Much can be learned from the structural and functional properties shared by the six members of the transient receptor potential vanilloid (TRPV) family of ion channels. In addition, the specializations of each for their physiological roles shed light on the workings of the entire family. This chapter will focus on gating of TRPV1–6 channels, with discussion of permeation, expression patterns, and physiological roles covered elsewhere.1–5

28.1 STRUCTURE OF TRPV CHANNELS

TRPV channels are members of the voltage-gated superfamily of ion channels with six-membrane-spanning domains, intracellular N- and C-termini, a (weak) voltage sensor domain in S1–S4, and a pore domain formed by S5–P–S6. Each functional TRPV channel is composed of four identical subunits.

Electron cryomicroscopy of TRPV16 and TRPV47 shows the structure of these channels to resemble that of Kv channels, 8 with a transmembrane structure of about 60–80 Å on a side with a hanging gondola about 100–110 Å tall. The N-termini of TRPV channels include an ankyrin repeat domain, a feature shared with TRPC channels. The x-ray crystal structures of the ankyrin repeat domains of TRPV1,9 TRPV2,10,11 TRPV4,12 and TRPV613 channels have been solved in which six ankyrin repeats are observed. In the structures of TRPV1 and TRPV4, an ATP molecule binds to the fingers formed by the first and second ankyrin repeats. Interestingly, although the structure of the ankyrin repeats of TRPV2 is extremely similar to that of the ankyrin repeats of TRPV1, N-terminal fragments of TRPV1 bind ATP in vitro but fragments of TRPV2 do not. Although ATP has no effect on TRPV1 or TRPV2 in excised patches,14 differences in the properties of the two channels may be useful in determining whether direct binding has a physiological role in regulating TRPV1.

28.2 TEMPERATURE DEPENDENCE OF GATING

TRPV1, TRPV2, TRPV3, and TRPV4 can all be activated by elevated temperature. Temperature dependence is typically discussed in terms of \( Q_{10} \), the ratio of the rate of a reaction at one temperature to that at another. Although all enzymes are temperature sensitive to some extent, with typical \( Q_{10} \) values around 2, TRPV1–4 have \( Q_{10} \) values in the range of 10–40.

Whether a discrete temperature-sensing domain exists in TRP channels is currently the subject of active debate. Different regions of the N-terminal domain15 as well as the C-terminal domain,16 the pore turret,17,18 and the pore/extracellular loop following the pore19,20 have all been suggested to contain a temperature sensor.

An alternative mechanism to a discrete temperature-sensitive domain is based on first principles of thermodynamics.21 Because proteins can have high heat capacity (\( \Delta C_p \)), enthalpy (\( \Delta H \)) and entropy (\( \Delta S \)) cannot be thought of as independent of temperature. A relatively simple derivation leads to a surprising U-shaped dependence of the conformational change on temperature (Figure 28.1). Applied to TRP channels, this means that all that are activated by heat may also activated by cold, and vice versa.

Whether we observe activation by heat or cold would depend on the temperature range for each turn of the U. Starting at the vertex (Figure 28.1, arrow), if the turn on the left is within the physiological range, then cold activation will be observed, whereas if the turn on the right is within the physiological range, then activation by heat is observed. This model-free framework predicts that, if it were practical to lower or raise the experimental temperature to any arbitrary value, then activation by both cold and heat would be observed for TRPV1–TRPV4. In this mechanism, a discrete temperature sensor would be simply
TRPV channels

A region of the protein with particularly high heat capacity. However, a discrete temperature sensor is not needed, as a change in heat capacity associated with the global conformational change would be sufficient to account for the steep temperature sensitivity of TRP channels.

### 28.3 REGULATION BY G-PROTEIN-COUPLED RECEPTORS

Increased sensitivity of pain-receptor neurons in the setting of inflammation is due, in part, to activation of G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases. In particular, Gq-coupled receptors, such as the bradykinin receptor, contribute to sensitization via their coupling to phospholipase C β (PLCβ). PLCβ hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to generate diacylglycerol and inositol trisphosphate. The diacylglycerol in turn activates protein kinase C. Phosphorylation of two serines, S502 and S800, potentiate activation of TRPV1 by capsaicin. Association of TRPV1 (and TRPV2, TRPV3, and TRPV4) with A kinase anchoring protein 79/150 (AKAP79/150) appears to play a role in bradykinin-mediated sensitization of TRPV1 by positioning PKC appropriately to phosphorylate TRPV1. Interestingly, AKAP79/150 may also play a role in desensitization. The list of GPCRs that can regulate TRPV1 is extensive and growing; additional signaling pathways may well be revealed.

