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## Review

# TRP channels interaction with lipids and its implications in disease<sup>☆</sup>

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## ABSTRACT

Transient receptor potential (TRP) proteins are a family of ion channels central for sensory signaling. These receptors and, in particular, those involved in thermal sensing are also involved in pain signaling. Noteworthy, thermosensory receptors are polymodal ion channels that respond to both physical and chemical stimuli, thus integrating different environmental clues. In addition, their activity is modulated by algogenic agents and lipidergic substances that are primarily released in pathological states. Lipids and lipid-like molecules have been found that can directly activate some thermosensory channels or modulate their activity by either potentiating or inhibiting it. To date, more than 50 endogenous lipids that can regulate TRP channel activity in sensory neurons have been described, thus representing the majority of known endogenous TRP channel modulators. Lipid modulators of TRP channels comprise lipids from a variety of metabolic pathways, including metabolites of the cyclooxygenase, lipoxygenase and cytochrome-P450 pathways, phospholipids and lysophospholipids. Therefore, TRP-channels are able to integrate and interpret incoming signals from the different metabolic lipid pathways. Taken together, the large number of lipids that can activate, sensitize or inhibit neuronal TRP-channels highlights the pivotal role of these molecules in sensory biology as well as in pain transduction and perception. This article is part of a Special Issue entitled: Lipid–protein interactions. Guest Editors: Amitabha Chattopadhyay and Jean-Marie Ruyschaert.

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## 1. Introduction

Survival of organisms critically depends on sensing environmental signals and transducing the information to the brain for proper response. For this purpose, the peripheral nervous system has specialized

neurons that contain the sensing molecular machinery, namely ion channels that respond to chemical and physical stimuli. An important family of sensory channels is the Transient Receptor Potential (TRP). These are non-selective cation channels, which are organized in six sub-families, namely Ankyrin (TRPA), Canonical (TRPC), Melastatin (TRPM), Mucolipin (TRPML), Polycystin (TRPP) and Vanilloid (TRPV) [1–4]. In sensory neurons, they contribute to a plethora of activities including thermal sensation, homeostasis of body temperature and pain [5,6]. In the central nervous system (CNS), TRP channels have been associated with neurogenesis, brain development and synaptic transmission [7,8]. Moreover, TRPV1 and TRPA1 have also been related to immunity, obesity and thermogenesis [9]. Therefore, it is not surprising that

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dysfunction of TRP channels is involved in the etiology of several diseases [10].

Structurally, TRP channels are tetrameric assemblies of basic subunits organized around a central aqueous pore. Akin to voltage-gated  $K^+$  channels, each subunit is composed of a membrane region containing 6 transmembrane segments. The recent structural model derived from cryo-electron microscopic images has clearly shown this molecular analogy [11]. TRP channels mainly differ in their cytosolic N- and C-terminal domains, which are involved in channel gating and mediating intracellular signaling. Indeed, most of TRP channels, if not all, are part of protein complexes known as signalplexes [12–14].

TRPs are activated by a myriad of physical and chemical stimuli such as temperature, pressure, voltage, irritant agents, inflammatory molecules, pH, osmolality changes, UVB radiation and a large list of natural chemical compounds [2,5,6,15–17]. TRP-evoked ionic currents are pivotal for the modulation of action potential in peripheral terminals, and to modify signaling pathways. Notably, TRP channel functionality is modulated by intracellular signaling pathways such as those mediated by protein kinase C (PKC), protein kinase A (PKA) and phospholipase C (PLC) [6,18]. This interplay makes TRP channels highly dynamic signal integrators whose activity depends on the cellular context and state.

As integral membrane proteins, TRP channels are highly sensitive to the lipid environment, although studies in reconstituted controlled systems are still scarce. Furthermore, some potent TRP agonists are lipids. The effect of lipids on TRP channels function has attracted the interest of the pharmaceutical industry and the academic research, as reflected by the number of research articles and reviews of this field [19–23].

A great variety of channels, including TRPs, is modulated by endogenous and exogenous lipids [21,24–29]. Cholesterol has been reported to potentiate or diminish the activity of ion channels [30]. Phosphatidylinositol 4-5-bisphosphate (PIP2) is a special case since it modulates the activity of many types of ion channels in a variety of modes [31–33]. A notable interest is currently centered on understanding the structure–function relations for TRP–lipid interactions. Information on the functional and structural interactions between lipids and TRP channels offers mechanistic insights into TRP-mediated cellular processes. Furthermore, such knowledge may shed light into pathological processes, and the structural information may be utilized to pave the way to develop TRP channel modulators with therapeutic potential. The history of lipid studies in the TRP field started in the late 1990s [34–36], and knowledge has thus far expanded to cover various aspects such as ligand binding, sensitivity shift, bilayer-protein interactions, and upstream metabotropic signaling. However, it has not been established yet whether TRP–lipid interactions act as second messengers, intercellular transmitters, or function to simply transform the plasma membrane properties affecting channel protein structure.

To date, more than 50 endogenous lipids that can regulate TRP channel activity in sensory neurons have been described, thus representing the majority of known endogenous TRP channel modulators (Fig. 1). Lipid modulators of TRP channels comprise lipids from a variety of metabolic pathways, including metabolites of the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome-P450 (CYP) pathways, phospholipids and lysophospholipids [37–40]. In addition, recent studies have identified lipid families previously not known to be connected to sensor signaling as modulators of TRP channels. These newly identified lipid modulators belong to hepoxilin A3 [41], 9-(S)-hydroxyoctadecadienoic acid (9-(S)-HODE) and 13-(S)-HODE from the LOX-pathway [42,43], 5(6)-epoxy-8Z,11Z,14Z-eicosatrienoic acid (5,6-EET) from the CYP-pathway and 20-hydroxyeicasatetraenoic acid (20-HETE) an  $\alpha$ -hydroxide of arachidonic acid (AA) [44,45], as well as the lysophosphatidic acid (LPA) [46]. Furthermore, molecules like the LOX-derived  $\omega$ -3 lipid Resolvin D1 (RvD1) and Resolvin D2 (RvD2) act as endogenous inhibitors of TRPA1, TRPV1, TRPV3 and TRPV4 [47], thus expanding the functionality of lipid-mediated TRP channel modulation. Therefore, TRP-channels are able to integrate and interpret incoming signals from the different metabolic lipid pathways. Taken

together, the large number of lipids that can activate, sensitize or inhibit neuronal TRP-channels highlights the pivotal role of these molecules in sensory biology as well as in pain transduction and perception. Here we review the current state of this exciting topic in classical thermoTRP biology, focusing on the structure–function data for TRP channels regulation by different lipid mediators such as lipid metabolites, phosphoinositides and components of lipid rafts. Novel thermosensory channels (TRPM2, TRPM3 and TRPM5) have been excluded because of yet limited data.

