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Transient receptor potential channels meet phosphoinositides

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Transient receptor potential (TRP) cation channels are unique cellular sensors that are involved in multiple cellular functions, ranging from transduction of sensory signals to the regulation of Ca^{2+} and Mg^{2+} homoeostasis. Malfunctioning of TRP channels is now recognized as the cause of several hereditary and acquired human diseases. At the time of cloning of the first *Drosophila* TRP channel, a close connection between gating and phosphatidylinositol phosphates (PIPs) was already recognized. In this review, we summarize current knowledge about the mechanisms of interaction between TRP channels and PIPs, and discuss the possible functional implications of TRP–PIP interactions to human physiology and pathophysiology.

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TRP channels regulated by phosphoinositides

Transient receptor potential (TRP) channels provide a close sensory interface between the environment and interior of cells. To fulfil this function, TRP channels require polymodality, that is, they are activated by a huge variety of stimuli, including physical factors such as temperature, voltage and mechanical forces, chemical factors such as pH and ions, mainly Ca^{2+} and Mg^{2+}, and various intracellular signalling pathways (Voets *et al*, 2005; Nilius *et al*, 2007; Venkatachalam and Montell, 2007). Although crystal structures of entire TRP channels are currently missing, it is generally accepted that TRP channels form homo- or heterotetramers and have a similar topology as voltage-gated K^+, Na^+ and Ca^{2+} channels. They contain six transmembrane (TM) segments per subunit with a pore region between TM5 and TM6, and intracellular amino (N) and carboxy (C) termini (Owsianik *et al*, 2006; Gaudet, 2008).

Transmembrane channels not only have an important function in the sensory system, but they are also involved in global Ca^{2+} and Mg^{2+} homoeostasis (Hoenderop and Bindels, 2008), and in various Ca^{2+}-dependent signalling processes that regulate cell growth, cell death, dendrite growth, cell cycle regulation, migration and many more (for reviews, see Bandell *et al*, 2007; Nilius *et al*, 2007; Venkatachalam and Montell, 2007; Talavera *et al*, 2008). In addition to their normal physiological functions, the involvement of TRP channels in the development of human diseases becomes an extremely important issue (Nilius *et al*, 2007).

Understanding the widespread physiological functions of TRP channels requires knowledge of the general aspects of their regulation and modulation. One exciting aspect of TRP channel regulation is the interaction with and modulation by plasma membrane phosphatidylinositol phosphates (PIPs), and in particular by phosphatidylinositol-4,5-biphosphate ($PI(4,5)P_2$). $PI(4,5)P_2$ is present in the cytoplasmic leaflet of the plasma membrane, where it constitutes about 1% of the lipid and forms the precursor of important signalling molecules such as inositol 1,4,5 trisphosphate (IP_3), diacylglycerol (DAG) and phosphatidylinositol 3,4,5 triphosphate ($PI(3,4,5)P_3$) (McLaughlin and Murray, 2005; Suh and Hille, 2007). Importantly, $PI(4,5)P_2$ itself exerts an effect as a signalling molecule that modulates the function of a variety of membrane proteins, including ion channels and transporters (Gamper and Shapiro, 2007; Suh and Hille, 2008). Rapid changes in $PI(4,5)P_2$ concentration occur upon activation of phospholipase C, leading to the formation of IP_3 and DAG. Moreover, different PIPs can occur in microdomains where they can be rapidly interconverted by an extremely dynamic signalling network of phospinositide phosphatases and kinases (Blero *et al*, 2007). At physiological PIPs are negatively charged (e.g., $PI(4,5)P_2$ has a valence of approximately −4) residues, allowing them to interact electrostatically rather non-specifically with positively charged residues on membrane proteins and with other cationic molecules. In addition, specialized PIP interaction sites have been identified, such as the pleckstrin homology (PH) domains, that function as a more specific PIP binding pocket.