#### 28.3.1 REGULATION BY PIP2

As controversial as is the mechanism of heat activation, the mechanism and role of regulation by the signaling lipid PIP2 are more so, with most of the work performed on TRPV1. Indeed, even whether PIP2 is an activator or an inhibitor of TRPV1 is subject to intense debate. It is therefore worth expanding on the evidence for activation and inhibition.

#### 28.3.2 PIP2 AS AN INHIBITOR OF TRPV1

The first proposal that PIP2 may inhibit TRPV1 was based on the sensitization of the channels by Gq-coupled GPCRs, described earlier. If Gq activation of PLCβ were sufficiently strong, the level of PIP2 in the inner leaflet of the plasma membrane might drop precipitously. Near-complete depletion of PIP2 by Gαq-coupled receptors is believed to regulate a number of other ion channels, including KIR and KCNQ channels. If PIP2 tonically inhibits TRPV1 in resting cells, and activation of Gq-coupled GPCRs activates PLCβ to deplete PIP2, then Gq-coupled GPCRs could indeed relieve the tonic inhibition, giving sensitization.

Three types of evidence have been presented to support PIP2 as a direct inhibitor of TRPV1. First, the application of an antibody against PIP2 to excised patches from cells expressing TRPV1 transiently increases the capsaicin-activated current. PIP2 is not inherently antigenic; beads coated to present many PIP2 head groups are used as the antigen. Furthermore, the specificity of the anti-PIP2 antibody is unknown. Taken at face value, however, the increased TRPV1 activity observed in response to sequestration of PIP2 by the anti-PIP2 antibody in inside-out excised patches supports a role of PIP2 as an inhibitor of TRPV1.

The second basis for PIP2 as an inhibitor of TRPV1 is that the application of bacterial phosphatidylinositol (PI)-PLC to patches increased both basal currents and capsaicin-activated currents. However, biochemical and structural studies have shown that bacterial PI-PLC, in contrast to mammalian PI-PLC, does not hydrolyze phosphatidylinositol phosphate (PIP) or PIP3. It does cleave PI robustly, however, raising the question of whether PI may inhibit TRPV1.

The third line of evidence supporting PIP2 as an inhibitor comes from experiments using purified TRPV1 reconstituted into synthetic liposomes of defined composition. The temperature and capsaicin dependence were measured in liposomes that included either no phosphoinositides, 4% PI, 4% phosphatidylinositol 4-phosphate (PI(4)P), 4% phosphatidylinositol 3-phosphate (PI(3)P), 4% phosphatidylinositol 3,4,5-trisphosphate (PIP3), or phosphatidylinositol 3,4,5-trisphosphate (PIP3). With the exception of PIP3, all the phosphoinositides significantly increased the threshold for activation by from about 27°C to about 38°C (Figure 28.2). PI, PI(4)P, and PIP2 also decreased the apparent affinity for capsaicin, whereas PIP3 did not.
Although the simplest explanation of these data is that phosphoinositides inhibit TRPV1, a number of experimental and conceptual concerns call this conclusion into question. The greatest of these concerns is the symmetrical nature of the liposome membrane, that is, what is the effect of having PIP2 on both sides of the channels? When tested with outside-out patches from cells expressing TRPV1, adding PIP2 to the extracellular leaflet robustly inhibited the capsaicin-activated currents (Figure 28.3). Although reconstituting channels into asymmetric bilayers would be required to put the case to rest definitively, the observation that PIP2 is activating when in the physiological leaflet of the plasma membrane only, and inhibits when present in both the inner and outer leaflets of both cell and synthetic membranes, suggests that the inhibition observed is not physiological.

