for TRPM8, see text for details. B. TRPV6 is constitutively active; Ca^{2+} flowing through the channel activates PLC and the ensuing PIP_2 depletion inactivates the channel, i.e. it stabilizes its activity at a lower steady state [88;107]. Channel inhibition by Ca^{2+} -calmodulin is also likely to contribute to channel inactivation, see text for details. This model is also possible for TRPV5, which is also dependent on PIP_2 and undergoes Ca^{2+} -induced inactivation, but several predictions of this model have not been tested yet on this channel. C. TRPM4 and 5 are impermeable to Ca^{2+} . Increased cytoplasmic Ca^{2+} activates these channels (fast effect), but it also activates PLC leading to the depletion of PIP_2 (slower effect). This sequence of events leads to a transient activation of these channels [14;70;78; 79].

Table 1

Regulation of TRPM, TRPV, TRPA and TRPP channels by phosphoinositides. Expression pattern is based on both data in the literature and the EST expression profile of the Unigene database. The list of regulators is not intended to be complete; it serves as an orientation for the reader about the function of the channels.

	Expression pattern	Regulation	Regulation by phosphoinositides
TRPM4	Widely expressed	i.c. Ca^{2+} activates	PIP_2 activates in excised patches [79]
TRPM5	Taste tissue, intestine	i.c. Ca^{2+} activates, heat	PIP_2 activates in excised patches [70;78]
TRPM7	Widely expressed	cAMP, shear stress, Mg^{2+} inhibits	PIP_2 activates in excised patches, PIP_2 depletion inhibits [47]. Role of PIP_2 depletion was challenged [51;52]
TRPM8	Sensory neurons, prostate	Cold, voltage, cooling agents (menthol, icilin)	PIP_2 activates in excised patches [14;59], PIP_2 depletion inhibits [14;37;39;59], PIP_2 depletion is involved in Ca^{2+} dependent desensitization / adaptation [14;37]
TRPV1	Sensory neurons, several other tissues	Heat $>43^\circ\text{C}$, low pH, capsaicin	PIP_2 and other phosphoinositides activate in excised patches [84-86] PIP_2 depletion is involved in Ca^{2+} -induced desensitization [85;89;91;92] PIP_2 may partially inhibits in intact cells [85;100;124]
TRPV5	Epithelial cells kidney distal tubule	Constitutively active; Ca^{2+} -induced inactivation	PIP_2 activates in excised patches [14;106]
TRPV6	Duodenum, kidney, placenta Various other cell types	Constitutively active; Ca^{2+} -induced inactivation	PIP_2 activates in excised patches [88] PIP_2 depletion inhibits, involved in Ca^{2+} -induced inactivation [88]
TRPA1	Sensory neurons: DRG, vagal ganglia	Mustard oil, allicin, acrolein, formaldehyde Noxious cold (?)	PIP_2 inhibits heterologous desensitization by capsaicin in whole-cell [90] PIP_2 activates in excised patches, inhibits desensitization in whole-cell [119] PIP_2 inhibits sensitization by PAR in whole-cell [117] PIP_2 inhibits in excised patches in the presence of PPPi , no effect w/o PPPi [87; 118] Depletion of PIP_2 with rapamycin-inducible phosphatase have no effect [60]
TRPP2	Kidney, widely expressed	Mechanosensor? mutation causes polycystic kidney disease	PIP_2 inhibits, depletion of PIP_2 by EGF activates [122]