As summarized in Table I, interactions with PIPs have been reported for a large number of TRP channels. In some cases, molecular details about the mode and specificity of the

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Table I TRPs and PIPs: putative binding sites and functional effects of interaction

	Effect of PIP_2	PIP interaction sites	References
TRPC3	+	CIRB domain	van Rossum *et al* (2005)
TRPC4	−	84 C-terminal AA; C-terminal PDZ domain and binding to F-actin domain required	Otsuguro *et al* (2008)
TRPC5	−/+	—	Kim *et al* (2008b), Trebak *et al* (2008)
TRPC6	+/−	CIRB domain	Kwon *et al* (2007)
TRPC7	+/−	—	Lemonnier *et al* (2008)
TRPV1	+/−	C-terminal region, TRP domain, depending on Pirt association	Prescott and Julius (2003), Stein *et al* (2006), Lukacs *et al* (2007), Rohacs (2007), Kim *et al* (2008a)
TRPV5	+	TRP domain	Lee *et al* (2005), Rohacs *et al* (2005), Thyagarajan *et al* (2008)
TRPV6	+	TRP domain	Thyagarajan *et al* (2008)
TRPM4	+	PH-like domain	Zhang *et al* (2005); Nilius *et al* (2006)
TRPM5	+	TRP domain	Liu and Liman (2003)
TRPM6	?	—	
TRPM7	+/−	—	Runnels *et al* (2002), Gwanyanya *et al* (2006), Langeslag *et al* (2006)
TRPM8	+	TRP domain	Liu and Qin (2005), Rohacs *et al* (2005)
TRPA1	+/−	—	Karashima *et al* (2008), Kim *et al* (2008c)
TRPP2	−	—	Ma *et al* (2005)

(+) refers to activation and (−) refers to inhibition by $PI(4,5)P_2$.

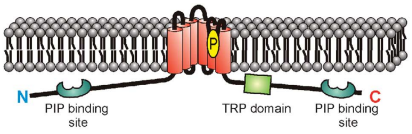
interaction have been reported. Although different PIPs may have comparable potencies in regulating TRP channels, it seems that $PI(4,5)P_2$ is the more crucial regulator, most likely due to its relative abundance in the plasma membrane. For example, TRPV1 was inhibited only by a recombinant $PI(4,5)P_2$-specific PH-domain construct, whereas a PH-domain that binds $PI(3,4,5)P_3$ or enzymatic production of PI(4)P were ineffective (Klein *et al*, 2008). However, there are also a worrisome number of conflicting reports for several TRP channels as to whether $PI(4,5)P_2$ activates or inhibits TRP channel gating. Apart from differences in experimental procedures and/or their interpretation, such apparently conflicting results may indicate the existence of more than one mode of $PI(4,5)P_2$-dependent regulation of a single TRP channel, leading to a bell-shaped $PI(4,5)P_2$ dependence. Obviously, a clearer molecular picture of the PIP–TRP channel interaction and a better understanding of the gating mechanisms will be required to fully understand the impact of the different PIPs on the different TRPs. Recently developed methods that allow rapid modulation of the PIPs in the plasma membrane without production of DAG and IP_3 or release of intracellular calcium represent powerful new tools that will facilitate research approaching the mechanistic and functional principles of TRP–PIP interactions (for recent reviews, see McLaughlin, 2006; Suh and Hille, 2008).

Mechanisms of TRP–PIP interactions

Full understanding of the molecular basis of the regulation of TRP channel function by PIPs requires detailed information about (1) the interaction site(s) of PIP on the channel or on associated proteins and (2) the mechanism whereby PIP-binding influences channel gating.