## 2. Lipid metabolites as modulators of TRP channel function

Original efforts to deorphanize TRPV1 channels [63] suggested that endocannabinoids, such as anandamide, were TRPV1 agonists [35]. Later, N-arachidonoyl-dopamine (NADA) and the N-oleyl-dopamine (OLDA) [38,60] were also determined to be TRPV1 agonists. Lipidergic endovanilloids may bind to the intracellular receptor side like capsaicin but compelling evidence is yet lacking. Similarly, nonpsychoactive cannabinoids such as cannabidiol and cannabitol have been described as TRPV2 agonists [64]. These results suggested that lipid metabolites may be critical modulators of TRP channel function.

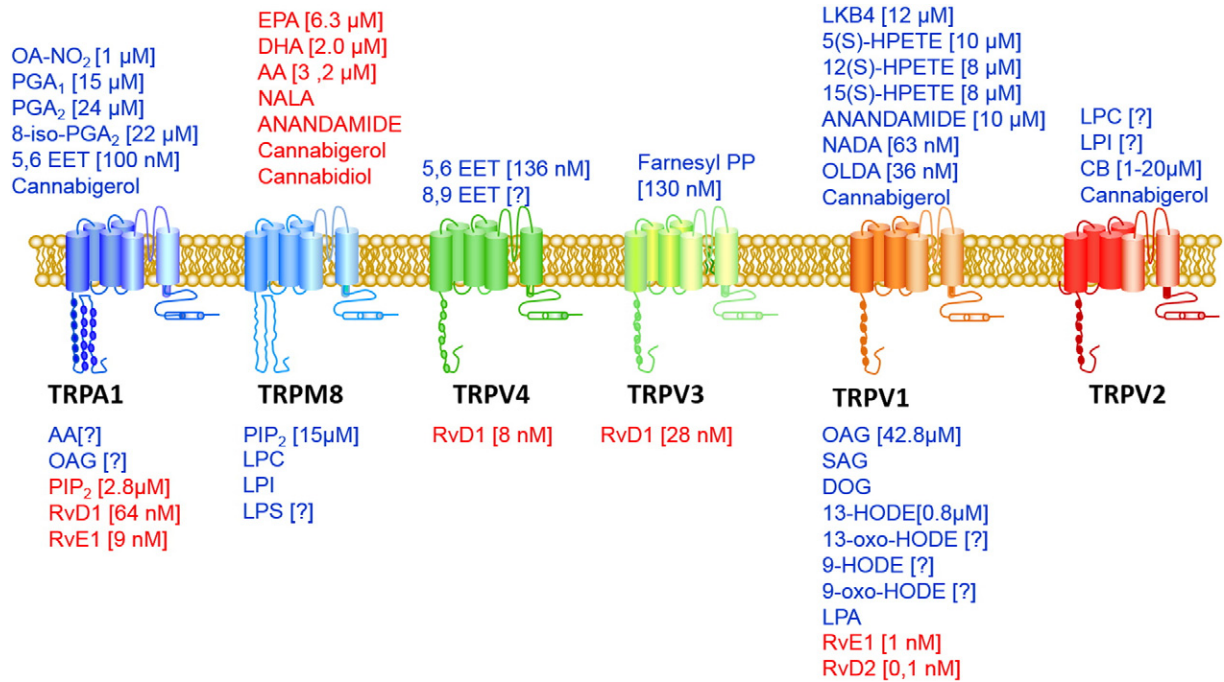
Lipids from cell membranes can be metabolized by Phospholipase  $A_2$  (PLA<sub>2</sub>) which release free polyunsaturated fatty acids (PUFAs) and lysophospholipids (LPLs) or by PLC that produce Diacylglycerol (DAG) and Inositol trisphosphate (IP<sub>3</sub>) (Fig. 2). PUFAs can be additionally metabolized by COX, LOX and CYP enzymes given a plethora of lipids molecules able to modulate thermoTRP channels activity [65]. LPLs produced by PLA<sub>2</sub> are also able to regulate the activity of TRP channels. For instance, LPA, increased upon tissue injury, activates TRPV1 through a direct mechanism by apparently interacting with the channel C terminus [46], indicating a key role of this lipid in pain transduction.

Other LPLs that have been shown to modulate TRP channels are lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), and lysophosphatidylserine (LPS) that alter the thermal sensitivity of TRPM8, raising the temperature threshold toward normal body temperature. This positive modulation by LPLs provides a potential physiological mechanism for sensitizing and activating TRPM8 in the absence of temperature variations [56,66]. However, direct binding of these lipidergic metabolites to TRPM8 has not yet been demonstrated; and, LPLs may just affect the plasma membrane physico-chemical properties and thus indirectly influence channel gating.

LPC and LPI are also able to induce calcium influx via TRPV2 channel [67]. This activation, which involves Gq/Go-protein and phosphatidylinositol-3,4 kinase (PI3,4K) signaling, appears to be mainly due to TRPV2 translocation to the plasma membrane and is dependent on the length of the side-chain and the nature of the lysophospholipid head-group. A potential pathological role of TRPV2 activation by LPC and LPI has been suggested in prostate cancer as it increases cell migration of the prostate cancer cell line PC3 [67].

