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Phosphoinositide signaling in somatosensory neurons

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Abstract

Somatosensory neurons of the dorsal root ganglia (DRG) and trigeminal ganglia (TG) are responsible for detecting thermal and tactile stimuli. They are also the primary neurons mediating pain and itch. A large number of cell surface receptors in these neurons couple to phospholipase C (PLC) enzymes leading to the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] and the generation of downstream signaling molecules. These neurons also express many different ion channels, several of which are regulated by phosphoinositides. This review will summarize the knowledge on phosphoinositide signaling in these neurons, with special focus on effects on sensory and other ion channels.

Introduction

The primary somatosensory neurons that mediate temperature and touch sensation as well as detect painful stimuli are dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons. They are pseudounipolar cells; their peripheral processes conduct nerve impulses from the skin or mucous membranes, and the shorter central processes convey information to the central nervous system. The nerve fibers the axons of DRG neurons form are classically categorized by diameter and conduction velocity, to unmyelinated C-fibers with the slowest conduction velocity, associated with temperature and pain detection, myelinated A δ fibers associated with pain and temperature sensation, A β fibers associated with mechanosensation, and A α sensory fibers that convey information from proprioceptors.

Intense mechanical and thermal stimuli evoke pain; this acute nociceptive pain is a major defense mechanism against tissue damage. Pain can become chronic via two general mechanisms: chronic inflammation sensitizes the pain pathway to otherwise non-painful stimuli, and either physical or chemical nerve damage leads to neuropathic pain. Chronic pain is a major unsolved medical problem, and has been a significant driving force behind research on this field (Basbaum et al., 2009). Skin diseases, and exposure of the skin to irritants often induce itch, which is also detected by DRG neurons, and also a significant medical problem (Bautista et al., 2014).

The cell bodies of DRG and TG neurons are relatively easy to isolate, and they survive well in cell culture, thus they are amenable to electrophysiological characterization using the

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patch clamp technique. The peripheral nerve endings, where physiological sensation actually happens, are not accessible for patch clamping. While nerve impulses can be detected that arise from these nerve termini, using for example the skin-nerve preparation (Zimmermann et al., 2009), these composite nerve signals do not convey sufficient information on the contribution of individual ion channel types. Most of our knowledge about ion channels in sensory neurons comes from electrophysiological characterization of cell bodies, and we assume that a similar set of ion channels are found in the nerve termini. This assumption has been confirmed by many genetic knockout studies, where deletion of ion channels described in the cell body, had the expected sensory phenotype, indicating that the ion channel is also present in the peripheral termini.

Sensory neurons express a large number of G-protein coupled receptors (GPCR-s), many of which couple to phospholipase C (PLC) enzymes via G_q proteins (Stone and Molliver, 2009). The best understood primary function of these receptors is sensitization of the sensory system upon inflammation (Linley et al., 2010). This sensitization is responsible for the well-known phenomenon of thermal hyperalgesia and mechanical allodynia, the increased sensitivity to heat, cold and mechanical stimuli during inflammation (Linley et al., 2010). G_q -coupled GPCR-s also play important roles in itch (Liu and Dong, 2015). In addition, PLC enzymes may also be stimulated by Ca^{2+} influx via ion channels (Rohacs et al., 2005).

This review will describe the various ion channels found in sensory DRG and TG neurons, and will focus on how phosphoinositide signaling influences them. We will discuss direct regulation of ion channels by phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], and the consequences of changes in phosphoinositide levels on ion channel activity. We will also summarize the effects of downstream signaling after PLC activation on neuronal ion channels.

DRG and TG neurons are very diverse. There is no single neuron in which all of the ion channels described below would be expressed. Various categorizations of peripheral sensory neurons have been proposed (Basbaum et al., 2009), detailed description of which is beyond the scope of this review. The most recent one of these, based on unbiased principle component analysis of RNA sequencing data from a large number of individual neurons, divided mouse DRG neurons to 11 neuronal sub-populations (Usoskin et al., 2015). The 5 neurofilament positive subpopulations in that study correspond to myelinated low threshold mechanoreceptors (NF1-3) and myelinated proprioceptors (NF4-5), the 3 non-peptidergic neuron populations (NP1-3) correspond to non-myelinated nociceptors and neurons responsible for itch, and the 2 peptidergic subpopulations correspond to non-myelinated (PEP1) and lightly myelinated (PEP2) peptidergic thermosensitive nociceptors. A larger tyrosine hydroxylase positive population (TH) corresponded to non-myelinated low-threshold mechanoreceptors (Li et al., 2011). The vast majority of GPCR-s and sensory ion channels discussed in this review are predominantly expressed in the NP and PEP groups, and to some extent in the TH group described in (Usoskin et al., 2015).

PLC-coupled GPCR signaling in sensory neurons

Cell surface receptors that couple to G_q/G_{11} proteins, activate PLC β enzymes that hydrolyze PI(4,5)P₂ into the two classical second messengers inositol 1,4,5-trisphosphate (IP₃), which releases Ca²⁺ from intracellular stores via binding to the IP₃ receptor (Mikoshiba, 2015) and diacylglycerol (DAG), which activates Protein Kinase C (PKC) (Figure 1). Consistent with the role of this pathway in pain sensitization, nociceptor specific dual deletion of G_q and G_{11} reduced hypersensitivity in both inflammatory and neuropathic pain models (Tappe-Theodor et al., 2012). On the RNA level, the highest expressing PLC β isoform in DRG neurons is PLC β 3. Genetic deletion of this enzyme significantly reduced histamine mediated itch (Han et al., 2006). Ca²⁺ signals induced by histamine in DRG neurons were eliminated in PLC β 3^{-/-} mice, but responses to other agonists, such as bradykinin or UTP were intact, showing the presence and importance of other PLC β isoforms. PLC β isoforms may also localize to the nucleus in brain cortical neurons, where they play distinct roles from their effects at the plasma membrane (Garcia del Cano et al., 2014), but this area of research is largely unexplored in peripheral sensory neurons.

DRG neurons express a variety of GPCR-s that are activated by inflammatory mediators, and many of these receptors couple to G_q and PLC β enzymes (Stone and Molliver, 2009). GPCR-s that activate PLC in DRG neurons include bradykinin B2 receptors, purinergic P2Y receptors, chemokine receptors, prostaglandin receptors (EP1), lysophosphatidic acid (LPA) receptors and proteinase-activated (PAR) receptors. As mentioned earlier, the best-known function of these receptors in DRG neurons is to sensitize the pain pathway. Examples for this sensitization will be discussed throughout this review.

Some of the highest expressing G_q coupled receptors in DRG neurons are the members of the Mas1-Related G protein-coupled receptor (Mrgpr) family (Liu and Dong, 2015). While the endogenous ligand for several of these receptors are not yet known, Mrgpr-s are involved in histamine independent itch (Liu and Dong, 2015). MrgprA3 was shown to be activated by chloroquine, an antimalarial drug that cause itch as a side effect (Liu et al., 2009). It was shown that chloroquine-induced itch involves the activation of TRPA1 channels (Wilson et al., 2011). MrgprD on the other hand was found to be activated by β -alanine, also known to induce itch (Liu et al., 2012). Mrgprd activation by β -alanine was also shown to inhibit KCNQ channels, which could also contribute to increased neuronal excitability (Crozier et al., 2007).

Changes in phosphoinositide levels upon GPCR activation

While stimulation of overexpressed G_q -coupled receptors usually induces clear reduction in phosphoinositide levels (Falkenburger et al., 2010; Borbiri et al., 2015), stimulation of endogenous receptors in different cell types show highly variable responses in decrease of PI(4,5)P₂ (van der Wal et al., 2001). To generate a clear IP₃-induced Ca²⁺ signal, hydrolysis of a relatively small fraction of PI(4,5)P₂ is sufficient and PI(4,5)P₂ levels do not necessarily have to decrease substantially during receptor stimulation. Surprisingly little is known on phosphoinositide dynamics in response to stimulating endogenous G_q coupled receptors in peripheral somatosensory neurons. It was proposed that bradykinin, while clearly activates

PLC, does not decrease PI(4,5)P₂ levels in rat DRG neurons (Liu et al., 2010). The authors found that bradykinin induced the translocation of the GFP-tagged PLCδ1-PH domain from the plasma membrane to the cytoplasm, a phenomenon often interpreted as depletion of PI(4,5)P₂. The PLCδ1-PH domain binds to PI(4,5)P₂ with high affinity and specificity, but it also binds IP₃ the product of the activation of PLC (Hirose et al., 1999). Thus it is debated how much PI(4,5)P₂ depletion and displacement by IP₃ contributes to the translocation of this construct (van der Wal et al., 2001; Xu et al., 2003; Szentpetery et al., 2009). When using the GFP-tagged phosphoinositide binding domain of the tubby protein, which does not bind IP₃, (Quinn et al., 2008) bradykinin did not induce significant translocation (Liu et al., 2010), indicating that bradykinin may not induce a substantial decrease in PI(4,5)P₂ levels. An earlier report found that stimulation of Protease activated PAR-2 receptors induce the translocation of PLCδ1-PH domain from the plasma membrane to the cytoplasm, indicating PLC activation and potential decrease in PI(4,5)P₂ levels (Linley et al., 2008).

In our work, we found that using the same tubby-GFP construct, bradykinin evoked a small but significant decrease in PI(4,5)P₂ levels in mouse DRG neurons (Lukacs et al., 2013b). This decrease was markedly smaller than that induced by capsaicin, which activates a Ca²⁺ sensitive PLC, most likely a PLCδ isoform by stimulating Ca²⁺ influx via TRPV1 channels, see next paragraph. Bradykinin, unlike capsaicin, did not induce any decrease in PI(4)P levels, as detected by a fluorescence based PI(4)P sensor (Lukacs et al., 2013b). While there is some quantitative discrepancy between the two papers, they agree that bradykinin activated PLC, but it only induced a small (or no) decrease in PI(4,5)P₂ levels (Liu et al., 2010; Lukacs et al., 2013b). Even though DRG neurons express a large number of G_q coupled receptors, to our knowledge, the effects of other endogenous receptors on phosphoinositide dynamics have not been tested so far.

Activation of PLC by Ca²⁺ influx

As mentioned before activation of TRPV1 channels by capsaicin in DRG neurons lead to a robust depletion of PI(4,5)P₂ and PI(4)P in a Ca²⁺ influx dependent manner (Lukacs et al., 2013b). Capsaicin also induced the formation of IP₃, showing that the mechanism is PLC activation (Lukacs et al., 2007). As we will discuss later, PLC-mediated phosphoinositide depletion plays an important role in Ca²⁺-dependent desensitization of TRPV1 (Lukacs et al., 2013b). Ca²⁺ influx via TRPM8 was also shown to deplete PI(4,5)P₂ (Rohacs et al., 2005; Daniels et al., 2009) in a Ca²⁺ dependent manner, accompanied by the formation of IP₃ (Yudin et al., 2011). Similar to TRPV1, Ca²⁺ -induced PLC activation plays a role desensitization of TRPM8 (Yudin and Rohacs, 2011), see also later. Depolarization of DRG neurons by 20 mM potassium chloride also induced depletion of PI(4,5)P₂ and PI(4)P in DRG neurons, even though to a lesser extent than capsaicin (Lukacs et al., 2013b). The Ca²⁺ signal evoked by potassium chloride was also smaller than that induced by capsaicin, when measured with a low affinity Ca²⁺ sensitive dye (Lukacs et al., 2013b).