At this point, there are experimental data suggesting the existence of multiple distinct types of PIP interaction sites in the TRP superfamily (Figure 1), all of which are characterized by an abundance of positively charged residues. First, a region containing eight positive charges in the C terminus of TRPV1 was identified as a possible $PI(4,5)P_2$ interaction site responsible for $PI(4,5)P_2$-mediated channel inhibition (Prescott and Julius, 2003). However, it should be noted that recent reports have cast doubts over the inhibitory effect of $PI(4,5)P_2$ on TRPV1, and the relevance of this putative $PI(4,5)P_2$ site remains controversial (Stein *et al*, 2006; Lishko *et al*, 2007; Lukacs *et al*, 2007; Rohacs, 2007; Kim *et al*, 2008a). Second, Rohacs *et al* (2005) have implicated the TRP domain, a C-terminal region adjacent to TM6 in the TRPV, TRPM and TRPC subfamilies, in the $PI(4,5)P_2$-dependent regulation of different TRP channels, including TRPM8, TRPV5 and TRPM5. Neutralization of conserved positive charges in this region caused a reduction in the apparent $PI(4,5)P_2$ affinity of these channels, suggesting that these residues interact directly with membrane PIPs. Third, evidence from mutagenesis experiments revealed a function of a more distal C-terminal region in the $PI(4,5)P_2$-dependent regulation of TRPM4 (Nilius *et al*, 2006). This region also contains a cluster of basic residues and can be considered as a PH-like domain because it contains the PH consensus sequence $(K/R)\text{-}X_n\text{-}(K/R)\text{-}X\text{-}(K/R)_2$ (were X is any amino acid). Analysis of the sequence of the TRP superfamily (Figure 1) reveals one or more such PH-like domains in the majority of mammalian TRP channels, but whether these domains bind PIPs and/or modulate channel function is currently unclear. Atomic structures and biochemical evidence would be required to determine whether regions in TRP channels containing the PH-like consensus region actually form a PH domain, which normally consists of a seven-stranded β sandwich formed by two orthogonal antiparallel β sheets and a C-terminal amphiphilic α-helix (Suh and Hille, 2008). Fourth, Kwon *et al* (2007) found that the PIPs, particularly $PI(3,4,5)P_3$ as well as IP_6, disrupt the interaction between calmodulin and the C terminus of TRPC6. Moreover, mutating basic residues in the calmodulin binding site affected PIP binding and channel function, suggesting that PIPs interact directly with the calmodulin-binding site. Fifth, Otsuguro *et al* (2008) studied the effects of $PI(4,5)P_2$ on two different splice isoforms of TRPC4 and found that TRPC4α is inhibited by $PI(4,5)P_2$, whereas TRPC4β, which lacks 84 amino acids (Δ84AA) in the C terminus, is $PI(4,5)P_2$ insensitive. This suggests that this stretch of 84 AA contains all or part of a $PI(4,5)P_2$ interaction site. Clearly, there is a large variability in the nature and location of the putative PIP-interacting sites, which may not be too surprising, given the broad functional diversity and considerable sequence variation within the TRP superfamily.

A



TRPC3 (848 aa)	*n* 1–171: MEGSPSLRRM . . . PDITPIILAAHCQ		
TRPC4 (977 aa)		*c* 781–864:	GNGKDKRK . . . ANQIFSVS
TRPC6 (931 aa)		*c* 853–865:	RQYQKIMKRLIKR
TRPV1 (839 aa)		*c* 786–805:	RHWKNFALVPLLREASARDR
TRPV5 (729 aa)	*TRP domain* 587-600:	RAQVVATTVMLERK	
TRPM4 (1214 aa)		*c* 1136–1150:	RARDKRESDSERLKR
TRPM5 (1165 aa)	*TRP domain* 994-1007:	KFQRYNL IVEYHER	
TRPM8 (1104 aa)	*TRP domain* 995-1008:	KFQRYFLVQEYCSR	

Putative PH-like domains in TRPs

[KR]-X(3–11)-[KR]-X-[KR]-[KR]