A number of other caveats obfuscate the case for inhibition. These include the change in shape of the temperature dependence in TRPV1 reconstituted into phosphoinositides-free membranes compared to those including phosphoinositides (but not PIP2; Figure 28.2). The relationship between the shape of the temperature vs. normalized current curve and the threshold for activation (Figure 28.2) is not understood, and the interpretation of changes in the shape of the temperature dependence of activation is unclear. In addition, no controls were described to show that the mole fraction of phosphoinositides in the TRPV1-containing liposomes reflected the mole fraction in the lipid mixture used to make the liposomes. This is an important point because charged phosphoinositides come out of the organic solvent (chloroform in this case) earlier than the other lipids used. Typically, this leaves separate patches of phosphoinositides and other lipids in the vessel, and a heterogeneous population is formed that does not fully mix even after many freeze–thaw cycles. The phosphoinositides with the highest charge, PIP3, are the most likely to be affected by incorporation artifacts, interesting given that the behavior of TRPV1 in PIP3-containing liposomes was distinct from that of TRPV1 in liposomes containing all other phosphoinositides.

Based, in part, on the experiments with reconstituted channels, a mechanism for inhibition of TRPV1 by phosphoinositides has been proposed. The distal C-terminal region contains a number of basic residues that are postulated to interact with the negatively charged phosphoinositides head groups. This interaction is proposed to stabilize an inhibited conformation of TRPV1. In the absence of phosphoinositides, the inhibited conformation is proposed to be less stable relative to the noninhibited conformation so that depletion of PIP2 would be observed an increase in open probability. The interaction between the basic residues and the membrane must be more than just electrostatic in this mechanism, because PI inhibited reconstituted TRPV1 but phosphatidic acid, with the same charge as PI, did not. In addition, a purely electrostatic interaction would be expected to be the strongest for PIP3, yet no effect of PIP3 was observed. A protein that binds PI, PI(3)P, PI(4)P, and PIP2, but not PIP3 or phosphatidic acid, would indeed be very interesting.

**Figure 28.3** Leaflet-specific regulation of TRPV1 by PIP2. (a) Application of PIP2 to the intracellular leaflet, via addition to the bath with an inside-out patch, causes activation of TRPV1. (b) Application of PIP2 to the extracellular leaflet, via addition to the bath with an outside-out patch, causes inhibition of TRPV1. (From Senning, E.N. et al., J Biol Chem. 289(16), 10999, 2014.)

**28.3.3 PIP2 AS AN ACTIVATOR OF TRPV1**

PIP2 has been proposed to play a role in Ca2+-dependent desensitization of TRPV1, TRPV2, and TRPV6.34–32 TRPV channels are highly permeable to Ca2+, a convenient property that allows Ca2+ imaging to be used to assay their activity in cells. However, too much Ca2+ influx is poorly tolerated, and cells have a number of mechanisms to return calcium to resting levels. Among them is desensitization of TRPV channels, a self-limiting response in which the Ca2+ coming in through the channels also signals to reduce their activity. The desensitizing signaling pathway has been proposed to involve the stimulation of PLC by Ca2+ and PLC-mediated depletion of PIP2. Depletion of the activator PIP2 from TRPV1 would be observed as a decrease in TRPV1 activity, that is, desensitization.

For a signal to be identified as mediating an effect, a number of criteria must be met. The signal must be necessary, sufficient, and appropriately localized and occur either prior to or simultaneous with the observed effect. To answer whether depletion of PIP2 is the signal that reduces TRPV1 activity in response to Ca2+, we can examine each of these criteria in turn.

Is PIP2 depletion necessary for desensitization? If so, then doping the plasma membrane’s inner leaflet with extra PIP2 should eliminate or reduce desensitization. Whole-cell voltage-clamp experiments in which PIP2 was dialyzed into the cell via the patch pipette showed that desensitization was significantly reduced.8 Is PIP2 depletion sufficient for desensitization? This question has been addressed in both excised patches and in whole cells. In excised patches, application of a lysine polymer inhibited capsaicin-activated currents. The cationic lysine polymer is thought to bind to anionic lipids head groups, effectively reducing...
the free concentration of these lipids. Inhibition of TRPV1 by sequestering anionic lipids with the lysine polymer is consistent with an activating effect of PIP₂, but the nonselective nature of the reagent prevents a definitive interpretation.