There are 13 isoforms of PLC in the human genome (Fukami et al., 2010). GPCRs activate PLCβ1-4 via Gα_q or Gβγ, and Receptor Tyrosine Kinases activate PLCγ1 and PLCγ2. While all PLC isoforms require some Ca²⁺ for activity, PLCδ-s, but not PLCβs or PLCγs, were shown to be activated by Ca²⁺ alone (Allen et al., 1997). The more recently discovered

PLC η 1 and PLC η 2 (Cockcroft, 2006), as well as the sperm specific PLC ζ are also highly Ca²⁺ sensitive, but these isoforms are not expressed in DRG neurons (Lukacs et al., 2013b; Thakur et al., 2014). PLC ϵ RNA can be detected in whole DRG-s, but it is essentially not expressed in DRG neurons (Thakur et al., 2014), thus the most likely isoforms to be activated by Ca²⁺ influx in DRG neurons are PLC δ s. PLC δ 4 is expressed several fold higher in DRG neurons than the other two PLC δ isoforms PLC δ 3 and PLC δ 1 (Lukacs et al., 2007; Hammer et al., 2010; Gerhold et al., 2013; Thakur et al., 2014). Accordingly, capsaicin-induced desensitization of TRPV1 was reduced in PLC δ 4^{-/-} mice, but the effect was relatively moderate, pointing to the possible contribution of other PLC(δ) isoforms to phosphoinositide depletion.

As mentioned earlier, activation of TRPV1 by capsaicin induced a much larger depletion of phosphoinositides in DRG neurons than stimulation of bradykinin receptors. Bradykinin receptor expression in adult mouse DRG neurons is quite low, while TRPV1 expresses at quite high levels (Thakur et al., 2014) thus it can be argued that the difference is due to low expression levels of the GPCR. Interestingly however, TRPV1 activation was also more efficient in phosphoinositide depletion than GPCR activation when TRPV1 was co-expressed with muscarinic M1 receptors and phosphoinositide sensors in HEK293 cells (Borbiro et al., 2015). The difference between the two stimuli were less pronounced than in DRG neurons; capsaicin and carbachol evoked comparable decreases in PI(4,5)P₂ even though the effect of capsaicin was faster and somewhat more complete. Carbachol on the other hand induced a much smaller decrease in PI(4)P levels than capsaicin (Borbiro et al., 2015). It is not clear why Ca²⁺-induced PLC δ activation is more efficient than PLC β activation in reducing PI(4)P levels, as most PLC isoforms prefer PI(4,5)P₂ over PI(4)P, some possible scenarios are discussed in (Lukacs et al., 2013b).

Phosphoinositides, especially PI(4,5)P₂ are required for the activity of many different ion channels (Suh and Hille, 2008; Logothetis et al., 2015). Thus the robust depletion of phosphoinositides induced by TRPV1 activation is likely to influence the activity of other ion channels. Accordingly, capsaicin has been shown to inhibit several different ion channels in DRG neurons, and for inhibition of KCNQ channels (Zhang et al., 2011) and for inhibition of TRPA1 (Akopian et al., 2007) inclusion of PI(4,5)P₂ in the whole cell patch pipette alleviated capsaicin-induced inhibition. For inhibition of Voltage-gated Ca²⁺ channels (Wu et al., 2006), P2X channels (Stanchev et al., 2009), HCN channels (Kwak, 2012) and for voltage gated Na⁺ channels (Liu et al., 2001), the role of phosphoinositides has not been tested, see details at the discussion of the individual channels. Local capsaicin evokes intense burning pain, but paradoxically, it is also used as a local analgesic, because of the desensitization of the nociceptor neurons, which includes many processes, including desensitization of the channel itself, and potentially inhibition of some of the other ion channels described here (Szallasi and Blumberg, 1999).

Downstream signaling – Protein Kinase C (PKC)

Protein Kinase C enzymes are classical serine threonine kinases with 10 family members in mammals (Newton, 2010). Most of them are activated downstream of PLC by DAG and conventional isoforms are also stimulated by Ca²⁺. Various PKC isoforms have been shown

to be involved in pain sensitization (Velazquez et al., 2007). PKC ϵ received the most attention; it has been shown to be involved in thermal hyperalgesia mediated by bradykinin-induced sensitization of the heat and capsaicin sensitive TRPV1 channels (Cesare et al., 1999). PKC ϵ deficient mice have reduced thermal and mechanical hyperalgesia induced by epinephrine, and a PKC ϵ selective inhibitory peptide reduced both enhancement of TTX resistant Na⁺ currents and carrageenan-induced hyperalgesia in rats (Khasar et al., 1999). Other PKC isoforms may also play roles in pain sensitization in a modality specific manner. Deletion of PKC β , PKC γ or PKC δ , but not PKC α , attenuated thermal hyperalgesia induced by complete Freund's adjuvant (CFA) injection (Zhao et al., 2011). Mechanical allodynia induced by CFA, or nociceptive behaviors induced by formalin, on the other hand were not affected by deletion of PKC β , PKC γ or PKC δ , or PKC α (Zhao et al., 2011), pointing to the potential modality dependent involvement of various PKC isoforms in sensitization of DRG neurons.

Ion channels primarily activated by PLC signaling

Activation of PLC does not only lead to Ca²⁺ release from intracellular stores and activation of PKC. Downstream signaling pathways also activate ion channels in the plasma membrane. We briefly discuss three groups of such channels below.

Store-operated orai1 channels

Orai1 channels are Ca²⁺-selective ion channels activated by the IP₃-induced depletion of the Ca²⁺ stores in the endoplasmic reticulum (ER) that occurs during PLC activation (Putney, 2007). Loss-of-function mutations of orai1 cause severe immunodeficiency in humans by interfering with lymphocyte function (Feske et al., 2006). Orai1 is activated by the ER membrane resident STIM1 protein that clusters in response to Ca²⁺ depletion and activates orai1 by direct interaction via its CAD domain (Park et al., 2009). Both orai1 and STIM1 are expressed in DRG neurons and store operated Ca²⁺ currents could be detected in these cells (Gemes et al., 2011). Furthermore store operated Ca²⁺ entry was increased in an axonal injury model (Gemes et al., 2011). These measurements were performed in isolated cell bodies of DRG neurons, where the ER is clearly present. It has also been shown that the nerve terminals of free nerve endings in the skin contain axonal reticulum, the equivalent of the ER, making it possible that store operated Ca²⁺ channels function not only in the cell bodies, but also at the nerve terminals (Kruger et al., 2003). Interestingly, heat over 35 C° has been shown to induce clustering of STIM1 and activation of store operated Ca²⁺ entry (Xiao et al., 2011). While the role of store operated orai1 channels in peripheral sensory functions is far from being elucidated, it was shown that a store-operated channel blocker had significant analgesic effects; but this may have been due to inhibiting orai1 channels in the dorsal horn of the spinal cord (Xia et al., 2014).

Store operated Ca²⁺ entry and orai1 function is positively influenced by phosphoinositides in various ways. The poly-basic region of STIM1 may interact with phosphoinositides and it was shown that even though phosphoinositides are not essential for, they contribute to STIM1 accumulation at ER-PM junctions (Walsh et al., 2010). Another study found that depletion of both PI(4,5)P₂ and PI(4)P using PI4K inhibitors, but not selective depletion of

PI(4,5)P₂ using an inducible 5-phosphatase, inhibited store operated Ca²⁺ entry (Korzeniowski et al., 2009). This topic was reviewed recently in more detail (Cao et al., 2015).

TRPC-channels

All TRPC channels are activated downstream of PLC (Putney, 2004). TRPC3, TRPC6 and TRPC7 are directly activated by DAG, an effect independent of PKC, but the activation mechanism of the rest of the family has not been fully elucidated despite almost 20 years of intensive research (Putney and Tomita, 2011; Rohacs, 2013). TRPC channels are ubiquitously expressed, with most cell types having multiple isoforms. DRG neurons are not exception, several TRPC family members are present in them, with TRPC3 RNA expressing at especially high levels (Gerhold et al., 2013; Vandewauw et al., 2013; Thakur et al., 2014). Accordingly, application of oleylacylglycerol (OAG), a DAG analogue induces clear Ca²⁺ signals in DRG neurons (Kress et al., 2008). TRPC3 has been proposed to mediate Ca²⁺ signals and ionic currents evoked by IgG immune complexes in rat DRG neurons (Qu et al., 2012). Also, knockdown of TRPC3 has been shown to inhibit Ca²⁺ signals evoked by UTP or activation of proteinase activated receptors (Alkhani et al., 2014). While the role of PLC-activated TRPC currents in DRG neurons has not been elucidated yet, it was shown that nerve injury up regulated TRPC4 in rat DRG-s (Wu et al., 2008). Interestingly, another article reported downregulation of TRPC3, 4 and 5 in a nerve injury model in rats (Staaf et al., 2009). Nerve growth factor has been shown to regulate expression level of TRPC3 (Luo et al., 2007). TRPC3 and TRPC6 are also good candidates to be activated by Mrgpr receptors; it was shown for example that in TRPA1 negative DRG neurons, chloroquine-induced Ca²⁺ signals were inhibited by antagonists of TRPC channels (Than et al., 2013).

Two additional functions for TRPC channels have been proposed in DRG neurons, which are independent of PLC-mediated activation. Various TRPC channels have been shown to be mechanically activated, but at least for TRPC1 and for TRPC6 this notion is debated (Gottlieb et al., 2008). TRPC1 was proposed to be a stretch activated ion channel (Maroto et al., 2005), and also to play a role in light touch sensation (Garrison et al., 2012). TRPC6 was also proposed to be activated by membrane stretch (Spasova et al., 2006). Combined deletion of TRPC3 and TRPC6 caused deficits in light touch (Quick et al., 2012), these deficits however were quite modest, especially compared to the essentially complete loss of light touch sensation caused by deletion of piezo2 channels in DRG neurons and skin cells (Ranade et al., 2014).

TRPC5 is expressed in DRG neurons, and was shown to be cold sensitive, and proposed to play a role in sensing noxious cold temperatures (Zimmermann et al., 2011). TRPC5^{-/-} mice however did not show significant behavioral deficits in response to cold, presumably because of compensation by other cold sensitive ion channels (Zimmermann et al., 2011). Most other studies however found very low RNA levels for TRPC5 compared TRPC3 in DRG neurons (Gerhold et al., 2013; Vandewauw et al., 2013; Thakur et al., 2014).

Phosphoinositides regulate TRPC channels in a complex fashion, discussion of which is beyond the scope of this review. Briefly, the emerging picture is that TRPC channels, just like most other TRP channels, require PI(4,5)P₂ for optimal functioning, and substantial

depletion of this lipid limits their activity (Imai et al., 2012; Itsuki et al., 2014). PI(4,5)P₂ may also exert an additional negative effect on some TRPC channels, see for example (Trebak et al., 2009), relief from which during PLC activation could potentially contribute to their activation. This topic has been recently reviewed in more detail (Rohacs, 2014).

Calcium-activated Chloride channels (Cl_{Ca})

Cl_{Ca} channels are encoded by members of the TMEM16 family; the first member that was characterized is TMEM16A (Schroeder et al., 2008), also called Anoctamin1 or Ano1 (Yang et al., 2008). Cl⁻ channels are usually inhibitory, but in DRG neurons they are excitatory, because the reversal potential of Cl⁻ is more positive than the resting membrane potential due to its relatively high intracellular concentration (Liu et al., 2010). Ano1 is expressed in DRG neurons, and it was shown that bradykinin exerts its excitatory effect on DRG neurons via activating Ano1 and inhibiting voltage gated KCNQ K⁺ channels (Liu et al., 2010). It was also shown that Ano1 is selectively activated by Ca²⁺ signals evoked by GPCR stimulation compared to Ca²⁺ influx via voltage gated Ca²⁺ channels, because of the colocalization of the receptors with Ano1 (Jin et al., 2013). Ca²⁺ influx via TRPV1 also activates Ano1, contributing to the depolarization induced by capsaicin (Takayama et al., 2015). Ano1 was reported to be inhibited by PI(4,5)P₂ (Pritchard et al., 2014), an effect that may potentially contribute to its activation by bradykinin, or capsaicin. Another article however found no effect of various interventions that affect phosphoinositide metabolism on Ano1 activity (Tian et al., 2013). Ano1 was also proposed to be a noxious heat sensor (Cho et al., 2012).