B

	TRPC1 (793 aa):		*c* 700–713:	KWICSHTSKGKVKR
			c 712–727:	KRQNSLKEWRNLKQKR
TRPC	TRPC3 (848 aa):	*n* 8–19: RRMTVMREKGRR	*c* 722–733:	RIVNFPKCRRRR
	TRPC4 (977 aa):		*c* 671–684:	KWIWTHLCKKKMRRK
			c 780–791:	KSDSEGNSKDKKK
	TRPC5 (973 aa):		*c* 684–694:	KRDPDGRRRRR
	TRPC6 (931 aa):	*n* 63–78: RLAH RRQTVLREKGRR	*c* 771–782:	KSLFYLLLKLKK
	TRPC7 (862 aa):	*n* 8–23: KNMQRRHTTLREKGRR		
		n 199–209: KCNECTEKQRK		
TRPV	TRPV3 (790 aa):	*n* 109–121: KEEQRRKKRRLKKR		
		n 358–369: KYILSREIKEKR		
	TRPV4 (871 aa):	*n* 114–125: RHHSSDNKRWRKK		
	TRPV6 (725 aa):	*n* 314–323: KELVSLKWKR		
TRPM	TRPM1 (1533 aa):	*n* 219–228: KYGAEVKLRR	*c* 1123–1132:	RLSGRCRKKR
		n 411–418: KATEKEKK	*c* 1433–1447:	KTMKSRSFVYSRGRK
		n 425–435: KGGRGKGKGKKKGKVK		
	TRPM2 (1503 aa):		*c* 1392–1404:	REPGEMLPRKLKR
	TRPM3 (1579 aa):	*n* 302–311: KYGAEVKLRR		
		n 621–636: KLLGMEDDIPLRRGRK		
	TRPM4 (1214 aa):	*n* 327–337: RQGEARDRIRR		
	TRPM5 (1165 aa):	*n* 713–726: RGPRAVFLLTRWRK	*c* 1059–1071:	KENFLSKMEKRRR
	TRPM6 (2022 aa):	*n* 258–267: KYGNEMKLRR	*c* 1572–1587:	KAKMLTKDRRLSKKKK
		n 575–588: KFKEKSIVLHKSRK		
	TRPM7 (1865 aa):	*n* 255–264: KYGAEVRLRR		
		n 588–602: RPKIDTVMEEGKKKR		
	TRPM8 (1104 aa):	*n* 4–14: RAARLSMRNRR		
		n 153–163: KNFALKPRMRK		
TRPP	TRPP2 (968 aa):		*c* 705–719:	RKGYHKALVKLKLKK
			c 866–877:	KLEIMERAKLKR
	TRPP3 (805 aa):		*c* 585–599:	KQGYNKTLLRLRLRK
	TRPP5 (624 aa):		*c* 516–530:	KQSYKNVLEKFRLKK
TRPML	TRPML1 (580 aa):	*n* 55–62: KFRAKGRK		
	TRPML2 (538 aa):	*n* 46–60: KFYFMSPCEKYRARR	*c* 541–556:	KESSAFLSCICCRRRK
	TRPML3 (553 aa):	*n* 52–59: KFWARGRK		
TRPA	TRPA1 (1119 aa):	*n* 591–604: KRKEVVLTIIRSKR		

Figure 1 PIP binding on TRP channels. (**A**) Known binding sites of PIPs on TRP channels are shown. The localization is indicated. (**B**) Putative PIP-binding site in PH-like domains in human TRP channels are shown for all TRP channels (note that no PH-like motifs were found for TRPV1 and TRPV2). From all human TRP channels, PH-like domains were identified. *n* and *c* mark the localization in the N- and C-terminus, respectively, together with the amino-acid positions. Positively charged residues are in blue. The length of each human TRP channel is indicated.

In addition, recent evidence indicates that $PI(4,5)P_2$-dependent regulation of TRPV1 occurs through an accessory protein, Pirt. $PI(4,5)P_2$ binding is dependent on a cluster of basic residues in the C terminus of Pirt, and this binding enhances TRPV1 channel activity (Kim *et al*, 2008a). It is highly possible that other TRP channels are regulated in a