More selective than a lysine polymer is the pleckstrin homology domain of PLCδ1 (PH-PLC). The isolated PH-PLC, lacking the enzymatic portion of the protein, binds to the headgroup of PIP₂ about 1000-fold better than to either PIP or PIP₃. Recombinant PH-PLC can thus be used as a highly selective, reversible tool to reduce the free concentration of PIP₂ in the membrane. Recombinant, purified PH-PLC was applied to excised patches from cells expressing TRPV1. PH-PLC produced a concentration-dependent, reversible inhibition of the capsaicin-activated currents. Neither boiled PH-PLC nor PH-PLC applied to outside-out patches altered the capsaicin-activated current. Moreover, a PIP₂-selective pleckstrin homology domain was ineffective.

The sufficiency of PIP₂ depletion in producing desensitization of TRPV1 was tested in whole cells by bypassing PLC to dephosphorylate PIP₂ with either a voltage-sensitive phosphatase or a chemically inducible lipid phosphatase. In both cases, PIP₂ was dephosphorylated at the 5-position of the inositol ring, giving PI(4)P and avoiding generation of diacylglycerol and inositol trisphosphate. Activation of both the voltage-sensitive phosphatase and the chemically inducible phosphatase decreased the capsaicin-activated current but see also Lukacs et al., who found the effect of the chemically induced phosphatase to depend on the capsaicin concentration used).

Ca²⁺-dependent desensitization in TRPV2 is indistinguishable from Ca²⁺-dependent desensitization in TRPV1. In contrast to TRPV1, however, the ankyrin repeat domain of TRPV2 does not bind ATP or Ca²⁺/calmodulin. These data suggest that Ca²⁺-dependent desensitization does not require interactions among the ankyrin repeat domains and ATP and/or Ca²⁺-calmodulin. However, Ca²⁺-dependent desensitization of TRPV2 does appear to involve depletion of PIP₂. To determine whether depletion of PIP₂ and Ca²⁺-dependent desensitization occurred with similar kinetics, simultaneous confocal imaging and whole-cell voltage clamp were performed on cells expressing both TRPV1 and the PH-PLC PIP₂-binding protein discussed earlier, genetically fused to GFP. The desensitization observed occurred with the same time course as depletion of PIP₂ (Figure 28.4).

If PIP₂ is viewed as a cofactor required for TRPV1 activation, it is reasonable to ask whether, under physiological conditions, the PIP₂ concentration in the membrane ever falls low enough to create a pool of PIP₂-free TRPV1 channels. Recent work quantifying the partition coefficient of a water-soluble PIP₂, diC₈-PIP₂, underscores the importance of this question. The EC₅₀ value for activation of TRPV1 by diC₈-PIP₂ has been reported to be in the range of 0.5–5 µM, depending on the cell type and the recording conditions. These concentrations are the free concentrations in solution, but the important concentration is that of diC₈-PIP₂ in the membrane. Using isotothermal titration calorimetry with liposomes whose composition was modeled after the intracellular leaflet of neuronal plasma membranes, an EC₅₀ of 1 µM translates into 0.002 mol% diC₈-PIP₂ in the intracellular leaflet. The apparent affinity of TRPV1 for natural PIP₂ is within even an order of magnitude of this value, then it is possible that the PIP₂ level in the cell membrane never falls sufficiently to remove PIP₂ from TRPV1 under physiological conditions.

PIP₂ appears to activate all the TRPV channels, TRPV1, TRPV2, TRPV4, TRPV5, and TRPV6, with the exception of TRPV3. TRPV3 was found to be activated by PIP₂ depletion in whole cells and excised patches and to be inhibited by the direct application of diC₈-PIP₂ to inside-out excised patches. This fascinating inversion of regulation polarity, inhibition instead of activation, is reminiscent of the voltage dependence of HCN channels, compared to other voltage-gated channels. Whether PIP₂ asserts a different conformational rearrangement in the PIP₂-binding site of TRPV3, compared to other TRPVs, or whether instead the coupling between the PIP₂-binding site and the pore gate is inverted, remains to be determined.

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