Diacylglycerol is a signaling molecule generated by Gq-coupled GPCR receptors. DAG activates TRPV1 in a membrane-delimited manner [59]. Multiple DAG analogues, 1-oleoyl-2-acetyl-sn-glycerol (OAG), 1-Stearyl-2-arachinonyl-sn-glycerol (SAG), and 1,2-dioctanoyl-sn-glycerol (DOG) have this capability but with different potencies [59]. This activation is likely due to direct binding of the lipid to the channel because DAG downstream signaling such as DAG lipase and TRPV1 phosphorylation by DAG-activated protein kinase is not involved. More relevant, a TRPV1 mutant channel unable to bind capsaicin does not react with DAG. Diacylglycerol is also a TRPA1 activator and may contribute to the downstream mechanism of GPCR-induced pain [48].

Endogenous TRPV1 lipid ligands also include LOX metabolites such as 12-(S)-hydroperoxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid 12-(S)-HPETE, 15-(S)-HPETE and leukotriene B<sub>4</sub> (LKB<sub>4</sub>) [36]. The signaling leading to LOX metabolites includes bradykinin (BK) stimulation of the B2 receptor and PLA2 activation [68]. BK-induced excitation of sensory neurons via TRPV1 has been proposed to involve mobilization



**Fig. 1.** Lipidergic metabolites that activate (blue) or inhibit (red) TRP channels. References: [48] for arachidonic acid and OAG (TRPA1); [49] for PGA1, PGA2 (TRPA1); [50] for nitrooleic acid (TRPA1); [51,52] for PIP2 (TRPA1); [45] for 5,6-EET (TRPA1); [53] for Resolvin D1 (TRPA1, TRPV3 and TRPV4); [47] for Resolvin D1, D2 and E1 (TRPA1, TRPV1); [54] for nitrooleic acid OA-NO<sub>2</sub> (TRPA1, TRPV1); [55] for PIP2 (TRPM8); [56] for LPC, LPI, LPS, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) (TRPM8); [57] for 5,6-EET and 8,9-EET epoxyeicosatrienoic acids (TRPV4, TRPA1); [58] for FPP (TRPV3); [36] for 12(S)-HPETE, 15(S)-HPETE, 5(S)-HETE and leukotriene B4 (TRPV1); [35,36] for anandamide (TRPV1); [59] for OAG (1-oleoyl-2-acetyl-sn-glycerol), SAG (1-Stearyl-2-arachinonyl-sn-glycerol), and DOG (1,2-dioctanoyl-sn-glycerol) (TRPV1); [42,43] for 13-HODE, 13-oxo-HODE, 9-HODE and 9-oxo-HODE (TRPV1); [60] for NADA and OLDA (TRPV1); [59] for OAG, (TRPV1); [61] for LPC (TRPV2); [62] for cannabinoids CB (TRPV2).

of arachidonic acid by PLA<sub>2</sub> and generation of 12-(S)HPETE [36,68]. LOX also produces 9-HODE and 13-HODE from linoleic acid [42,43]. HODEs selectively act on TRPV1. This cascade seems to be initiated by noxious heat and contributes to heat sensitivity. Since HODEs are released during cell injury, their production could represent a mechanism for inflammatory hyperalgesia and mechanical allodynia [42,43].

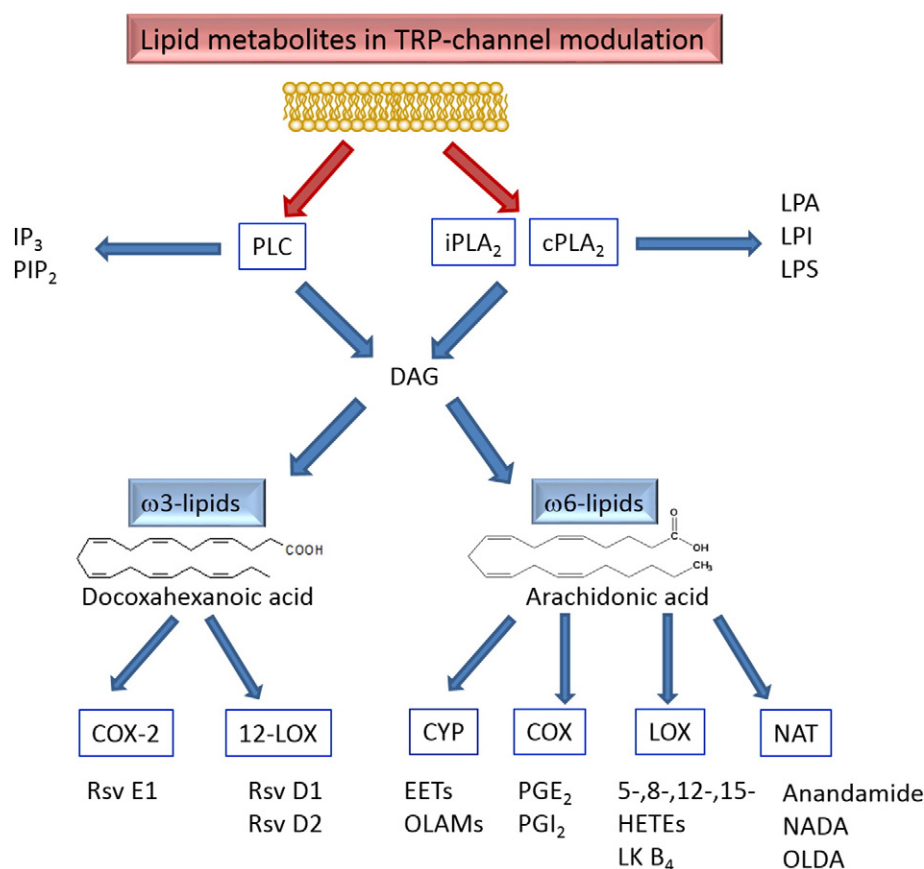
In addition to LOX metabolites, pro-inflammatory lipidergic mediators that affect TRPV1 activity include prostaglandins [69]. Their effect is mediated through different intracellular pathways, which culminate in the phosphorylation of the channel through PKC or PKA [70]. Furthermore, prostaglandins like PGA<sub>1</sub>, PGA<sub>2</sub>, 8-iso-PGA<sub>2</sub>, 15-deoxy-PGJ<sub>2</sub> and D12-PGJ<sub>2</sub>, formed by non-enzymatic dehydration of the respective PGs (PGD<sub>2</sub>, PGE<sub>2</sub> and PGE<sub>1</sub>), are also TRPA1 ligands and directly gate the channel to cause acute nociception [50,71,72]. Based on the recent evidence that TRPA1 antagonists alleviate visceral nociception, the blockade of cyclopentone prostaglandin formation may represent a novel avenue for therapeutic intervention in inflammatory visceral pain [72]. Other prostaglandins like PGE2 activate GPCRs leading to TRPV4 channel phosphorylation and activation [73].