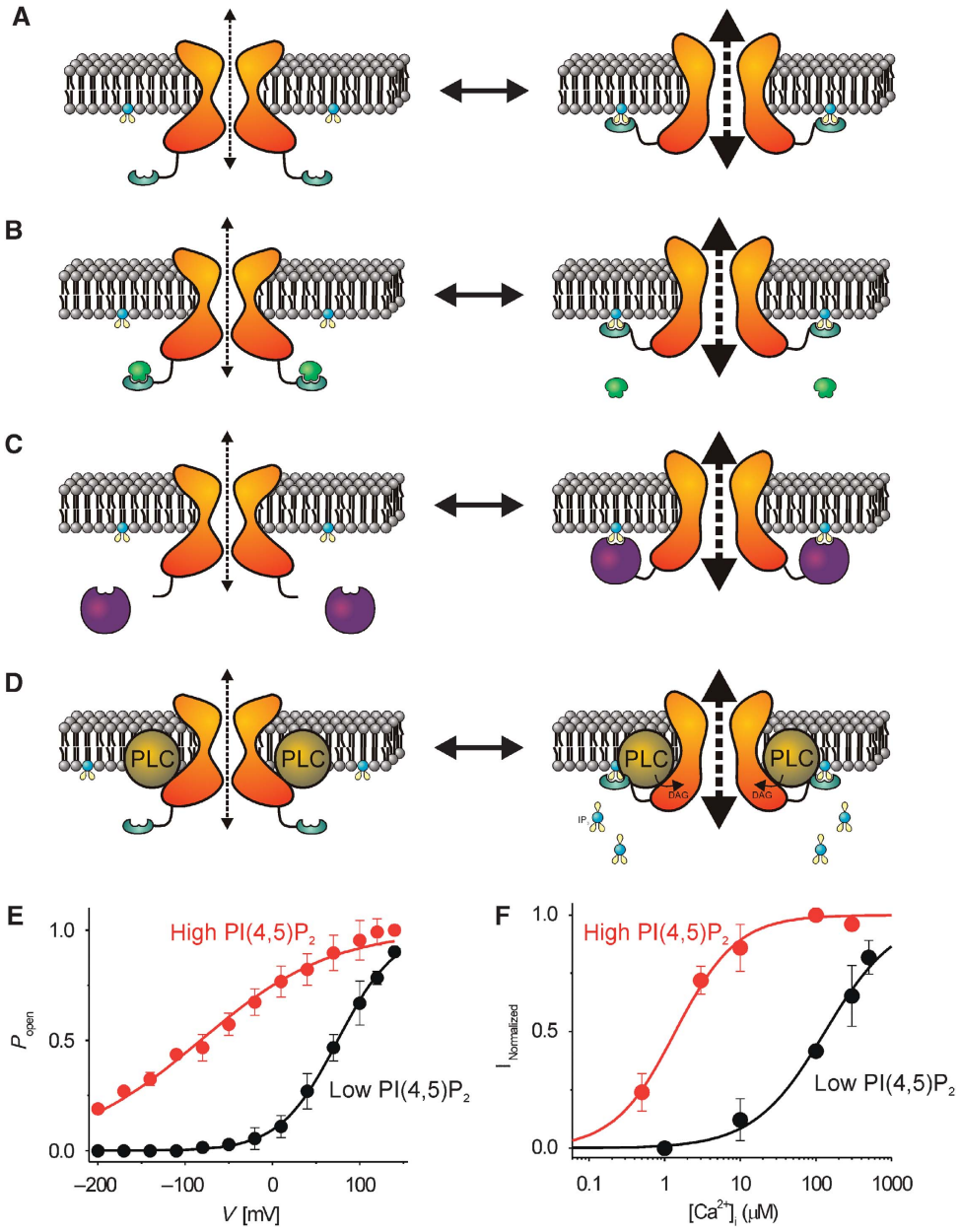


Figure 2 Mechanisms causing functional changes in TRP channel activity. (**A**) Binding of intracellular parts of the channel to $PI(4,5)P_2$ increases the probability of the channel spent in an open configuration. (**B**) $PI(4,5)P_2$ interacts with a normally inhibitory factor thereby activating the channel. (**C**) $PI(4,5)P_2$ binding requires an adaptor protein, and only in this configuration, channel activity can be modulated. (**D**) A TRP channel associates with an agonist-delivering PLC forming a signalplex. For details, see text. Note that these mechanisms might also be able to initiate inhibition of the channel. (**E**) Application of $PI(4,5)P_2$ to excised patches containing TRPM4 dramatically shifts the voltage dependence of channel activation towards more negative potentials, thereby activating the physiological membrane potentials of the channels (for details, see Nilius *et al*, 2006). (**F**) Activation of TRPM4 by elevation of the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ is sensitized by the application of $PI(4,5)P_2$ (Nilius *et al*, 2006).

similar way through interaction with a PIP-binding accessory subunit. The results obtained with Pirt also indicate that defining PIP-binding sites purely based on mutagenesis of TRP channel proteins is not unequivocal, as mutations that alter the interaction between the channel and a PIP-binding accessory subunit would lead to a change in the apparent PIP affinity of the channel.

So how does binding of $PI(4,5)P_2$ and other PIPs influence channel gating? We are currently far from understanding the structural rearrangements that occur upon PIP binding and TRP channel gating, but on the basis of the available results, four main mechanisms of action can be distinguished (Figure 2). Note that although these four mechanisms are depicted in such a way that $PI(4,5)P_2$ enhances channel opening, inhibitory effects of $PI(4,5)P_2$ may work along similar lines.