PI3K signaling in DRG neurons

DRG neurons also express Receptor Tyrosine Kinases, such as the Nerve Growth Factor (NGF) receptor. NGF exerts trophic effects on neonatal and fetal DRG neurons and plays important roles in their development. NGF also exerts acute pro-inflammatory effects, for example it was shown to sensitize TRPV1 channels, which was proposed to proceed via activating PLC and the depletion of PI(4,5)P₂ (Chuang et al., 2001). Later however several papers concluded that acute potentiation by NGF proceeds via increased trafficking of the TRPV1 protein in a PI3K sensitive manner (Bonnington and McNaughton, 2003; Zhang et al., 2005; Stein et al., 2006).

Ion channels in sensory neurons

Sensory neurons respond to mechanical, chemical or thermal stimuli. The primary output of the peripheral processes of DRG neurons is generation of action potentials that will eventually trigger the release of glutamate in the central terminals to stimulate secondary neurons in the dorsal horn of the spinal cord. Ion channels that generate these electrical signals in primary sensory neurons can be roughly divided to two categories. The ion channels that set the resting membrane potential, generate the action potential and initiate transmitter release, such as background K⁺ channels, voltage gated Na⁺, K⁺ and Ca²⁺ channels, are not specific to DRG neurons. The ion channels that respond to thermal, chemical or mechanical cues and initiate action potentials by depolarizing the cell membrane are often specifically expressed in sensory neurons, and they are usually called

sensory ion channels. Many sensory ion channels belong to the Transient Receptor Potential (TRP) family (Clapham, 2003), but not all sensory channels are TRPs, and not all TRPs are sensory (Wu et al., 2010). Sensory channels are often multimodal, and can be activated by multiple stimuli. This division, while useful didactically, is somewhat arbitrary, as some non-sensory ion channels can also be modulated by temperature or mechanical forces (Maingret et al., 2000; Hao et al., 2013).

Sensory TRP channels

The regulation of TRP channels by the PLC pathway (Rohacs, 2013), by phosphoinositides (Rohacs, 2014) and by GPCR-s in general (Veldhuis et al., 2015) have been recently reviewed. Here we briefly describe the TRP and other sensory channels found in DRG and TG neurons and discuss their regulation by the phosphoinositide signaling pathway.

TRPV1

TRPV1 is a heat- and capsaicin-activated ion channel; it is probably the most intensively studied sensory ion channel (Bevan et al., 2014). Its role as a physiological heat sensor is somewhat debated (Dhaka et al., 2006), but it is very well established to be important for thermal hyperalgesia (Vriens et al., 2014b). Thermal hyperalgesia is mediated by sensitization of TRPV1 by inflammatory mediators, such as bradykinin or prostaglandins. Sensitization refers to the shift in the activation curve of the channel to heat, capsaicin and low extracellular pH, that makes it easier for these stimuli to open the channel. The role of PKC in acute sensitization of TRPV1 is very well established (Numazaki et al., 2002; Bhawe et al., 2003; Lukacs et al., 2013b). Depletion of PI(4,5)P₂ and relief from inhibition by this lipid has been also proposed to play a role in acute sensitization (Chuang et al., 2001; Cao et al., 2013), but this effect is probably auxiliary to PKC (Lukacs et al., 2013b), and somewhat controversial. As we have recently reviewed this topic in detail (Rohacs, 2015), its detailed discussion is beyond the scope of this review.

The activity of TRPV1 similar to most other TRP channels depend on the presence of phosphoinositides (Stein et al., 2006; Lukacs et al., 2007; Klein et al., 2008; Lukacs et al., 2013a; Poblete et al., 2014), mainly PI(4,5)P₂, with a potential contribution of PI(4)P in a cellular context (Rohacs, 2014). These channels undergo desensitization during prolonged exposure to capsaicin. Several different Ca²⁺ dependent mechanisms, such as calmodulin (Numazaki et al., 2003; Rosenbaum et al., 2004; Lishko et al., 2007) and calcineurin (Docherty et al., 1996; Mohapatra and Nau, 2005) have been proposed to play a role in desensitization, but depletion of PI(4,5)P₂ and PI(4)P by Ca²⁺-induced PLC activation is emerging as a key mechanism (Liu et al., 2005; Lishko et al., 2007; Lukacs et al., 2007; Lukacs et al., 2013b) reviewed in (Rohacs, 2015).

TRPV2

TRPV2 was originally described as a noxious heat sensor (Caterina et al., 1999). TRPV2 RNA is expressed in DRG neurons at high levels (comparable to that of TRPV1) (Gerhold et al., 2013; Vandewauw et al., 2013; Thakur et al., 2014), and TRPV2 protein was also detected in medium and larger DRG neurons (Caterina et al., 1999). Interestingly, mice

lacking this channel have normal thermosensation (Park et al., 2011), but they are more susceptible to perinatal death, and have impaired macrophage phagocytosis (Link et al., 2010). TRPV2 activity depends on PI(4,5)P₂ and its depletion has been proposed to play a role in desensitization to its chemical agonist 2-APB in an expression system (Mercado et al., 2010). It was recently proposed that TRPV2 is an intracellular ion channel, upregulated by NGF, playing a role in neurite outgrowth (Cohen et al., 2015).

TRPV3

TRPV3 was identified as a sensor of moderately warm temperatures in keratinocytes (Peier et al., 2002b; Xu et al., 2002). This channel is often considered a thermo-TRP, but it is essentially not expressed in DRG neurons (Gerhold et al., 2013; Vandewauw et al., 2013; Thakur et al., 2014; Vriens et al., 2014b). While initial reports suggested the involvement of this channel in behavioral responses to warm temperatures, this effect is highly dependent on genetic background and sex (Vriens et al., 2014b). The most likely physiological role of TRPV3 is to regulate skin and hair development (Nilius et al., 2014). Consistent with this idea, mice lacking TRPV3 has altered skin barrier formation and curly hair (Cheng et al., 2010), whereas humans with a gain of function mutation of TRPV3 develop Olmsted syndrome that is characterized by skin and hair abnormalities, characterized by palmoplantar and periorificial keratoderma, alopecia and severe itching (Lin et al., 2012). TRPV3 was shown to be negatively regulated by PI(4,5)P₂ (Doerner et al., 2011).

TRPV4

TRPV4 is a mechanically activated ion channel playing important roles in osmosensation (Liedtke and Friedman, 2003). As opposed to the fast and presumably direct activation of piezo channels by membrane stretch, TRPV4 activation by osmotic swelling is slow, and was proposed to proceed via a lipid mediators generated by phospholipase A2 cytochrome P450 epoxygenase (Vriens et al., 2004). This channel is also activated by heat via a mechanism distinct from that induced by swelling (Vriens et al., 2004). TRPV4 was proposed to be regulated by PI(4,5)P₂ both positively (Garcia-Elias et al., 2013) and negatively (Takahashi et al., 2014). Mutations in TRPV4 cause human disease with a puzzling variety of manifestations including skeletal dysplasias and degeneration of motor neurons (Nilius and Voets, 2013). TRPV4 is also activated by moderate heat but it probably does not play a significant role in thermosensation (Vriens et al., 2014b). TRPV4 is expressed in adult mouse DRG neurons at very low levels compared to other sensory TRP channels (Thakur et al., 2014; Vriens et al., 2014b).

TRPM3

TRPM3 is a heat activated ion channel expressed in DRG neurons where it was proposed to play a role as a noxious heat sensor (Vriens et al., 2011). Like most thermo TRP channels, TRPM3 also has chemical activators, the most often used one being pregnenolone sulphate (Wagner et al., 2008). Clotrimazole, when co-applied with pregnenolone sulphate, opens an alternative permeation pathway with different pore properties (Vriens et al., 2014a). TRPM3 channel activity induced by pregnenolone sulphate in heterologous expression systems was shown to require the presence of PI(4,5)P₂ (Badheka, 2015; Toth et al., 2015).

Understanding the regulation of TRPM3 by phosphoinositide signaling in DRG neurons will require further studies.

TRPM8

TRPM8 is a cold- and menthol-activated ion channel expressed in DRG and TG neurons (McKemy et al., 2002; Peier et al., 2002a). Genetic deletion of this channel in mice clearly and unambiguously altered sensitivity to moderately cold temperatures (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). TRPM8 was shown to require PI(4,5)P₂ for activity both in cellular systems (Liu and Qin, 2005; Rohacs et al., 2005; Varnai et al., 2006; Daniels et al., 2009), and in planar lipid bilayers (Zakharian et al., 2009; Zakharian et al., 2010; El-Arabi et al., 2012), demonstrating that the lipid is a direct activator of these channels. In the presence of extracellular Ca²⁺, TRPM8 activity decreases during continuous stimulation by menthol or cold (McKemy et al., 2002), a phenomenon termed desensitization or adaptation. Ca²⁺-induced activation of PLC has been shown to play an important role in this phenomenon (Rohacs et al., 2005; Daniels et al., 2009; Yudin et al., 2011). The most abundantly expressed Ca²⁺ sensitive PLC isoform in DRG neurons is PLCδ4 (Lukacs et al., 2013b), and we recently found that small TRPM8 positive DRG neurons in PLCδ4^{-/-} mice show larger cold- and menthol-induced currents and the animals were more sensitive to evaporative cold (Yudin and Rohacs, 2015), showing the importance of PLCδ4 in the regulation of TRPM8 *in vivo*.

TRPA1

TRPA1 was originally described as a noxious cold sensor (Story et al., 2003), but its regulation by cold is controversial (Jordt et al., 2004; Bautista et al., 2006; Kwan et al., 2006; Karashima et al., 2009), and may be species dependent (Chen et al., 2013), see recent reviews on TRPA1 for more details (Vriens et al., 2014b; Zygmunt and Hogestatt, 2014). TRPA1 is well established to be a noxious chemical sensor, activated by mustard oil, acrolein, formaldehyde and many other compounds mainly by covalent modification (Jordt et al., 2004; Macpherson et al., 2007). This channel plays important roles not only in DRG neurons, but also in sensory nerves innervating the airways, playing important roles in detecting irritants (Bessac et al., 2008). As mentioned earlier, TRPA1 is also involved in chloroquine-induced itch, via activation by G_q-coupled MrgprA3 receptors (Wilson et al., 2011).

Phosphoinositides were also proposed to regulate this channel, but the picture in this regard is as confusing as with cold activation, reviewed in (Rohacs, 2014). Both activating (Karashima et al., 2008) and inhibitory effects (Kim et al., 2008) of PI(4,5)P₂ were proposed, but the channel was not inhibited by a rapamycin-inducible 5-phosphatase (Wang et al., 2008), and the purified reconstituted channel was active without PI(4,5)P₂ or other negatively charged lipids in planar lipid bilayers raising doubts about its phosphoinositide dependence (Moparathi et al., 2014). Activation of TRPV1 by capsaicin on the other hand inhibited TRPA1, and inclusion of PI(4,5)P₂ in the patch pipette alleviated this effect, suggesting dependence on PI(4,5)P₂ (Akopian et al., 2007). Bradykinin was shown to potentiate TRPA1 (Bandell et al., 2004).

Ligand activated and chemosensitive ion channels

Most thermo TRP channels discussed in the previous chapter have chemical activators, and TRPA1 is clearly functions as a noxious chemical sensor. Below we will briefly discuss two additional groups of ion channels that are present in DRG neurons, and are regulated by the chemical composition of the extracellular fluid.

P2X-ATP receptors

Extracellular ATP is an important signaling molecule, playing roles in many cellular processes. ATP acts on either G-protein coupled P2Y receptors, or directly activates P2X non-selective cation channels, which we briefly discuss here. The dominant P2X isoform expressed in DRG neurons is P2X3, with substantial expression of P2X2 and P2X4 (Thakur et al., 2014).