CYP-derived endogenous signaling lipids, the epoxyeicosatrienoic acids (EETs) and epoxyderivatives of arachidonic acid, have also been identified to interact with thermosensory TRP channels. Lipids 5,6-EET and to a lesser extent 8,9-EET have been identified to directly activate TRPV4 in vascular endothelial cells, promoting vasorelaxation [74]. Moreover, at high concentrations (>10 μM), 5,6-EET can activate TRPV4 in colonic afferents where it may cause visceral hyperalgesia [75,76]. However, in lumbal DRG L4 and L5 neurons, 5,6-EET causes Ca<sup>2+</sup> transients independently of TRPV4 and acts as an endogenous TRPA1 activator. 5,6-EET is synthesized in dorsal root ganglia and the dorsal horn of the spinal cord during acute pain and causes mechanical hypersensitivity by activating TRPA1 on primary afferent terminals in the L4–L5 section of the dorsal horn [45]. Also, 8,9-EET is able to sensitize AITC-induced TRPA1 responses in DRG-neurons through still unknown mechanisms [45].

Nitrate fatty acids such as 9-nitrooleic acid (OA-NO<sub>2</sub>) that are generated during inflammation by phospholipids and nitric oxide (NO) are other endogenous lipidergic TRPA1 and TRPV1 activators [54]. The effect of OA-NO<sub>2</sub> on afferent neurons is initially excitatory; however, prolonged exposure to OA-NO<sub>2</sub> may desensitize the TRPV1 and TRPA1 channels and, in turn, suppress nociceptive and inflammatory responses [54]. It has been shown that subcutaneous injection of OA-NO<sub>2</sub> into a rat hindpaw induced delayed and prolonged nociceptive behavior. These results raise the possibility that OA-NO<sub>2</sub> might be useful clinically to reduce neurogenic inflammation and certain types of painful sensations by desensitizing TRPA1 expressed in nociceptive afferents [77].

It is interesting that resolvins, the most potent endogenous lipid ligands described thus far, selectively inhibit the TRPV1 and TRPA1 gating. RvD2 and RvE1 inhibit the capsaicin-activated currents at IC50 values of 0.1 nM and 1 nM concentrations, respectively [47], and AITC-activated currents with IC50 values of 2 nM and 9 nM, respectively. These results suggest distinct roles of resolvins in regulating TRP channels and identify RvD2 as a very potent endogenous inhibitor of inflammatory pain [47].

Another lipid that modulates thermosensory channels is the cholesterol intermediate lipid farnesyl pyrophosphate (FPP). This lipid is the sole known endogenous TRPV3 activator and made its effect via direct channel interaction. FPP has a strong potency and it produces a hyperalgesic effect *in vivo* [58]. FPP synthase is inhibited by nitrogen containing biphosphonates, which proved to be useful in certain types of bone cancer and neuropathic pain [80]. In contrast, RvD1 inhibits peripherally expressed TRPV3 leading to a modality-specific antinociception. Thus, stimulating the endogenous production of RvD1 or the extraneous administration of it and even its possible synthetic analogues might help reverse TRPV3-mediated pain states [53]. Since TRPV3 has been implicated in inflammatory pain [58] and skin disorders [81], manipulation of TRPV3 activity by FPP synthase blockers or RvD1 may be exploited for therapeutic purposes. Taken together, TRP channels have broad spectrum of chemoceptive sensitivity unlike the canonical ligand gated ion



**Fig. 2.** Lipid metabolites implicated in TRP channel modulation. Abbreviations: Phospholipase C (PLC), phospholipase A (PLA<sub>2</sub>), iPLA<sub>2</sub>: Ca<sup>2+</sup> insensitive, cPLA<sub>2</sub>: cytosolic, cytochrome P450 (CYP), cyclooxygenases (COX), lipoxygenases (LOX), HETEs: hydroxyeicosatetraenoic acids, EETs: epoxyeicosatrienoic acids, OLAMs: oxidized linoleic acid metabolites, leukotriene (LK), resolvin (Rsv) prostaglandins (PG), N-arachidonoyldopamine (NADA), N-oleoyldopamine (OLDA).

channels. This feature of TRP channels makes them the key transducer molecules to signal a wide range of stimuli from physiological to noxious stimuli.

### 3. Membrane micro-domains as modulators of TRP channels

One of the most studied membrane micro-domains are lipid rafts which are domains rich in cholesterol, sphingomyelin and gangliosides that play an important role modulating the activity of ion channels as the nicotinic acetylcholine channel [82–86]. A similar central role has been reported for TRPM8 channels [29], whose activity is critically regulated by lipid raft association. Menthol- and cold-mediated responses of TRPM8 were potentiated when the association of the channel with lipid rafts was prevented. In addition, lipid raft disruption shifted the threshold for TRPM8 activation to a warmer temperature [29].

Intriguingly, several studies conducted with TRPV1 have provided controversial results. Depletion of cholesterol, upon incubation with methyl β-cyclodextrin (MCD), did not change heat activation currents in TRPV1 expressed in *Xenopus laevis* oocytes, while in dorsal root ganglion neurons the amplitude of capsaicin-activated currents was significantly reduced [87]. In contrast, it was reported that <sup>3</sup>(H)RTX binding to TRPV1 receptors was not modulated by cholesterol depletion in rat C6 glioma cells [88]. However, disruption of lipid rafts by depleting any of its cardinal constituents, namely cholesterol, sphingomyelin or gangliosides by pharmacological tools blocked TRPV1 gating by various agonists [89]. Inhibition of Ca<sup>2+</sup> responses occurred when cholesterol was depleted by MCD [87,90], or when extracellular sphingomyelin molecules were broken down by sphingomyelinase [91], and also when the cells were incubated with D-PDMP or myricin which reduce the biosynthesis of gangliosides by inhibiting ceramide glucosylation

[92]. However, capsaicin-induced TRPV1 single channel currents in HEK293-transfected cells were not changed after cholesterol depletion, but diminished with cholesterol addition. R579D and F758Q substitutions in the S5 helix of TRPV1 reduced this cholesterol inhibitory effect [93]. Thus, the role of hydrophobic interactions in drug/agonist binding to the TRP channel/lipid raft interface might be more important in drug action than it has previously been taken into account [29,89,94].