In the first and most straightforward model, specific intracellular parts of the channel are attracted towards the plasma membrane due to the direct interaction with $PI(4,5)P_2$, leading to a stabilization of the open state (Figure 2A). This is similar to what has been proposed for $PI(4,5)P_2$-dependent gating of inward-rectifier K^+ channels, where results from structural, functional and molecular modelling studies suggest that $PI(4,5)P_2$ exerts tangential force on

the N- and C-termini to open the channel (Logothetis *et al*, 2007; Suh and Hille, 2008).

In the second mechanism, modulation of a TRP channel occurs through a competition between PIPs and other accessory proteins for binding to the same site on the TRP channel (Figure 2B). This model is based on results obtained for TRPC6, where binding of calmodulin to a C-terminal site inhibits the channel, whereas removal of calmodulin by PIPs results in channel activation (Kwon *et al*, 2007). Indeed, also for TRPC3, 6 and 7, $PI(4,5)P_2$ is bound to a site localized in the C-terminal calmodulin-IP_3-receptor-binding site (Patterson *et al*, 2002; van Rossum *et al*, 2005; Kwon *et al*, 2007).

The third mechanism depicts the situation where PIPs interact indirectly with the TRP channel, through a PIP-binding accessory protein, as in the case of the Pirt-dependent modulation of TRPV1 by $PI(4,5)P_2$ (Kim *et al*, 2008a) (Figure 2C). The consequences of this interaction may be similar to what we described for the model in Figure 2A. In case of TRPC4, inhibition by $PI(4,5)P_2$ depends on the association of the channel with actin cytoskeleton depending on a C-terminal PDZ-binding motif (Thr-Thr-Arg-Leu) that links TRPC4 to F-actin through NHERF and ezrin. Deletion of this site prevents inhibitory effects of $PI(4,5)P_2$ (Otsuguro *et al*, 2008).

The fourth mechanism might exploit TRP-channel interaction with an agonist-delivering enzyme (Figure 2D). Such a mechanism can be hypothesized for TRPC3 (van Rossum *et al*, 2005), where interaction between channel and $PI(4,5)P_2$ involves an intermolecular PH-like domain, with the N-terminal part provided by PLCγ1 and the C-terminal part provided by TRPC3. The close association between TRPC3, PLCγ1 and $PI(4,5)P_2$ may be considered as a signalplex, causing localized production of the channel agonist DAG and controlling the surface expression of the channel (van Rossum *et al*, 2005).

Independent of the structures and mechanisms underlying the PIP–TRP channel interactions, it is interesting to evaluate the consequences of this interaction on the basic electrophysiological properties of the channel. In most cases, it seems that the interaction with specific PIPs such as $PI(4,5)P_2$ is not absolutely required for channel opening, but rather influences the relative stability of open and closed states and/or influences the channels' sensitivity to different activating stimuli (Liu and Liman, 2003; Rohacs *et al*, 2005; Nilius *et al*, 2006; Stein *et al*, 2006). This is in detail exemplified by the effects of $PI(4,5)P_2$ on the Ca^{2+}-activated, voltage-dependent TRP channel TRPM4. In inside-out patches, TRPM4 activity decays rapidly, due to the loss of $PI(4,5)P_2$ from the excised membrane patch (Nilius *et al*, 2006). Addition of $PI(4,5)P_2$ to the cytosolic side of the patch then results in an $\sim$100-fold increase in the affinity of TRPM4 for Ca^{2+} (Figure 2E) and a significant leftward shift of the voltage-dependent activation curve (Figure 2F) (Nilius *et al*, 2006). The effect of $PI(4,5)P_2$ on the voltage dependence of TRP channels such as TRPM4 and TRPM8 is reminiscent to results obtained for the bacterial voltage-gated K^+ KvAP (Voets *et al*, 2004a; Rohacs *et al*, 2005; Nilius *et al*, 2006; Schmidt *et al*, 2006). To be operational, the voltage sensor KvAP requires negatively charged phospholipids, such as phosphatidylglycerol, (Schmidt *et al*, 2006), which provide a stabilizing environment for the positively charged arginines of the KvAP voltage sensor. Recent evidence indicates that the voltage sensor of TRP channels is located in the region encompassing S4, similar to that of classical voltage-gated cation channels (Voets *et al*, 2007). Moreover, *in silico* modelling efforts suggest an interaction between $PI(4,5)P_2$ and basic residues in the S4-S5 linker of TRPV1 (Brauchi *et al*, 2007). A direct interaction between $PI(4,5)P_2$ and the voltage sensor may also explain why the apparent gating charge of TRPM4 decreases from 0.7 equivalent charges upon $PI(4,5)P_2$ depletion to $\sim$0.35 equivalent charges in the presence of $PI(4,5)P_2$ (Nilius *et al*, 2006).