ATP can be released from cells via plasma membrane transport proteins such as pannexins or CALHM channels (Taruno et al., 2013). Keratinocytes for example may release ATP via connexin hemichannels (Barr et al., 2013), and heat activation of TRPV3 in keratinocytes was proposed to transmit information via ATP release (Mandadi et al., 2009), a notion consistent with reduced non-noxious warmth sensitivity of P2X3^{-/-} mice (Souslova et al., 2000). ATP can also be released from damaged cells in a non-specific manner, and P2X3^{-/-} mice showed reduced responses to formalin-induced pain. Responses to noxious thermal and mechanical stimuli however were normal in P2X3^{-/-}, but the animals also showed reduced inflammatory hyperalgesia (Souslova et al., 2000).

Several P2X isoforms were shown to require PI(4,5)P₂ and to be inhibited by depletion of PI(4,5)P₂ (Fujiwara and Kubo, 2006; Bernier et al., 2008a; Bernier et al., 2008b); this topic was reviewed recently (Bernier et al., 2013). It was shown that in rat DRG neurons activation of P2Y2 receptors inhibit P2X3 via PI(4,5)P₂ depletion (Mo et al., 2013). Capsaicin also inhibits P2X3 in TRPV1 expressing cells (Stanchev et al., 2009), and in rat DRG neurons (Piper and Docherty, 2000) but the involvement of phosphoinositides were not examined.

Acid Sensing Ion Channels (ASIC)

ASIC channels are distant relatives of the Epithelial Na⁺ channels ENaC. They are activated by extracellular acidification and they are expressed in DRG neurons, but their role in sensory functions is controversial (Wemmie et al., 2013). Despite the well documented regulation of the related ENaC family by phosphoinositides (Pochynyuk et al., 2008), there is no report on phosphoinositide regulation of ASIC channels.

Mechanosensitive ion channels

DRG neurons have been shown to display mechanically activated (MA) currents with various inactivation/adaptation kinetics (Hu and Lewin, 2006; Hao and Delmas, 2010; Rugiero et al., 2010). The molecular identity of these ion channels has been a mystery until relatively recently (Sachs, 2010). TRPA1, TRPV4 and ASICs have been proposed earlier to play roles, but the data were controversial (Arnadottir and Chalfie, 2010; Sachs, 2010). The

recently identified Piezo2 ion channels are the well-accepted molecular counterparts of the Rapidly Adapting MA current in DRG neurons (Coste et al., 2010), but the proteins that mediate Slowly and Intermediate Adapting MA current have not yet been identified.

Piezo2

Piezo 2 and its close relative Piezo1 are bona fide mechanosensitive ion channels that generate large mechanically-induced ionic currents when expressed in heterologous systems (Volkers et al., 2014). Genetic deletion of Piezo2 in DRG neurons and in Merkel cell results in a profound loss of gentle touch sensation in mice (Ranade et al., 2014). Piezo2 currents have been shown to be potentiated by activation of bradykinin receptors with the involvement of PKC (Dubin et al., 2012). Rapidly adapting MA current in DRG neurons were also shown to be potentiated by activation of P2Y receptors by extracellular UTP and ATP (Lechner and Lewin, 2009). Activation of TRPV1 by capsaicin on the other hand resulted in a profound inhibition of Piezo2 currents in DRG neurons expressing both TRPV1 and Piezo2. This inhibition was reproduced both in heterologously expressed Piezo1 and Piezo2, and the effect was alleviated by the inclusion of either PI(4,5)P₂ or PI(4)P in the patch pipette (Borbiro et al., 2015).

Ion channels involved in setting the resting membrane potential, generation of action potentials and transmitter release

Two-pore (2P) K⁺ channels

2P K⁺ channels are also called background K⁺ channels, because they are constitutively open, thus they are important determinants of the resting membrane potential (Enyedi and Czirjak, 2010). These channels are also regulated by a large number of factors, including temperature (Maingret et al., 2000), mechanical forces (Honore et al., 2006) and various signaling pathways (Enyedi and Czirjak, 2010). Changes in the activity of 2P channels can affect the resting membrane potential, leading to increased, or decreased excitability. Several 2P channels are expressed in sensory neurons (Thakur et al., 2014) and 2P channels have been shown to significantly contribute to the resting membrane potential in these cells (Du et al., 2014). Genetic deletion of TREK-1 (KCNK2) resulted in enhanced sensitivity to mechanical stimuli and painful heat (Alloui et al., 2006). TREK-1 is positively modulated by heat, and closure of a background channel upon cooling was proposed to play a role in cold transduction (Viana et al., 2002), but TREK-1^{-/-} mice showed normal cold sensitivity (Alloui et al., 2006). TRESK (KCNK18) (Czirjak et al., 2004) is also expressed in DRG neurons (Dobler et al., 2007), and its inhibition by hydroxy- α -sanshool was proposed to be responsible for the tingling pungency of Szechuan peppers (Bautista et al., 2008). Interestingly, functional knockout of TRESK channels in mice resulted in no change in resting membrane potential in DRG neurons, but it increased cellular excitability (Dobler et al., 2007). It was shown that in peripheral axotomy, TRESK channels are downregulated, and this contributes to increased excitability (Tulleuda et al., 2011).

TASK, TREK-1 and TRAAK channels were proposed to be regulated by PI(4,5)P₂ (Chemin et al., 2005; Lopes et al., 2005), but their inhibition by PLC coupled agonists have been shown to be mediated by direct binding of G_q subunits, rather than PI(4,5)P₂ depletion (Chen

et al., 2006). More recently, it was proposed that PLC-coupled agonists inhibit TASK channel via DAG (Wilke et al., 2014). We are not aware of any data on regulation of 2P channels in DRG neurons by phosphoinositide signaling.

Voltage gated K⁺ channels

Voltage gated K⁺ (Kv) channels are the most diverse superfamily of ion channels (Yu and Catterall, 2004) and DRG neurons express many different Kv-s (Du and Gamper, 2013; Thakur et al., 2014). Here I will focus on regulation of the KCNQ (Kv7.x) channels, also called M-current, which are very well established to depend on PI(4,5)P₂ and to be regulated by changes in phosphoinositide levels (Suh and Hille, 2002; Zhang et al., 2003). KCNQ channels are low voltage activated, and contribute to setting the resting membrane potential in DRG neurons (Du et al., 2014), thus their inhibition has a significant effect on cellular excitability.

KCNQ2 (Kv7.2) is the major KCNQ transcript in DRG neurons, it is mainly expressed in small diameter neurons, and its expression level was shown to decrease upon nerve injury contributing to increased excitability in neuropathic pain (Rose et al., 2011). Activation of proteinase-activated PAR-2 receptors was shown to decrease KCNQ mediated M-currents in DRG neurons, and this inhibition required both the depletion of PI(4,5)P₂ and the increase in cytoplasmic Ca²⁺ (Linley et al., 2008). Bradykinin was also shown to inhibit M-currents in DRG neurons (Liu et al., 2010). As mentioned earlier, chloroquine also inhibits KCNQ channels via the activation of MrgprA3 receptors in a PLC dependent manner (Crozier et al., 2007). Finally, activation of TRPV1 by capsaicin was shown to inhibit KCNQ channels via PI(4,5)P₂ depletion (Zhang et al., 2011).

Voltage gated Na⁺ (VDNaC) channels

VDNaC channels generate the upstroke of action potentials; they are targets for local anesthetics such as lidocaine, and mutations in some of them cause syndromes with inability to sense pain (Waxman and Zamponi, 2014). There is no data currently on phosphoinositide sensitivity of VDNaC channels, and very little is known on their regulation by this pathway. Capsaicin was shown to inhibit VDNaC in TG neurons, and the extent of inhibition was proportional to the inward current evoked by capsaicin, but the role of phosphoinositide signaling was not examined (Liu et al., 2001). Stimulation of NK-1 receptors was reported to enhance the activity of tetrodotoxin-resistant Nav1.8 channel in small diameter DRG neurons, in a manner dependent on PKC ϵ , an effect that may contribute to inflammatory hyperalgesia (Cang et al., 2009).

Voltage gated Ca²⁺ channels (VGCC)

The classical characterization of VGCC to T, N and L-type is based on work in DRG neurons (Nowycky et al., 1985). Unbiased high throughput RNA sequencing show high levels of expression of Cav2.2 (N-type), Cav1.2 (L-type), Cav3.2 and 3.3 (T-type) in DRG neurons (Thakur et al., 2014). N-type VGCC has been shown by multiple approaches to require PI(4,5)P₂ for activity (Wu et al., 2002; Gamper et al., 2004; Suh et al., 2010), reviewed in (Gamper and Rohacs, 2012). Cav1.2 and Cav1.3 L-type channels were also

reported to be partially inhibited by PI(4,5)P₂ depletion, but T-type channels were not affected (Suh et al., 2010).

N-type VGCC are well known to be rapidly inhibited by G_i-coupled GPCR-s via Gβγ subunits. Activation of G_q coupled receptors, however, also exerts a slower inhibition on these channels via PI(4,5)P₂ depletion in superior cervical ganglion cells (Gamper et al., 2004). N-type VGCC in DRG neurons have been reported to be inhibited by two different G_q coupled receptors MrgprC (Li et al., 2014) and purinergic P2Y receptors (Gerevich et al., 2004). The role of phosphoinositide depletion however was not examined in either of these cases. There are several reports showing that capsaicin inhibits VGCC in TRPV1 positive DRG neurons (Bleakman et al., 1990; Hagenacker et al., 2005; Wu et al., 2005, 2006) (Docherty et al., 1991; Comunanza et al., 2011), but the role of phosphoinositide signaling was not examined in any of the papers. Given the clear dependence of N-type channels on PI(4,5)P₂ and the robust depletion of PI(4,5)P₂ induced by TRPV1 activation (Lukacs et al., 2013b) this mechanism is feasible, at least for inhibition of N-type channels.

Hyperpolarization-activated Cyclic Nucleotide-gate (HCN) channels

HCN channels are found in neurons and other excitable tissues that fire action potentials rhythmically. HCN1 and HCN2 channels are expressed in DRG neurons (Acosta et al., 2012), HCN2 was shown to play a role in mechanical, but not heat hyperalgesia in chronic inflammation (Schnorr et al., 2014), and HCN2 levels were increased after axotomy (Smith et al., 2015). Capsaicin was shown to inhibit HCN channels in TRPV1 positive DRG neurons in a Ca²⁺ dependent manner (Kwak, 2012); the role of phosphoinositides were not addressed, but the effect was inhibited by cyclosporine, implying the role of calcineurin. HCN channels were proposed to be positively regulated by PI(4,5)P₂ by shifting the activation of the channel to more depolarized voltages (Zolles et al., 2006). In another publication, however, activation of PLC coupled receptors resulted in a shift of activation gating to more negative voltages, the opposite of what was expected if phosphoinositides positively regulate HCN channels (Pian et al., 2007).

Conclusions

This review attempted to discuss signaling through phosphoinositides in peripheral sensory neurons, with focus on effects on ion channels. I tried to be comprehensive, yet brief, a combination inevitably leading to oversimplifications. The role of PLC signaling in acute inflammatory sensitization has been very well established, and the role of this signaling pathway in certain forms of itch is emerging. The diversity of sensory neurons and the pleiotropic nature of phosphoinositide signaling make the picture complex however. There is an abundance of information available on this topic, but a lot of dots need to be connected by future research to have a clearer picture on the details in many cases.