Apart from lipid rafts, the influence of other membrane micro-domains has not been described. Similarly, whether lipidic metabolites exert part of their modulating TRP activity by creating such micro-domains in the membrane is yet elusive, but very exciting notion.

### 4. Modulation of TRP channel function by phosphoinositides

The most studied lipid modulator of TRP channel activity is phosphoinositides, which appear to be a master regulator of this family of ion channels. The regulatory role of phosphoinositides on ion channels was first demonstrated by the PI(4,5)P<sub>2</sub> requirement of the cardiac K<sub>ATP</sub> channels [95]. Subsequent publications indicated that members of inwardly-rectifying and voltage-gated K<sup>+</sup> and voltage-gated Ca<sup>2+</sup> channel families were also regulated by PI(4,5)P<sub>2</sub> (see [19,96]). TRP channels are also subjected to phosphoinositide regulation. This regulation could be by direct binding or by indirect mechanisms involving interacting proteins whose function depends on PIP<sub>2</sub> binding. A good example of that is the Erzin-PIP<sub>2</sub> interaction that strengthens actin binding to the cellular membrane and modulates cell adhesion and morphology [97]. Similarly, it has been proposed that the membrane protein Pirt can modulate TRPV1 activity through a PIP<sub>2</sub>-dependent mechanism [52]. This mode of regulation has not been intensively

studied. Therefore, it is plausible that future studies unveil new indirect PIP2-mediated modulators of TRP channels.

The direct effect of PI(4,5)P2 on channel activity has been well characterized for TRPM8. Menthol-evoked currents in TRPM8 inside-out patch clamp recordings run down as consequence of the phosphatases that eliminate PI(4,5)P2. Similarly, sequestering membrane PIP2 with polylysins or anti-PI(4,5)P2 antibody inactivates the channel [55, 98]. In cells, the inactivation of TRPM8 by the rapamycin-induced 5' phosphatase or with *Ciona intestinalis* Voltage Sensitive Phosphatase (ciVSP) further support that membrane PIP2 depletion inactivates TRPM8 [99,100]. Conversely, recovering PI(4,5)P2 levels with the short acyl chain counterpart diC8-PIP2 or by stimulating the conversion of PI(4)P into PI(4,5)P2 with MgATP, recovers the activity of the cold receptor in excised patches [55,98,100]. In terms of specificity the finding that TRPM8 activity in excised patches or in artificial bilayers is not supported by PI(4)P, PI(3,5)P2 or PI(3,4,5) underscores a strict requirement for PI(4,5)P2 [101,102]. The requirement of PIP2 for channels activity has also been reported for TRPM4, TRPM5, TRPM6, TRPM7 and TRPV5 [55,103–107].

The scenario reported for TRPV1 is markedly different. In this case the hydrolysis of PI(4,5)P2 favors channel function by relieving tonic channel inhibition or by the action of the generated second messengers [24,108,109]. However, recent findings on TRPV1 underscore that besides its inhibitory role, PI(4,5)P2 is also a necessary cofactor for channel activation. PI(4,5)P2 and PI(4)P enhance the activity of TRPV1 [110–113] and its depletion decrease channel activity [25,111,114]. Furthermore, rundown of PIP2 is determinant for desensitization upon prolonged exposure to an activating stimulus [25,110,115]. At variance with TRPM8, TRPV1 channel activity can be supported, although at a lower extent, by PI(4)P, PI(3,4)P2 and PIP(3,4,5)P3 and other phospholipids such as phosphatidylglycerol [26,110]. Negative charged lipids such as oleoyl-CoA or 1,2-dioleoyl-*sn*-glycero-3-((N-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl) (DGS-NTA) can also hold TRPV1 function as well, implying a low selectivity of TRPV1 in phosphoinositide binding [26]. Noteworthy, the dependence of the TRP channel function on specific phosphoinositides offers new layers of regulation. First, it imposes a temporal constrain to the channel response such that a sustained signal can be easily terminated. And, second, the different content on phosphoinositides of the subcellular membranes spatially restricts the channel activity to the specific membranes or membrane regions [96].

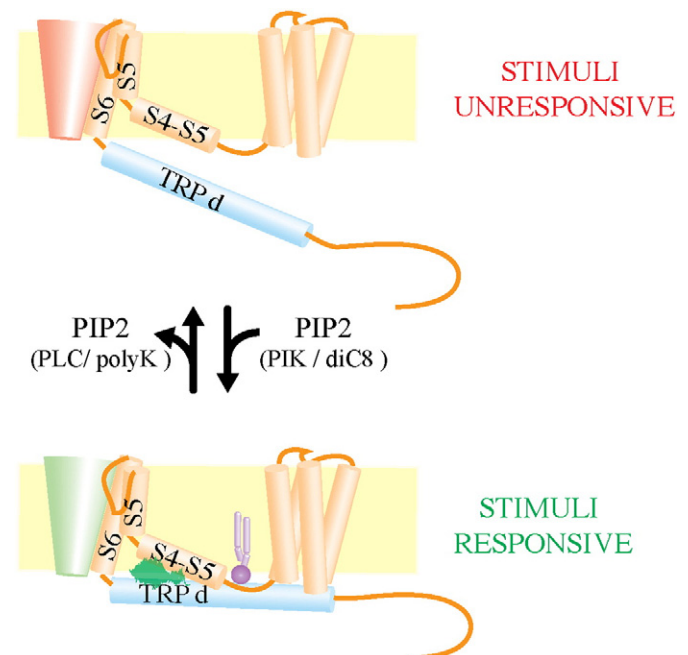
Virtually any stretch of positive residues can interact with PI(4,5)P2. Several of such sequences can be found in almost all TRP channels but not all of them mediate PIP2 regulation. Functionally relevant binding sites have been characterized by combining molecular biology techniques such as site directed mutagenesis with patch clamp recordings. In TRPM8, positively charged amino acids in the TRPbox, a 6-mer conserved domain in the C-terminus near the internal channel gate, mediate PIP2 effect. When K995, R998 and R1008 are mutated, the sensitivity for PI(4,5)P2 decreases accompanied with channel blockade by PIP2 depletion [55]. Mutations in the N-end of the C-terminus, the so-called TRP domain, also affect the sensitivity for PIP2 in TRPM5, TRPV1 and TRPV5 [55,116,117]. However, they have little effect, if any, on TRPM4 suggesting the existence of other PIP2 binding sites for channel modulation. Indeed, TRPM4–PIP2 interacting region is located in the PH-like domain at the distal C-terminus of the channel [103].