(Patho)physiological implications of PIP–TRP interactions

We are only starting to understand the importance of PIP–TRP interactions, but the various functions of TRP channels in sensory and non-sensory processes predict that alterations in cellular PIP levels will have diverse (patho)physiological consequences.

Desensitization

Somatosensory processes including thermosensing and pain are known to exhibit desensitization, meaning that the perceived intensity of an invariant stimulus decreases with time. There are several lines of evidence that alterations of cellular $PI(4,5)P_2$ levels have an important function in this process. A nice example is the $PI(4,5)P_2$-dependent regulation of TRPM8, the main sensor for innocuous cold. Ca^{2+} influx through the activated TRPM8 channel causes depletion of the cellular $PI(4,5)P_2$ pool through stimulation of a Ca^{2+}-sensitive PLC, thereby gradually reducing TRPM8 activity. This may contribute to the well-known adaptation to a mildly cold stimulus. Similarly, the $PI(4,5)P_2$-dependent regulation of TRPV1, one of the most important ion channels for pain sensation, may have important effects on pain perception. TRPV1 rapidly desensitizes upon stimulation with agonists such as capsaicin, and recovery from desensitization requires resynthesis of $PI(4,5)P_2$ (Liu and Qin, 2005). $PI(4,5)P_2$ breakdown is also crucially involved in the desensitization of receptor-operated channels, whose activation is coupled to activation of PLC-coupled receptors. For example, desensitization of TRPC5 following activation by muscarinic receptor stimulation is slowed by intracellularly applied $PI(4,5)P_2$. This type of regulation may be important for muscarinic fine-tuning of smooth muscle contraction (Kim *et al*, 2008b).

Excitability

There are indications that voltage-dependent non-selective cation channels, most likely mediated by TRPM4 and/or TRPM5, have an important function in regulating neuronal excitability (Egorov *et al*, 2002; Crowder *et al*, 2007). For example, neurons in the brain stem preBötzinger complex generate discharge bursts of action potentials that drive the inspiratory phase of breathing. Modulation of $PI(4,5)P_2$ levels alter the inspiratory drive potentials, which has been attributed to modulation of TRPM4/TRPM5 (Crowder *et al*, 2007).

Trafficking

Binding of PIPs to TRPs has been implicated in TRP channel trafficking to the plasma membrane. For example, EGF receptor-induced translocation of TRPC5 depends on the