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REFERENCES

- Acosta C, McMullan S, Djouhri L, Gao L, Watkins R, Berry C, Dempsey K, Lawson SN. HCN1 and HCN2 in Rat DRG neurons: levels in nociceptors and non-nociceptors, NT3-dependence and influence of CFA-induced skin inflammation on HCN2 and NT3 expression. *PLoS One*. 2012; 7:e50442. [PubMed: 23236374]
- 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]
- Alkhani H, Ase AR, Grant R, O'Donnell D, Groschner K, Seguela P. Contribution of TRPC3 to store-operated calcium entry and inflammatory transductions in primary nociceptors. *Mol Pain*. 2014; 10:43. [PubMed: 24965271]
- Allen V, Swigart P, Cheung R, Cockcroft S, Katan M. Regulation of inositol lipid-specific phospholipase cdelta by changes in Ca^{2+} ion concentrations. *Biochem J*. 1997; 327:545–552. Pt 2. [PubMed: 9359428]
- Alloui A, Zimmermann K, Mamet J, Duprat F, Noel J, Chemin J, Guy N, Blondeau N, Voilley N, Rubat-Coudert C, Borsotto M, Romey G, Heurteaux C, Reeh P, Eschaliere A, Lazdunski M. TREK-1, a K^+ channel involved in polymodal pain perception. *EMBO J*. 2006; 25:2368–2376. [PubMed: 16675954]
- Arnadottir J, Chalfie M. Eukaryotic mechanosensitive channels. *Annu Rev Biophys*. 2010; 39:111–137. [PubMed: 20192782]
- Badheka D, Borbiri I, Rohacs T. Transient Receptor Potential Melastatin 3 is a phosphoinositide dependent ion channel. *Journal of General Physiology*. 2015; 146:65–77. [PubMed: 26123195]
- Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron*. 2004; 41:849–857. [PubMed: 15046718]
- Barr TP, Albrecht PJ, Hou Q, Mongin AA, Strichartz GR, Rice FL. Air-stimulated ATP release from keratinocytes occurs through connexin hemichannels. *PLoS One*. 2013; 8:e56744. [PubMed: 23457608]
- Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell*. 2009; 139:267–284. [PubMed: 19837031]
- Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell*. 2006; 124:1269–1282. [PubMed: 16564016]
- Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature*. 2007; 448:204–208. [PubMed: 17538622]
- Bautista DM, Sigal YM, Milstein AD, Garrison JL, Zorn JA, Tsuruda PR, Nicoll RA, Julius D. Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. *Nat Neurosci*. 2008; 11:772–779. [PubMed: 18568022]
- Bautista DM, Wilson SR, Hoon MA. Why we scratch an itch: the molecules, cells and circuits of itch. *Nat Neurosci*. 2014; 17:175–182. [PubMed: 24473265]
- Bernier LP, Ase AR, Chevallier S, Blais D, Zhao Q, Boue-Grabot E, Logothetis D, Seguela P. Phosphoinositides regulate P2X4 ATP-gated channels through direct interactions. *J Neurosci*. 2008a; 28:12938–12945. [PubMed: 19036987]
- Bernier LP, Ase AR, Seguela P. Post-translational regulation of P2X receptor channels: modulation by phospholipids. *Front Cell Neurosci*. 2013; 7:226. [PubMed: 24324400]
- Bernier LP, Ase AR, Tong X, Hamel E, Blais D, Zhao Q, Logothetis DE, Seguela P. Direct modulation of P2X1 receptor-channels by the lipid phosphatidylinositol 4,5-bisphosphate. *Mol Pharmacol*. 2008b; 74:785–792. [PubMed: 18523136]
- 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]
- Bevan S, Quallo T, Andersson DA. Trpv1. *Handb Exp Pharmacol*. 2014; 222:207–245. [PubMed: 24756708]

- Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau R.W.t. Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). *Proc Natl Acad Sci U S A*. 2003; 100:12480–12485. [PubMed: 14523239]
- Bleakman D, Brorson JR, Miller RJ. The effect of capsaicin on voltage-gated calcium currents and calcium signals in cultured dorsal root ganglion cells. *Br J Pharmacol*. 1990; 101:423–431. [PubMed: 1701680]
- Bonnington JK, McNaughton PA. Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. *J Physiol*. 2003; 551:433–446. [PubMed: 12815188]
- Borbiro I, Badheka D, Rohacs T. Activation of TRPV1 channels inhibit mechanosensitive Piezo channel activity by depleting membrane phosphoinositides. *Sci Signal*. 2015; 8:ra15. [PubMed: 25670203]
- Cang CL, Zhang H, Zhang YQ, Zhao ZQ. PKCepsilon-dependent potentiation of TTX-resistant Nav1.8 current by neurokinin-1 receptor activation in rat dorsal root ganglion neurons. *Mol Pain*. 2009; 5:33. [PubMed: 19563686]
- Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D. TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron*. 2013; 77:667–679. [PubMed: 23439120]
- Cao X, Choi S, Maleth JJ, Park S, Ahuja M, Muallem S. The ER/PM microdomain, PI(4,5)P₂ and the regulation of STIM1-Orai1 channel function. *Cell Calcium*. 2015; 58:342–348. [PubMed: 25843208]
- Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature*. 1999; 398:436–441. [PubMed: 10201375]
- Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA. Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. *Neuron*. 1999; 23:617–624. [PubMed: 10433272]
- Chemin J, Patel AJ, Duprat F, Lauritzen I, Lazdunski M, Honore E. A phospholipid sensor controls mechanogating of the K⁺ channel TREK-1. *EMBO J*. 2005; 24:44–53. [PubMed: 15577940]
- Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B, Kim D. Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat Commun*. 2013; 4:2501. [PubMed: 24071625]
- Chen X, Talley EM, Patel N, Gomis A, McIntire WE, Dong B, Viana F, Garrison JC, Bayliss DA. Inhibition of a background potassium channel by Gq protein alpha-subunits. *Proc Natl Acad Sci U S A*. 2006; 103:3422–3427. [PubMed: 16492788]
- Cheng X, Jin J, Hu L, Shen D, Dong XP, Samie MA, Knoff J, Eisinger B, Liu ML, Huang SM, Caterina MJ, Dempsey P, Michael LE, Dlugosz AA, Andrews NC, Clapham DE, Xu H. TRP channel regulates EGFR signaling in hair morphogenesis and skin barrier formation. *Cell*. 2010; 141:331–343. [PubMed: 20403327]
- Cho H, Yang YD, Lee J, Lee B, Kim T, Jang Y, Back SK, Na HS, Harfe BD, Wang F, Raouf R, Wood JN, Oh U. The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. *Nat Neurosci*. 2012; 15:1015–1021. [PubMed: 22634729]
- Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P₂-mediated inhibition. *Nature*. 2001; 411:957–962. [PubMed: 11418861]
- Clapham DE. TRP channels as cellular sensors. *Nature*. 2003; 426:517–524. [PubMed: 14654832]
- Cockcroft S. The latest phospholipase C, PLCeta, is implicated in neuronal function. *Trends Biochem Sci*. 2006; 31:4–7. [PubMed: 16310357]
- Cohen MR, Johnson WM, Pilat JM, Kiselar J, DeFrancesco-Lisowitz A, Zigmond RE, Moiseenkova-Bell VY. NGF regulates TRPV2 via ERK signaling to enhance neurite outgrowth in developing neurons. *Mol Cell Biol*. 2015
- Colburn RW, Lubin ML, Stone DJ Jr. Wang Y, Lawrence D, D'Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N. Attenuated cold sensitivity in TRPM8 null mice. *Neuron*. 2007; 54:379–386. [PubMed: 17481392]

- Comunanza V, Carbone E, Marcantoni A, Sher E, Ursu D. Calcium-dependent inhibition of T-type calcium channels by TRPV1 activation in rat sensory neurons. *Pflugers Arch.* 2011; 462:709–722. [PubMed: 21904821]
- Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science.* 2010; 330:55–60. [PubMed: 20813920]
- Crozier RA, Ajit SK, Kaftan EJ, Pausch MH. MrgD activation inhibits KCNQ/M-currents and contributes to enhanced neuronal excitability. *J Neurosci.* 2007; 27:4492–4496. [PubMed: 17442834]
- Czirjak G, Toth ZE, Enyedi P. The two-pore domain K⁺ channel, TRESK, is activated by the cytoplasmic calcium signal through calcineurin. *J Biol Chem.* 2004; 279:18550–18558. [PubMed: 14981085]
- Daniels RL, Takashima Y, McKemy DD. Activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositol 4,5-bisphosphate. *J Biol Chem.* 2009; 284:1570–1582. [PubMed: 19019830]
- 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]
- Dhaka A, Viswanath V, Patapoutian A. Trp ion channels and temperature sensation. *Annu Rev Neurosci.* 2006; 29:135–161. [PubMed: 16776582]
- Dobler T, Springauf A, Tovornik S, Weber M, Schmitt A, Sedlmeier R, Wischmeyer E, Doring F. TRESK two-pore-domain K⁺ channels constitute a significant component of background potassium currents in murine dorsal root ganglion neurones. *J Physiol.* 2007; 585:867–879. [PubMed: 17962323]
- Docherty RJ, Robertson B, Bevan S. Capsaicin causes prolonged inhibition of voltage-activated calcium currents in adult rat dorsal root ganglion neurons in culture. *Neuroscience.* 1991; 40:513–521. [PubMed: 2027470]
- 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]
- Doerner JF, Hatt H, Ramsey IS. Voltage- and temperature-dependent activation of TRPV3 channels is potentiated by receptor-mediated PI(4,5)P₂ hydrolysis. *J Gen Physiol.* 2011; 137:271–288. [PubMed: 21321070]
- Du X, Gamper N. Potassium channels in peripheral pain pathways: expression, function and therapeutic potential. *Curr Neuropharmacol.* 2013; 11:621–640. [PubMed: 24396338]
- Du X, Hao H, Gigout S, Huang D, Yang Y, Li L, Wang C, Sundt D, Jaffe DB, Zhang H, Gamper N. Control of somatic membrane potential in nociceptive neurons and its implications for peripheral nociceptive transmission. *Pain.* 2014; 155:2306–2322. [PubMed: 25168672]
- Dubin AE, Schmidt M, Mathur J, Petrus MJ, Xiao B, Coste B, Patapoutian A. Inflammatory signals enhance piezo2-mediated mechanosensitive currents. *Cell Rep.* 2012; 2:511–517. [PubMed: 22921401]
- El-Arabi AM, Salazar CS, Schmidt JJ. Ion channel drug potency assay with an artificial bilayer chip. *Lab Chip.* 2012; 12:2409–2413. [PubMed: 22549308]
- Enyedi P, Czirjak G. Molecular background of leak K⁺ currents: two-pore domain potassium channels. *Physiol Rev.* 2010; 90:559–605. [PubMed: 20393194]
- Falkenburger BH, Jensen JB, Hille B. Kinetics of M1 muscarinic receptor and G protein signaling to phospholipase C in living cells. *J Gen Physiol.* 2010; 135:81–97. [PubMed: 20100890]
- Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature.* 2006; 441:179–185. [PubMed: 16582901]
- Fujiwara Y, Kubo Y. Regulation of the desensitization and ion selectivity of ATP-gated P2X₂ channels by phosphoinositides. *J Physiol.* 2006; 576:135–149. [PubMed: 16857707]
- Fukami K, Inanobe S, Kanemaru K, Nakamura Y. Phospholipase C is a key enzyme regulating intracellular calcium and modulating the phosphoinositide balance. *Prog Lipid Res.* 2010; 49:429–437. [PubMed: 20553968]