A group of positive residues downstream the TRPbox of TRPV1 and TRPV2 has been shown to interact with PIP2, implying that it may be shared by other TRP channels and serve as a general regulatory region in this family of ion channels [112]. It is also worth mentioning that not all PIP2 binding sites reside in the C-terminus of the channel. A case in point is TRPV4 who has the PIP2 binding site in the cytosolic N-terminus. It has been proposed that PIP2 binding in TRPV4 favors the conformational rearrangements that lead to the activation of the channel by hypotonicity and heat [118].

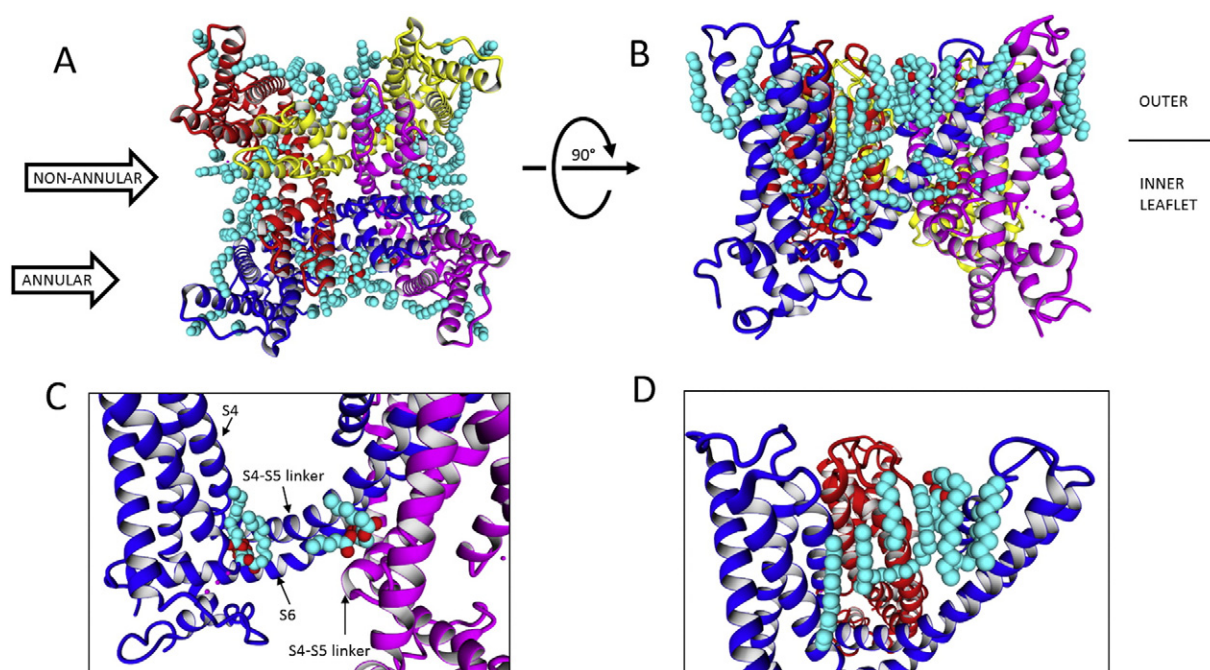
Structure–function studies pinpoint to the TRP domain as a pivotal region for channel gating and PIP2 modulation as it contains residues central for PIP2 binding [119–122]. The functional relevance of this domain has been further substantiated by its conformation in the proposed high resolution TRPV1 structural model [11]. Recent studies have further demonstrated the importance of the TRP domain on allosteric channel activation. Mutations targeting W697 in the TRP box of TRPV1 resulted in a complete loss of voltage sensitivity while retaining capsaicin response [121]. Interestingly, the W697 is interacting with S4–S5 loop considered central for voltage sensing [11,121]. In TRPM8, mutations Y981E and Y981K destabilized the gate resulting in constitutive channel opening [122]. Interestingly, subsequent mutations on the S6-TRP box linker restored the regulated activity mainly by re-establishing coupling of stimuli sensing and pore opening. Mutations in the TRP domain with greater functional impact reside at the predicted interaction interface between the TRP helix and the loop S4–S5 [122], suggesting that the TRP helix/S4–S5 loop interaction is central for transmitting different stimuli to the gate. Remarkably, these regions have also intense PIP2 contacts with residues in the S4–S5 loop such as those suggested for TRPV1 [116,117]. Thus, PIP2 binding can strengthen the TRP helix/S4–S5 loop interaction favoring coupling of the putative voltage sensor and the channel pore. Noteworthy, S4–S5 loop/PIP2 contacts have been shown to control the pore opening in Kv channels [123]. Taking together, it is tempting to propose that by tethering the TRP domain to the membrane, PIP2 promotes and strengthens the TRP helix/S4–S5 loop interaction setting the structural framework for the allosteric regulation of channel gating (Fig. 3).