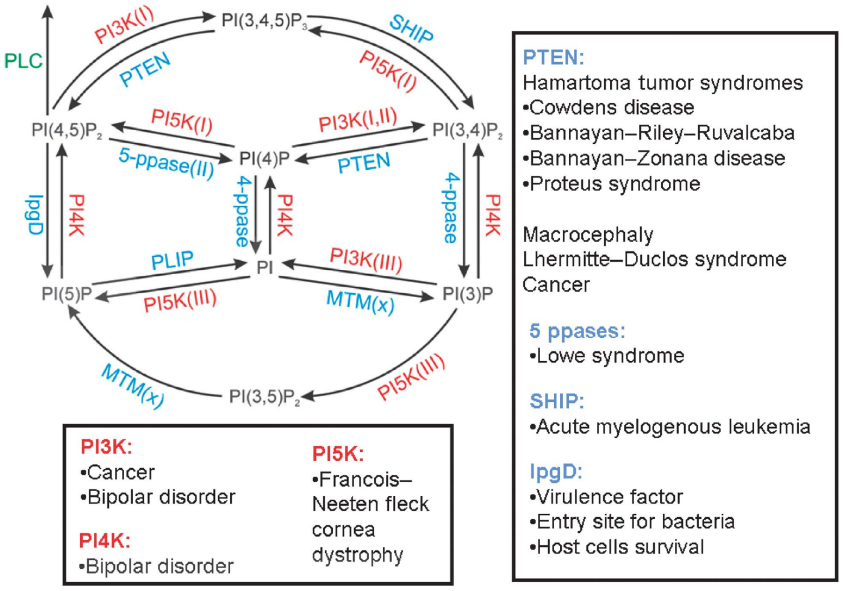


Figure 3 Diseases linked to PIP metabolism. A scheme of the PIP metabolism including some critical kinases (PIxK, in red) and phosphatases (x-ppase in blue) is shown. The boxes shown refer to defective enzyme functions that can lead to human diseases. For details, see Blero *et al*, 2007 and Krauss and Haucke, 2007. (SHIP, P5-phosphatase; PTEN, tyrosine P5-phosphatase; MTM, myotubularins (1–7), i.e., P3-phosphatases; IpgD, invasion plasmid gene D P4 phosphatase).

rac1-mediated activation of PIP(5) kinase, leading to the production of $PI(4,5)P_2$ (Bezzerides *et al*, 2004). Likewise, the interaction between $PI(4,5)P_2$ and the intermolecular PH-like domain formed by TRPC3 and PLCγ1 regulates the incorporation of TRPC3 into the plasma membrane (van Rossum *et al*, 2005).

Feedback regulation

Transient receptor potential channels are involved in the transepithelial transport of both Ca^{2+} (TRPV5 and TRPV6) and Mg^{2+} (TRPM6 and TRPM7). To maintain a balance between apical divalent cation influx (through TRP channels) and basolateral cation extrusion, the activity of these TRP channels is tightly regulated by Ca^{2+}- or Mg^{2+}-dependent feedback mechanisms. Ca^{2+}-dependent inactivation of TRPV5 and TRPV6 involves Ca^{2+}- and PLC-dependent reduction of cellular $PI(4,5)P_2$ levels (Gordon-Shaag *et al*, 2008; Thyagarajan *et al*, 2008), whereas Mg^{2+}-dependent inactivation of TRPM6 and TRPM7 may be partially due to screening of the negative charges of the $PI(4,5)P_2$ by intracellular Mg^{2+} (Runnels *et al*, 2002; Voets *et al*, 2004b; Kozak *et al*, 2005).

Human diseases

In the case of inwardly rectifying Kir channel, mutations that directly affect channel–$PI(4,5)P_2$ interactions have been shown to cause hereditary diseases. Mutation of arginine at position 218 in Kir 2.1 to either glutamine or tryptophane causes Andersen's s syndrome (Plaster *et al*, 2001), whereas mutations in Kir1.1 (R311Q and R311W) are responsible for genetic defects associated with the antenatal variant of Bartter's syndrome (Schulte *et al*, 1999). At present, no such mutations have been identified in TRP channels, but the increasing number of diseases associated with genetic defects in TRP channel genes urges further research in this direction (Nilius, 2007; Nilius *et al*, 2007).

In addition, several diseases are known to be due to defects in enzymes involved in PIP metabolism (Halstead *et al*, 2005). In view of the often dramatic effects of $PI(4,5)P_2$ on TRP channels, it must be considered that alterations in the availability of $PI(4,5)P_2$ could translate into deregulation of the TRP channel activity and thereby contribute to the pathological process (for reviews, see Blero *et al*, 2007; Krauss and Haucke, 2007). For example, there is evidence linking bipolar disorder to altered $PI(4,5)P_2$ signalling (Halstead *et al*, 2005), which might include TRP channel dysfunction (Chahl, 2007; Nilius *et al*, 2007). Lowe syndrome (mental retardation, lens cataract, glaucoma, growth defects and renal dysfunction) is linked to a decreased PI-5-phosphatase function, which leads to increased plasma membrane $PI(4,5)P_2$ content and most likely dysregulation of many TRP channels. Other examples in which disturbances in the PIP metabolism are linked to diseases are shown in Figure 3 and comprise tumour syndromes, cornea diseases, forms of leukaemia and disorders connected to endocytosis.

Conclusions

From the data reviewed here, it can be concluded that regulation by PIPs represents a general mechanism for modulation of the majority, if not all, of TRP channels. Only a few TRP channel–PIP interactions, however, have been studied in detail. With the recent advances in the molecular tools to study and modulate PIP metabolism, we foresee that some urgent questions regarding the mechanisms and (patho)physiological implications of this form of TRP channel regulation will be answered in the coming years.

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