- Gamper N, Reznikov V, Yamada Y, Yang J, Shapiro MS. Phosphatidylinositol [correction] 4,5-bisphosphate signals underlie receptor-specific Gq/11-mediated modulation of N-type Ca^{2+} channels. *J Neurosci*. 2004; 24:10980–10992. [PubMed: 15574748]
- Gamper N, Rohacs T. Phosphoinositide sensitivity of ion channels, a functional perspective. *Subcell Biochem*. 2012; 59:289–333. [PubMed: 22374095]
- Garcia del Cano G, Montana M, Aretxabala X, Gonzalez-Burguera I, Lopez de Jesus M, Barrondo S, Salles J. Nuclear phospholipase C-beta1 and diacylglycerol LIPASE-alpha in brain cortical neurons. *Adv Biol Regul*. 2014; 54:12–23. [PubMed: 24076015]
- Garcia-Elias A, Mrkonjic S, Pardo-Pastor C, Inada H, Hellmich UA, Rubio-Moscardo F, Plata C, Gaudet R, Vicente R, Valverde MA. Phosphatidylinositol-4,5-bisphosphate-dependent rearrangement of TRPV4 cytosolic tails enables channel activation by physiological stimuli. *Proc Natl Acad Sci U S A*. 2013; 110:9553–9558. [PubMed: 23690576]
- Garrison SR, Dietrich A, Stucky CL. TRPC1 contributes to light-touch sensation and mechanical responses in low-threshold cutaneous sensory neurons. *J Neurophysiol*. 2012; 107:913–922. [PubMed: 22072513]
- Gemes G, Bangaru ML, Wu HE, Tang Q, Weihrauch D, Koopmeiners AS, Cruikshank JM, Kwok WM, Hogan QH. Store-operated Ca^{2+} entry in sensory neurons: functional role and the effect of painful nerve injury. *J Neurosci*. 2011; 31:3536–3549. [PubMed: 21389210]
- Gerevich Z, Borvendeg SJ, Schroder W, Franke H, Wirkner K, Norenberg W, Furst S, Gillen C, Illes P. Inhibition of N-type voltage-activated calcium channels in rat dorsal root ganglion neurons by P2Y receptors is a possible mechanism of ADP-induced analgesia. *J Neurosci*. 2004; 24:797–807. [PubMed: 14749424]
- Gerhold KA, Pellegrino M, Tsunozaki M, Morita T, Leitch DB, Tsuruda PR, Brem RB, Catania KC, Bautista DM. The star-nosed mole reveals clues to the molecular basis of mammalian touch. *PLoS One*. 2013; 8:e55001. [PubMed: 23383028]
- Gottlieb P, Folgering J, Maroto R, Raso A, Wood TG, Kurosky A, Bowman C, Bichet D, Patel A, Sachs F, Martinac B, Hamill OP, Honore E. Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Arch*. 2008; 455:1097–1103. [PubMed: 17957383]
- Hagenacker T, Spletstoesser F, Greffrath W, Treede RD, Busselberg D. Capsaicin differentially modulates voltage-activated calcium channel currents in dorsal root ganglion neurones of rats. *Brain Res*. 2005; 1062:74–85. [PubMed: 16269136]
- Hammer P, Banck MS, Amberg R, Wang C, Petznick G, Luo S, Khrebtukova I, Schroth GP, Beyerlein P, Beutler AS. mRNA-seq with agnostic splice site discovery for nervous system transcriptomics tested in chronic pain. *Genome Res*. 2010; 20:847–860. [PubMed: 20452967]
- Han SK, Mancino V, Simon MI. Phospholipase Cbeta 3 mediates the scratching response activated by the histamine H1 receptor on C-fiber nociceptive neurons. *Neuron*. 2006; 52:691–703. [PubMed: 17114052]
- Hao J, Delmas P. Multiple desensitization mechanisms of mechanotransducer channels shape firing of mechanosensory neurons. *J Neurosci*. 2010; 30:13384–13395. [PubMed: 20926665]
- Hao J, Padilla F, Dandonneau M, Lavebratt C, Lesage F, Noel J, Delmas P. Kv1.1 channels act as mechanical brake in the senses of touch and pain. *Neuron*. 2013; 77:899–914. [PubMed: 23473320]
- Hirose K, Kadowaki S, Tanabe M, Takeshima H, Iino M. Spatiotemporal dynamics of inositol 1,4,5-trisphosphate that underlies complex Ca^{2+} mobilization patterns. *Science*. 1999; 284:1527–1530. [PubMed: 10348740]
- Honore E, Patel AJ, Chemin J, Suchyna T, Sachs F. Desensitization of mechano-gated K2P channels. *Proc Natl Acad Sci U S A*. 2006; 103:6859–6864. [PubMed: 16636285]
- Hu J, Lewin GR. Mechanosensitive currents in the neurites of cultured mouse sensory neurones. *J Physiol*. 2006; 577:815–828. [PubMed: 17038434]
- Imai Y, Itsuki K, Okamura Y, Inoue R, Mori MX. A self-limiting regulation of vasoconstrictor-activated TRPC3/C6/C7 channels coupled to $\text{PI}(4,5)\text{P}_2$ -diacylglycerol signalling. *J Physiol*. 2012; 590:1101–1119. [PubMed: 22183723]

- Itsuki K, Imai Y, Hase H, Okamura Y, Inoue R, Mori MX. PLC-mediated PI(4,5)P₂ hydrolysis regulates activation and inactivation of TRPC6/7 channels. *J Gen Physiol.* 2014; 143:183–201. [PubMed: 24470487]
- Jin X, Shah S, Liu Y, Zhang H, Lees M, Fu Z, Lippiat JD, Beech DJ, Sivaprasadarao A, Baldwin SA, Zhang H, Gamper N. Activation of the Cl[−] channel ANO1 by localized calcium signals in nociceptive sensory neurons requires coupling with the IP3 receptor. *Sci Signal.* 2013; 6:ra73. [PubMed: 23982204]
- Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature.* 2004; 427:260–265. [PubMed: 14712238]
- 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]
- Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, Nilius B, Voets T. TRPA1 acts as a cold sensor in vitro and in vivo. *Proc Natl Acad Sci U S A.* 2009; 106:1273–1278. [PubMed: 19144922]
- Khasar SG, Lin YH, Martin A, Dadgar J, McMahon T, Wang D, Hundle B, Aley KO, Isenberg W, McCarter G, Green PG, Hodge CW, Levine JD, Messing RO. A novel nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice. *Neuron.* 1999; 24:253–260. [PubMed: 10677042]
- 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:C92–99. [PubMed: 18495815]
- Klein RM, Ufret-Vincenty CA, Hua L, Gordon SE. Determinants of molecular specificity in phosphoinositide regulation. Phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂) is the endogenous lipid regulating TRPV1. *J Biol Chem.* 2008; 283:26208–26216. [PubMed: 18574245]
- Korzeniowski MK, Popovic MA, Szentpetery Z, Varnai P, Stojilkovic SS, Balla T. Dependence of STIM1/Orai1-mediated calcium entry on plasma membrane phosphoinositides. *J Biol Chem.* 2009; 284:21027–21035. [PubMed: 19483082]
- Kress M, Karasek J, Ferrer-Montiel AV, Scherbakov N, Haberberger RI. TRPC channels and diacylglycerol dependent calcium signaling in rat sensory neurons. *Histochem Cell Biol.* 2008; 130:655–667. [PubMed: 18663462]
- Kruger L, Kavookjian AM, Kumazawa T, Light AR, Mizumura K. Nociceptor structural specialization in canine and rodent testicular "free" nerve endings. *J Comp Neurol.* 2003; 463:197–211. [PubMed: 12815757]
- Kwak J. Capsaicin Blocks the Hyperpolarization-Activated Inward Currents via TRPV1 in the Rat Dorsal Root Ganglion Neurons. *Exp Neurobiol.* 2012; 21:75–82. [PubMed: 22792028]
- Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron.* 2006; 50:277–289. [PubMed: 16630838]
- Lechner SG, Lewin GR. Peripheral sensitisation of nociceptors via G-protein-dependent potentiation of mechanotransduction currents. *J Physiol.* 2009; 587:3493–3503. [PubMed: 19505980]
- Li L, Rutlin M, Abaira VE, Cassidy C, Kus L, Gong S, Jankowski MP, Luo W, Heintz N, Koerber HR, Woodbury CJ, Ginty DD. The functional organization of cutaneous low-threshold mechanosensory neurons. *Cell.* 2011; 147:1615–1627. [PubMed: 22196735]
- Li Z, He SQ, Xu Q, Yang F, Tiwari V, Liu Q, Tang Z, Han L, Chu YX, Wang Y, Hin N, Tsukamoto T, Slusher B, Guan X, Wei F, Raja SN, Dong X, Guan Y. Activation of MrgC receptor inhibits N-type calcium channels in small-diameter primary sensory neurons in mice. *Pain.* 2014; 155:1613–1621. [PubMed: 24813294]
- Liedtke W, Friedman JM. Abnormal osmotic regulation in *trpv4*^{−/−} mice. *Proc Natl Acad Sci U S A.* 2003; 100:13698–13703. [PubMed: 14581612]
- Lin Z, et al. Exome sequencing reveals mutations in TRPV3 as a cause of Olmsted syndrome. *Am J Hum Genet.* 2012; 90:558–564. [PubMed: 22405088]

- Link TM, Park U, Vonakis BM, Raben DM, Soloski MJ, Caterina RJ. TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. *Nat Immunol.* 2010; 11:232–239. [PubMed: 20118928]
- Linley JE, Rose K, Ooi L, Gamper N. Understanding inflammatory pain: ion channels contributing to acute and chronic nociception. *Pflugers Arch.* 2010; 459:657–669. [PubMed: 20162302]
- Linley JE, Rose K, Patil M, Robertson B, Akopian AN, Gamper N. Inhibition of M current in sensory neurons by exogenous proteases: a signaling pathway mediating inflammatory nociception. *J Neurosci.* 2008; 28:11240–11249. [PubMed: 18971466]
- 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]
- Liu B, Linley JE, Du X, Zhang X, Ooi L, Zhang H, Gamper N. The acute nociceptive signals induced by bradykinin in rat sensory neurons are mediated by inhibition of M-type K^+ channels and activation of Ca^{2+} -activated Cl^- -channels. *J Clin Invest.* 2010; 120:1240–1252. [PubMed: 20335661]
- 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]
- 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]
- Liu L, Oortgiesen M, Li L, Simon SA. Capsaicin inhibits activation of voltage-gated sodium currents in capsaicin-sensitive trigeminal ganglion neurons. *J Neurophysiol.* 2001; 85:745–758. [PubMed: 11160509]
- Liu Q, Dong X. The role of the Mrgpr receptor family in itch. *Handb Exp Pharmacol.* 2015; 226:71–88. [PubMed: 25861775]
- Liu Q, Sikand P, Ma C, Tang Z, Han L, Li Z, Sun S, LaMotte RH, Dong X. Mechanisms of itch evoked by beta-alanine. *J Neurosci.* 2012; 32:14532–14537. [PubMed: 23077038]
- Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, Ru F, Guan Y, Weng HJ, Geng Y, Udem BJ, Kollarik M, Chen ZF, Anderson DJ, Dong X. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell.* 2009; 139:1353–1365. [PubMed: 20004959]
- Logothetis DE, Petrou VI, Zhang M, Mahajan R, Meng XY, Adney SK, Cui M, Baki L. Phosphoinositide Control of Membrane Protein Function: A Frontier Led by Studies on Ion Channels. *Annu Rev Physiol.* 2015; 77:81–104. [PubMed: 25293526]
- Lopes CM, Rohacs T, Czirjak G, Balla T, Enyedi P, Logothetis DE. PIP_2 hydrolysis underlies agonist-induced inhibition and regulates voltage gating of two-pore domain K^+ channels. *J Physiol.* 2005; 564:117–129. [PubMed: 15677683]
- Lukacs V, Rives JM, Sun X, Zakharian E, Rohacs T. Promiscuous activation of transient receptor potential vanilloid 1 channels by negatively charged intracellular lipids, the key role of endogenous phosphoinositides in maintaining channel activity. *J Biol Chem.* 2013a; 288:35003–35013. [PubMed: 24158445]
- Lukacs V, Thyagarajan B, Varnai P, Balla A, Balla T, Rohacs T. Dual regulation of TRPV1 by phosphoinositides. *J Neurosci.* 2007; 27:7070–7080. [PubMed: 17596456]
- Lukacs V, Yudin Y, Hammond GR, Sharma E, Fukami K, Rohacs T. Distinctive changes in plasma membrane phosphoinositides underlie differential regulation of TRPV1 in nociceptive neurons. *Journal of Neuroscience.* 2013b; 33:11451–11463. [PubMed: 23843517]
- Luo W, Wickramasinghe SR, Savitt JM, Griffin JW, Dawson TM, Ginty DD. A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. *Neuron.* 2007; 54:739–754. [PubMed: 17553423]
- Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature.* 2007; 445:541–545. [PubMed: 17237762]
- Maingret F, Lauritzen I, Patel AJ, Heurteaux C, Reyes R, Lesage F, Lazdunski M, Honore E. TREK-1 is a heat-activated background K^+ channel. *EMBO J.* 2000; 19:2483–2491. [PubMed: 10835347]