## 5. TRP channels contain putative lipid binding sites

A central question arises: are there binding sites for lipids in TRP channels? Can the channel structure reveal specific lipid docking sites? Although it has been reported that lipids modulated the channel activity of TRP channels, there is no information regarding the presence of lipid binding sites in the receptor. This limitation likely arises from



**Fig. 3.** Possible action of PIP2 on TRP channels. PIP2 binding close to the channel pore allows the interactions of the S4–S5 linker with the TRP domain. Variations of PIP2 levels regulate channels responsiveness by uncoupling the sensing domains from the channel gate. The drawing represents a single subunit of the tetramer. The pivotal regions for stimuli coupling are named in the picture. The S4–S5 loop (S4–S5)/TRP domain (TRPd) interactions are represented by a green cloud and the PIP2 moiety is colored in purple.



**Fig. 4.** TRPV1 in complex with lipids transferred from Kv1.2/Kv2.1 potassium channel. A. Extracellular view of the TRPV1 showing annular and non-annular lipids. Protein chains are represented in ribbon and color blue, red, yellow and magenta. Lipids in ball representation, with carbon and oxygen colored cyan and red, respectively. B. Side view of TRPV1 showing the transmembrane  $\alpha$ -helices and the inner and outer membrane leaflets. C. Detail of non-annular lipid involved in intrasubunit interactions, putatively assigned to PIP2 binding site. D. Detail of other non-annular lipids in the inner and outer membrane leaflets.

scarce high-resolution structural information available on TRP channels, which is restricted to the cytosolic ankyrin domains and peptides from the C-terminus. The Ankyrin-repeat domain (ARD) from the cytoplasmic N-terminus of different TRP channels has been characterized by X-ray crystallography unraveling binding sites for regulatory molecules [124–128]. At variance, obtaining structures for the full-length channel is cumbersome as these proteins are hard to crystallize and the crystals diffract poorly. Alternatively, electron cryo-microscopy structures of full-length TRPV1, TRPV2, TRPV4 and TRPA1 have disclosed the molecular architecture and subunit organization [127,129–131], although at a low resolution (13.6 Å at most). Recently, however, a breakthrough in TRP channel structure was reached. Using single particle electron cryo-microscopy, a 3.4 Å structural model of TRPV1 lacking the distal part of the C-terminus was reported for the closed and open states [11,132]. Unfortunately, none of these structures were determined in complex with lipids.

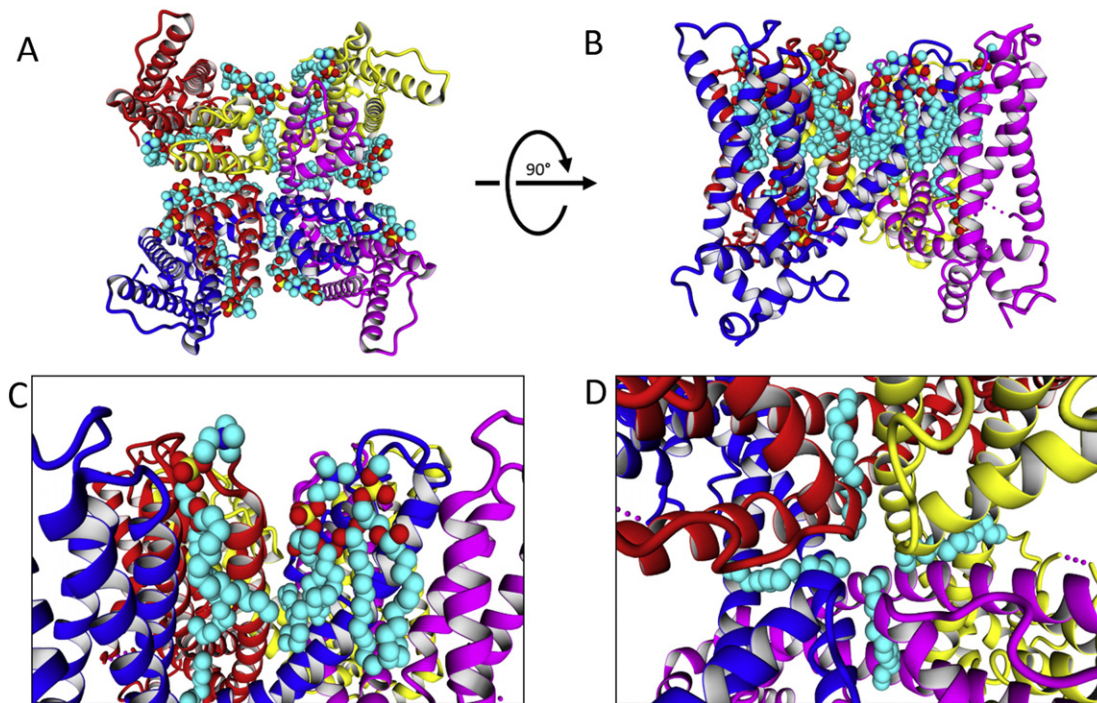
Thus, to uncover putative lipid binding sites on TRP channels, we used the structural information available for other ion channels. Hence, we superposed the TRPV1 EM structural model on representative Kv, Nav and Cav ion channels structures obtained in complex with lipids. Then, the lipids were transferred to the TRPV1 structural model to determine whether the TRP channel exhibited similar binding sites. Small clashes between lipid fragments and TRPV1 residues were removed by molecular steepest descent minimization.

Fig. 4 shows different views of the TRPV1 channel with lipids transferred from the Kv1.2/Kv2.1 (paddle-chimera) Kv channel (code 2R9R) [133]. A top view of TRPV1 in complex with transferred lipids (Fig. 4A) shows a similar arrangement of annular and non-annular lipid around the channel, denser in the outer leaflet (Fig. 4B). Annular lipids fit well on TRPV1 in both leaflets. In the inner leaflet two phospholipidic fragments are in deep contact with the S4–S5 linker (Fig. 4C), a region of the channel that couples voltage-sensor domain and the pore for channel gating [134–136]. TRPV1 conformation is compatible with two phospholipids, one is in deep intrasubunit contacts with the S4–S5 linker (Fig. 4C, left), the TRP domain and the S1–S4 domain. The other interaction is mediated by inter-subunit contacts with S5 and the beginning of S4–S5 linker (Fig. 4C, right). The polar head group of

both phospholipids resides at different depths in the membrane, which has been related to the perturbation that protein exerts on the membrane at lipid–protein interface [133]. This unusual position for a phospholipid is related to specific favorable contacts with the protein, probably influencing its structure and function [137].