- Mandadi S, Sokabe T, Shibasaki K, Katanosaka K, Mizuno A, Moqrich A, Patapoutian A, Fukumi-Tominaga T, Mizumura K, Tominaga M. TRPV3 in keratinocytes transmits temperature information to sensory neurons via ATP. *Pflugers Arch.* 2009; 458:1093–1102. [PubMed: 19669158]
- Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP. TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol.* 2005; 7:179–185. [PubMed: 15665854]
- McKemy DD, Neuhauser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature.* 2002; 416:52–58. [PubMed: 11882888]
- Mercado J, Gordon-Shaag A, Zagotta WN, Gordon SE. Ca^{2+} -dependent desensitization of TRPV2 channels is mediated by hydrolysis of phosphatidylinositol 4,5-bisphosphate. *J Neurosci.* 2010; 30:13338–13347. [PubMed: 20926660]
- Mikoshiba K. Role of IP_3 receptor signaling in cell functions and diseases. *Adv Biol Regul.* 2015; 57:217–227. [PubMed: 25497594]
- Mo G, Peleshok JC, Cao CQ, Ribeiro-da-Silva A, Seguela P. Control of P2X3 channel function by metabotropic P2Y2 utp receptors in primary sensory neurons. *Mol Pharmacol.* 2013; 83:640–647. [PubMed: 23249537]
- 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]
- Moparthi L, Survery S, Kreir M, Simonsen C, Kjellbom P, Hogestatt ED, Johanson U, Zygmunt PM. Human TRPA1 is intrinsically cold- and chemosensitive with and without its N-terminal ankyrin repeat domain. *Proc Natl Acad Sci U S A.* 2014; 111:16901–16906. [PubMed: 25389312]
- Newton AC. Protein kinase C: poised to signal. *Am J Physiol Endocrinol Metab.* 2010; 298:E395–402. [PubMed: 19934406]
- Nilius B, Biro T, Owsianik G. TRPV3: time to decipher a poorly understood family member! *J Physiol.* 2014; 592:295–304. [PubMed: 23836684]
- Nilius B, Voets T. The puzzle of TRPV4 channelopathies. *EMBO Rep.* 2013; 14:152–163. [PubMed: 23306656]
- Nowycky MC, Fox AP, Tsien RW. Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature.* 1985; 316:440–443. [PubMed: 2410796]
- 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]
- Numazaki M, Tominaga T, Toyooka H, Tominaga M. Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. *J Biol Chem.* 2002; 277:13375–13378. [PubMed: 11884385]
- Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, Lewis RS. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell.* 2009; 136:876–890. [PubMed: 19249086]
- Park U, Vastani N, Guan Y, Raja SN, Koltzenburg M, Caterina MJ. TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. *J Neurosci.* 2011; 31:11425–11436. [PubMed: 21832173]
- Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A. A TRP channel that senses cold stimuli and menthol. *Cell.* 2002a; 108:705–715. [PubMed: 11893340]
- Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Bevan S, Patapoutian A. A heat-sensitive TRP channel expressed in keratinocytes. *Science.* 2002b; 296:2046–2049. [PubMed: 12016205]
- Pian P, Bucci A, Decostanzo A, Robinson RB, Siegelbaum SA. Modulation of cyclic nucleotide-regulated HCN channels by PIP_2 and receptors coupled to phospholipase C. *Pflugers Arch.* 2007; 455:125–145. [PubMed: 17605039]
- Piper AS, Docherty RJ. One-way cross-desensitization between P2X purinoceptors and vanilloid receptors in adult rat dorsal root ganglion neurones. *J Physiol.* 2000; 523:685–696. Pt 3. [PubMed: 10718747]

- Poblete H, Oyarzun I, Olivero P, Comer J, Zuniga M, Sepulveda RV, Baez-Nieto D, Gonzalez Leon C, Gonzalez-Nilo F, Latorre R. Molecular Determinants of Phosphatidylinositol 4,5Bisphosphate (PI(4,5)P₂) Binding to Transient Receptor Potential V1 (TRPV1) Channels. *J Biol Chem*. 2014; 290:2086–2098. [PubMed: 25425643]
- Pochynnyuk O, Bugaj V, Stockand JD. Physiologic regulation of the epithelial sodium channel by phosphatidylinositides. *Curr Opin Nephrol Hypertens*. 2008; 17:533–540. [PubMed: 18695396]
- Pritchard HA, Leblanc N, Albert AP, Greenwood IA. Inhibitory role of phosphatidylinositol 4,5-bisphosphate on TMEM16A-encoded calcium-activated chloride channels in rat pulmonary artery. *Br J Pharmacol*. 2014; 171:4311–4321. [PubMed: 24834965]
- Putney JW Jr. The enigmatic TRPCs: multifunctional cation channels. *Trends Cell Biol*. 2004; 14:282–286. [PubMed: 15183184]
- Putney JW Jr. Recent breakthroughs in the molecular mechanism of capacitative calcium entry (with thoughts on how we got here). *Cell Calcium*. 2007; 42:103–110. [PubMed: 17349691]
- Putney JW, Tomita T. Phospholipase C signaling and calcium influx. *Adv Enzyme Regul*. 2011; 52:152–164.
- Qu L, Li Y, Pan X, Zhang P, LaMotte RH, Ma C. Transient receptor potential canonical 3 (TRPC3) is required for IgG immune complex-induced excitation of the rat dorsal root ganglion neurons. *J Neurosci*. 2012; 32:9554–9562. [PubMed: 22787041]
- Quick K, et al. TRPC3 and TRPC6 are essential for normal mechanotransduction in subsets of sensory neurons and cochlear hair cells. *Open Biol*. 2012; 2:120068. [PubMed: 22724068]
- Quinn KV, Behe P, Tinker A. Monitoring changes in membrane phosphatidylinositol 4,5-bisphosphate in living cells using a domain from the transcription factor tubby. *J Physiol*. 2008; 586:2855–2871. [PubMed: 18420701]
- Ranade SS, Woo SH, Dubin AE, Moshourab RA, Wetzel C, Petrus M, Mathur J, Begay V, Coste B, Mainquist J, Wilson AJ, Francisco AG, Reddy K, Qiu Z, Wood JN, Lewin GR, Patapoutian A. Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature*. 2014; 516:121–125. [PubMed: 25471886]
- Rohacs T. Regulation of Transient Receptor Potential channels by the phospholipase C pathway. *Advances in Biological Regulation*. 2013; 53:341–355. [PubMed: 23916247]
- Rohacs T. Phosphoinositide regulation of TRP channels. *Handb Exp Pharmacol*. 2014; 233:1143–1176. [PubMed: 24961984]
- Rohacs T. Phosphoinositide regulation of TRPV1 revisited. *Pflugers Arch*. 2015; 467:1851–1869. [PubMed: 25754030]
- Rohacs T, Lopes CM, Michailidis I, Logothetis DE. PI(4,5)P₂ regulates the activation and desensitization of TRPM8 channels through the TRP domain. *Nat Neurosci*. 2005; 8:626–634. [PubMed: 15852009]
- Rose K, Ooi L, Dalle C, Robertson B, Wood IC, Gamper N. Transcriptional repression of the M channel subunit Kv7.2 in chronic nerve injury. *Pain*. 2011; 152:742–754. [PubMed: 21345591]
- Rosenbaum T, Gordon-Shaag A, Munari M, Gordon SE. Ca²⁺/calmodulin modulates TRPV1 activation by capsaicin. *J Gen Physiol*. 2004; 123:53–62. [PubMed: 14699077]
- Rugiero F, Drew LJ, Wood JN. Kinetic properties of mechanically activated currents in spinal sensory neurons. *J Physiol*. 2010; 588:301–314. [PubMed: 19948656]
- Sachs F. Stretch-activated ion channels: what are they? *Physiology (Bethesda)*. 2010; 25:50–56. [PubMed: 20134028]
- Schnorr S, Eberhardt M, Kistner K, Rajab H, Kasser J, Hess A, Reeh P, Ludwig A, Herrmann S. HCN2 channels account for mechanical (but not heat) hyperalgesia during long-standing inflammation. *Pain*. 2014; 155:1079–1090. [PubMed: 24525276]
- Schroeder BC, Cheng T, Jan YN, Jan LY. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell*. 2008; 134:1019–1029. [PubMed: 18805094]
- Smith T, Al Otaibi M, Sathish J, Djouhri L. Increased expression of HCN2 channel protein in L4 dorsal root ganglion neurons following axotomy of L5- and inflammation of L4-spinal nerves in rats. *Neuroscience*. 2015; 295:90–102. [PubMed: 25813712]
- Souslova V, Cesare P, Ding Y, Akopian AN, Stanfa L, Suzuki R, Carpenter K, Dickenson A, Boyce S, Hill R, Nebunius-Oosthuizen D, Smith AJ, Kidd EJ, Wood JN. Warm-coding deficits and

- aberrant inflammatory pain in mice lacking P2X3 receptors. *Nature*. 2000; 407:1015–1017. [PubMed: 11069182]
- Spasova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL. A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A*. 2006; 103:16586–16591. [PubMed: 17056714]
- Staaf S, Oerther S, Lucas G, Mattsson JP, Ernfors P. Differential regulation of TRP channels in a rat model of neuropathic pain. *Pain*. 2009; 144:187–199. [PubMed: 19446956]
- Stanchev D, Blosa M, Milius D, Gerevich Z, Rubini P, Schmalzing G, Eschrich K, Schaefer M, Wirkner K, Illes P. Cross-inhibition between native and recombinant TRPV1 and P2X3 receptors. *Pain*. 2009; 143:26–36. [PubMed: 19223122]
- 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]
- Stone LS, Molliver DC. In search of analgesia: emerging roles of GPCRs in pain. *Mol Interv*. 2009; 9:234–251. [PubMed: 19828831]
- Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell*. 2003; 112:819–829. [PubMed: 12654248]
- Suh BC, Hille B. Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. *Neuron*. 2002; 35:507–520. [PubMed: 12165472]
- Suh BC, Hille B. PIP₂ is a necessary cofactor for ion channel function: how and why? *Annu Rev Biophys*. 2008; 37:175–195. [PubMed: 18573078]
- Suh BC, Leal K, Hille B. Modulation of high-voltage activated Ca(2+) channels by membrane phosphatidylinositol 4,5-bisphosphate. *Neuron*. 2010; 67:224–238. [PubMed: 20670831]
- Szallasi A, Blumberg PM. Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol Rev*. 1999; 51:159–212. [PubMed: 10353985]
- Szentpetery Z, Balla A, Kim YJ, Lemmon MA, Balla T. Live cell imaging with protein domains capable of recognizing phosphatidylinositol 4,5-bisphosphate; a comparative study. *BMC Cell Biol*. 2009; 10:67. [PubMed: 19769794]
- Takahashi N, Hamada-Nakahara S, Itoh Y, Takemura K, Shimada A, Ueda Y, Kitamata M, Matsuoka R, Hanawa-Suetsugu K, Senju Y, Mori MX, Kiyonaka S, Kohda D, Kitao A, Mori Y, Suetsugu S. TRPV4 channel activity is modulated by direct interaction of the ankyrin domain to PI(4,5)P₂. *Nat Commun*. 2014; 5:4994. [PubMed: 25256292]
- Takayama Y, Uta D, Furue H, Tominaga M. Pain-enhancing mechanism through interaction between TRPV1 and anoctamin 1 in sensory neurons. *Proc Natl Acad Sci U S A*. 2015; 112:5213–5218. [PubMed: 25848051]
- Tappe-Theodor A, Constantin CE, Tegeder I, Lechner SG, Langeslag M, Lepczynsky P, Wirotanseng RI, Kurejova M, Agarwal N, Nagy G, Todd A, Wettschureck N, Offermanns S, Kress M, Lewin GR, Kuner R. Gα_{q/11} signaling tonically modulates nociceptor function and contributes to activity-dependent sensitization. *Pain*. 2012; 153:184–196. [PubMed: 22071319]
- Taruno A, Vingdteux V, Ohmoto M, Ma Z, Dvoryanchikov G, Li A, Adrien L, Zhao H, Leung S, Abernethy M, Koppel J, Davies P, Civan MM, Chaudhari N, Matsumoto I, Hellekant G, Tordoff MG, Marambaud P, Foscett JK. CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. *Nature*. 2013; 495:223–226. [PubMed: 23467090]
- Thakur M, Crow M, Richards N, Davey GI, Levine E, Kelleher JH, Agley CC, Denk F, Harridge SD, McMahon SB. Defining the nociceptor transcriptome. *Front Mol Neurosci*. 2014; 7:87. [PubMed: 25426020]
- Than JY, Li L, Hasan R, Zhang X. Excitation and modulation of TRPA1, TRPV1, and TRPM8 channel-expressing sensory neurons by the pruritogen chloroquine. *J Biol Chem*. 2013; 288:12818–12827. [PubMed: 23508958]
- Tian Y, Schreiber R, Wanitchakool P, Kongsuphol P, Sousa M, Uliyakina I, Palma M, Faria D, Traynor-Kaplan AE, Fragata JI, Amaral MD, Kunzelmann K. Control of TMEM16A by