PIP2 has been described as a special case since it modulates the activity of many types of ion channels in a variety of modes [31–33], including TRPV1 [116,117]. The current understanding of the structural requirements for PIP2 binding comes from potassium inward rectifying channels. The crystal structure of Kir2.2 shows that the phosphoinositide binds at the interface between the transmembrane domain and the C-terminal domain. A closer look at the binding site discloses hints for PIP2 interaction: the acyl chains are accommodated by hydrophobic surfaces of the transmembrane region and the negative charged phosphate interact with positively R and K residues in tether helix [138]. Brauchi et al. [116] hypothesized that the polar head group of PIP2 could contact with the TRP domain through basic residues (R701 and K710), and the acyl chain could mediate the interaction between two adjacent subunits. In the model, the location of the PIP2 head group is compatible with the interaction with R701 (and probably with K710) in the TRP domain, and with R575 in the S4–S5 linker.

Fig. 5 depicts TRPV1 channel in complex with lipids transferred from a Cav ion channel (code 4MS2). Non-annular lipids also fit well on TRPV1 intersubunit spaces, as observed for lipids transferred from Kv channels (Fig. 4). A detailed view of the outer leaflet indicates that non-annular lipids occupy three kinds of intersubunit grooves, including regions between S1–S4 (blue chain) and pore (red chain), inter-pore (red and blue chains), and between pore (blue chain) and the S1–S4 (magenta chain) domains (Fig. 5C). Polar head groups appear to interact with proximal basic residues such as K535, K603, K656, and R534, while acyl chains appear to occupy hydrophobic clefts at the transmembrane level. The most noticeable feature of the TRPV1 in complex with lipids is that TRPV1 conformation is also compatible with lateral pore fenestrations observed for Nav and Cav channels [139–141]. These lateral portals revealed a hydrophobic access pathway to the central cavity (Fig. 5D), which could explain the effect observed for some general and local anesthetics, which has been shown to activate different TRP



**Fig. 5.** TRPV1 in complex with lipids transferred from a Cav channel. A. Extracellular view of the TRPV1 showing lipids derived from the crystal (code 4MS2). Representation similar with Fig. 4. B. Side view of TRPV1 showing the transmembrane  $\alpha$ -helices and the lipids fragments, mainly located in the outer leaflets. C. Detail of annular lipid involved in intersubunit interactions in hydrophobic clefts of three protein subunits (blue, red and magenta). D. Detail of the hydrophobic portals connecting the membrane and the central pore cavity, as observed for closed Nav and Cav channels.

channels [142,143]. The size of the portal cavities in different ion channels has been related to the different-sized drugs or hydrophobic molecules gaining access to the central cavity during different stages of pore gating [140]. In the case of TRPV1, superposition with Cav and Nav channels denoted a shorter cavity ( $4 \times 4 \text{ \AA}$  in TRPV1 vs  $8 \times 10 \text{ \AA}$  in Nav) due to a displacement of the selectivity filter and pore-helix that reduces the portal entrance to the inner cavity. Consequently, this lipid binding site could be restricted in TRPV1 to acyl chain-like molecules rather than to di-acylated molecules such as phospholipids, or other higher size hydrophobic molecules. Furthermore, the portal entering is very close to the vanilloid binding site, as described for capsaicin and resiniferatoxin in TRPV1 [132]. In addition, the intersubunit region connecting S5 from one subunit and the beginning of S4–S5 from the adjacent subunit is compatible with the proposed docking-generated model for cholesterol binding [28], since transference of lipids to TRPV1 locates phospholipids (Fig. 4C) or acyl chains in this position. Taken together, these observations indicate that, at least in TRPV1 channels, there are well-defined binding sites for lipids, suggesting a critical role of these molecules in TRPV1 gating. In addition, the modeled structures indicate that TRPV1 channels may accommodate a variety for lipid molecules, thus augmenting the modulatory mechanisms of channel gating. These observations for TRPV1 may be also extended to other TRP channels, as the transmembrane region is quite conserved within the TRP channel superfamily.

## 6. Outlook

Sensory TRP ion channels are crucial molecular components of pain transduction. Identification of their natural ligands has provided insights into the molecular basis of peripheral pain. Coincidentally, lipid-derived substances are emerging as important biological mediators. The diversity of endogenous lipids as modulators for sensory TRP-channels demonstrates the complexity of lipid signaling in nociceptive processing. Studies on the modulation of TRP channel function by lipidergic endogenous agents have contributed to our understanding

of the interaction between sensory nerves and diseased or damaged tissues, which is central in the generation and maintenance of pain. However, there are still many unsolved issues. Matching clinical pain types with TRP subtypes remains a challenge, particularly outside of TRPV1. For example, the role of TRPV2, a noxious heat receptor, to pain generation remains elusive. Its lipid interaction is also unclear. In addition, better information on the ligand binding site (amino acids) is needed to establish the pharmacological rationale for optimizing synthetic therapeutic agents targeting TRPs. Future studies are needed to systematically identify the physiological role for each lipid group in pain perception by lipidomic approaches.

Unveiling the lipidergic structures that modulate TRP function may allow for novel lead compound synthesis for analgesics aimed at TRPs. As lipids participate in other biological phenomena including inflammation, it is possible to pursue the development of multi-target drugs. Depending on TRP expressing sites and their differential functionalities, other beneficial effects in different disciplines such as dermatology or cardiovascular physiology can be also envisioned. The advantage of using potent lipid modulators of TRPs, such as resolvins, for the treatment of inflammatory pain over conventional NSAIDs is that they can resolve inflammation and pain by restoring cellular/tissue homeostasis using endogenous signaling pathways. Collectively, an expansion of our knowledge on lipidergic ligands and the molecular nature of their interactions with sensory TRP channels will improve our understanding of peripheral pain mechanisms and contribute to clinical intervention for associated diseases.

## Conflict of interest

The authors declare no conflict of interest.

## Transparency document

The [Transparency document](#) associated with this article can be found, in the online version.

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