- INO-4995 and other inositolphosphates. *Br J Pharmacol.* 2013; 168:253–265. [PubMed: 22946960]
- Toth BI, Konrad M, Ghosh D, Mohr F, Halaszovich CR, Leitner MG, Vriens J, Oberwinkler J, Voets T. Regulation of the transient receptor potential channel TRPM3 by phosphoinositides. *J Gen Physiol.* 2015; 146:51–63. [PubMed: 26123194]
- 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.* 2009; 457:757–769. [PubMed: 18665391]
- Tulleuda A, Cokic B, Callejo G, Saiani B, Serra J, Gasull X. TRESK channel contribution to nociceptive sensory neurons excitability: modulation by nerve injury. *Mol Pain.* 2011; 7:30. [PubMed: 21527011]
- Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, Hjerling-Leffler J, Haeggstrom J, Kharchenko O, Kharchenko PV, Linnarsson S, Ernfors P. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci.* 2015; 18:145–153. [PubMed: 25420068]
- van der Wal J, Habets R, Varnai P, Balla T, Jalink K. Monitoring agonist-induced phospholipase C activation in live cells by fluorescence resonance energy transfer. *J Biol Chem.* 2001; 276:15337–15344. [PubMed: 11152673]
- Vandewauw I, Owsianik G, Voets T. Systematic and quantitative mRNA expression analysis of TRP channel genes at the single trigeminal and dorsal root ganglion level in mouse. *BMC Neurosci.* 2013; 14:21. [PubMed: 23410158]
- 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 living cells. *J Cell Biol.* 2006; 175:377–382. [PubMed: 17088424]
- Velazquez KT, Mohammad H, Sweitzer SM. Protein kinase C in pain: involvement of multiple isoforms. *Pharmacol Res.* 2007; 55:578–589. [PubMed: 17548207]
- Veldhuis NA, Poole DP, Grace M, McIntyre P, Bunnett NW. The G protein-coupled receptor-transient receptor potential channel axis: molecular insights for targeting disorders of sensation and inflammation. *Pharmacol Rev.* 2015; 67:36–73. [PubMed: 25361914]
- Viana F, de la Pena E, Belmonte C. Specificity of cold thermotransduction is determined by differential ionic channel expression. *Nat Neurosci.* 2002; 5:254–260. [PubMed: 11836533]
- Volkers L, Mechoukhi Y, Coste B. Piezo channels: from structure to function. *Pflugers Arch.* 2014
- Vriens J, Held K, Janssens A, Toth BI, Kerselaers S, Nilius B, Vennekens R, Voets T. Opening of an alternative ion permeation pathway in a nociceptor TRP channel. *Nat Chem Biol.* 2014a; 10:188–195. [PubMed: 24390427]
- Vriens J, Nilius B, Voets T. Peripheral thermosensation in mammals. *Nat Rev Neurosci.* 2014b; 15:573–589. [PubMed: 25053448]
- Vriens J, Owsianik G, Hofmann T, Philipp SE, Stab J, Chen X, Benoit M, Xue F, Janssens A, Kerselaers S, Oberwinkler J, Vennekens R, Gudermann T, Nilius B, Voets T. TRPM3 is a nociceptor channel involved in the detection of noxious heat. *Neuron.* 2011; 70:482–494. [PubMed: 21555074]
- Vriens J, Watanabe H, Janssens A, Droogmans G, Voets T, Nilius B. Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. *Proc Natl Acad Sci U S A.* 2004; 101:396–401. [PubMed: 14691263]
- Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Dufer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J. Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. *Nat Cell Biol.* 2008; 10:1421–1430. [PubMed: 18978782]
- Walsh CM, Chvanov M, Haynes LP, Petersen OH, Tepikin AV, Burgoyne RD. Role of phosphoinositides in STIM1 dynamics and store-operated calcium entry. *Biochem J.* 2010; 425:159–168. [PubMed: 19843011]
- 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; 283:32691–32703. [PubMed: 18775987]

- Waxman SG, Zamponi GW. Regulating excitability of peripheral afferents: emerging ion channel targets. *Nat Neurosci.* 2014; 17:153–163. [PubMed: 24473263]
- Wemmie JA, Taugher RJ, Kreple CJ. Acid-sensing ion channels in pain and disease. *Nat Rev Neurosci.* 2013; 14:461–471. [PubMed: 23783197]
- Wilke BU, Lindner M, Greifenberg L, Albus A, Kronimus Y, Bunemann M, Leitner MG, Oliver D. Diacylglycerol mediates regulation of TASK potassium channels by Gq-coupled receptors. *Nat Commun.* 2014; 5:5540. [PubMed: 25420509]
- Wilson SR, Gerhold KA, Bifolck-Fisher A, Liu Q, Patel KN, Dong X, Bautista DM. TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci.* 2011; 14:595–602. [PubMed: 21460831]
- Wu D, Huang W, Richardson PM, Priestley JV, Liu M. TRPC4 in rat dorsal root ganglion neurons is increased after nerve injury and is necessary for neurite outgrowth. *J Biol Chem.* 2008; 283:416–426. [PubMed: 17928298]
- Wu L, Bauer CS, Zhen XG, Xie C, Yang J. Dual regulation of voltage-gated calcium channels by PtdIns(4,5)P₂. *Nature.* 2002; 419:947–952. [PubMed: 12410316]
- Wu LJ, Sweet TB, Clapham DE. International Union of Basic and Clinical Pharmacology. LXXXVI. Current progress in the mammalian TRP ion channel family. *Pharmacol Rev.* 2010; 62:381–404. [PubMed: 20716668]
- Wu ZZ, Chen SR, Pan HL. Transient receptor potential vanilloid type 1 activation down-regulates voltage-gated calcium channels through calcium-dependent calcineurin in sensory neurons. *J Biol Chem.* 2005; 280:18142–18151. [PubMed: 15746091]
- Wu ZZ, Chen SR, Pan HL. Signaling mechanisms of down-regulation of voltage-activated Ca²⁺ channels by transient receptor potential vanilloid type 1 stimulation with olvanil in primary sensory neurons. *Neuroscience.* 2006; 141:407–419. [PubMed: 16678970]
- Xia J, Pan R, Gao X, Meucci O, Hu H. Native store-operated calcium channels are functionally expressed in mouse spinal cord dorsal horn neurons and regulate resting calcium homeostasis. *J Physiol.* 2014; 592:3443–3461. [PubMed: 24860175]
- Xiao B, Coste B, Mathur J, Patapoutian A. Temperature-dependent STIM1 activation induces Ca²⁺ influx and modulates gene expression. *Nat Chem Biol.* 2011; 7:351–358. [PubMed: 21499266]
- Xu C, Watras J, Loew LM. Kinetic analysis of receptor-activated phosphoinositide turnover. *J Cell Biol.* 2003; 161:779–791. [PubMed: 12771127]
- Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D, Ge P, Lilly J, Silos-Santiago I, Xie Y, DiStefano PS, Curtis R, Clapham DE. TRPV3 is a calcium-permeable temperature-sensitive cation channel. *Nature.* 2002; 418:181–186. [PubMed: 12077604]
- Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, Park SP, Lee J, Lee B, Kim BM, Raouf R, Shin YK, Oh U. TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature.* 2008; 455:1210–1215. [PubMed: 18724360]
- Yu FH, Catterall WA. The VGL-chnome: a protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci STKE.* 2004; 2004:re15. [PubMed: 15467096]
- Yudin Y, Lukacs V, Cao C, Rohacs T. Decrease in phosphatidylinositol 4,5-bisphosphate levels mediates desensitization of the cold sensor TRPM8 channels. *J Physiol.* 2011; 589:6007–6027. [PubMed: 22005680]
- Yudin Y, Rohacs T. Regulation of TRPM8 channel activity. *Mol Cell Endocrinol.* 2011; 353:68–74. [PubMed: 22061619]
- Yudin Y, Rohacs T. The role of PLC δ 4 in regulation of TRPM8 channels in sensory neurons. *The Journal of Neuroscience Abstract.* 2015
- Zakharian E, Cao C, Rohacs T. Gating of transient receptor potential melastatin 8 (TRPM8) channels activated by cold and chemical agonists in planar lipid bilayers. *J Neurosci.* 2010; 30:12526–12534. [PubMed: 20844147]
- Zakharian E, Thyagarajan B, French RJ, Pavlov E, Rohacs T. Inorganic polyphosphate modulates TRPM8 channels. *PLoS One.* 2009; 4:e5404. [PubMed: 19404398]
- Zhang H, Craciun LC, Mirshahi T, Rohacs T, Lopes CM, Jin T, Logothetis DE. PIP₂ activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. *Neuron.* 2003; 37:963–975. [PubMed: 12670425]

- 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]
- Zhang XF, Han P, Neelands TR, McGaraughty S, Honore P, Surowy CS, Zhang D. Coexpression and activation of TRPV1 suppress the activity of the KCNQ2/3 channel. *J Gen Physiol.* 2011; 138:341–352. [PubMed: 21844219]
- Zhao C, Leitges M, Gereau R.W.t. Isozyme-specific effects of protein kinase C in pain modulation. *Anesthesiology.* 2011; 115:1261–1270. [PubMed: 22042410]
- Zimmermann K, Hein A, Hager U, Kaczmarek JS, Turnquist BP, Clapham DE, Reeh PW. Phenotyping sensory nerve endings in vitro in the mouse. *Nat Protoc.* 2009; 4:174–196. [PubMed: 19180088]
- Zimmermann K, Lennerz JK, Hein A, Link AS, Kaczmarek JS, Delling M, Uysal S, Pfeifer JD, Riccio A, Clapham DE. Transient receptor potential cation channel, subfamily C, member 5 (TRPC5) is a cold-transducer in the peripheral nervous system. *Proc Natl Acad Sci U S A.* 2011; 108:18114–18119. [PubMed: 22025699]
- Zolles G, Klocker N, Wenzel D, Weisser-Thomas J, Fleischmann BK, Roeper J, Fakler B. Pacemaking by HCN channels requires interaction with phosphoinositides. *Neuron.* 2006; 52:1027–1036. [PubMed: 17178405]
- Zygmunt PM, Hogestatt ED. Trpa1. *Handb Exp Pharmacol.* 2014; 222:583–630. [PubMed: 24756722]

Figure 1.
Phosphoinositide metabolism