The transient receptor potential (TRP) ion channel family was the last major ion channel family to be discovered. The prototypical member (dTRP) was identified by a forward genetic approach in Drosophila, where it represents the transduction channel in the photoreceptors, activated downstream of a Gq-coupled PLC. In the meantime 29 vertebrate TRP isoforms are recognized, distributed amongst seven subfamilies (TRPC, TRPV, TRPM, TRPML, TRPP, TRPA, TRPN). They subserve a wide range of functions throughout the body, most notably, though by no means exclusively, in sensory transduction and in vascular smooth muscle. However, their precise physiological roles and mechanism of activation and regulation are still only gradually being revealed. Most TRP channels are subject to multiple modes of regulation, but a common theme amongst the TRPC/V/M subfamilies is their regulation by lipid messengers. Genetic evidence supports an excitatory role of diacylglycerol (DAG) for the dTRP’s, although curiously only DAG metabolites (PUFAs) have been found to activate the Drosophila channels. TRPC2,3,6 and 7 are widely accepted as DAG-activated channels, although TRPC3 can also be regulated via a store-operated mechanism. More recently PIP$_2$ has been shown to be required for activity of TRPV5, TRPM4,5,7 and 8, whilst it may inhibit TRPV1 and the dTRPs. Although compelling evidence for a direct interaction of DAG with the TRPC channels is lacking, mutagenesis studies have identified putative PIP$_2$-interacting domains in the C-termini of several TRPV and TRPM channels.

By 1990 the founding members of most of the major functionally identified channel families had been molecularly identified. However, there was still a diverse range of non-voltage-gated cation channels, such as second-messenger operated channels (SMOCs), store-operated channels (SOCs or $I_{crac}$), calcium-activated non-selective cation channels (CAN channels) and a variety of sensory transduction channels, including those responsible for taste, hearing and temperature sensation, for which there were no molecular candidates. Although between them, their properties would not necessarily have suggested that they might belong to the same family, it turns out that at least one further major ion channel family had yet to be discovered, which now seems likely to account for most of these. This is the transient receptor potential (TRP) ion channel family, first discovered in Drosophila, and which is notable for its diversity of function and almost ubiquitous expression. In the meantime ~30 vertebrate isoforms have been isolated, but their full range of physiological functions is still only gradually being revealed, whilst their gating mechanisms in most cases remain poorly defined. Although it is the most recently discovered major class of ion channel families, the TRP family has already been extensively studied, with many thousands of papers published in its relatively brief, recognized, lifetime. Probably no other ion channel family has been so extensively reviewed in recent years, and this article will be restricted to an overview of the history of their discovery and diversity, before reviewing recent evidence on how TRP channels are regulated by a variety of lipids, which have emerged as major regulators of their activity.

History of TRP discovery

Drosophila TRP. The first ion channels to be sequenced, including the ACh receptor and voltage-gated Na$^+$ and Ca$^{2+}$ channels, were isolated by the classic approach of protein purification and microsequencing (reviewed in Hille, 2001). However, ion channels are often expressed at low levels, and therefore alternative approaches, which
make no demands on protein abundance or high-affinity ligands, were required to identify most of the other major classes of ion channel. In this respect, genetic approaches have proved particularly powerful, as exemplified by the voltage-gated K⁺ channel family, first identified by the cloning of the Drosophila behavioural mutant gene, Shaker (Kamb et al. 1987; Papazian et al. 1987; Tempel et al. 1987). Similarly, the Drosophila trp mutant was first isolated as a spontaneously occurring mutation, in which flies appeared blind in a simple optomotor behavioural test, whilst the photoreceptor’s response to light decayed to baseline during prolonged bright illumination (Cosens & Manning, 1969; Minke et al. 1975). When the responsible gene was finally cloned some 20 years later (Montell & Rubin, 1989), it was found to encode a novel transmembrane protein. Since mutants still had an apparently normal light response to weak illumination, it was originally considered unlikely that TRP represented the light-sensitive channel, although Minke & Selinger (1991) speculated that it might represent a Ca²⁺ transporter, since they thought that the trp mutant phenotype represented a defect in refilling intracellular Ca²⁺ stores (but see below for an alternative interpretation). However, by using a newly developed patch clamp preparation of dissociated photoreceptors (Hardie, 1991), Hardie & Minke (1992) were able to directly demonstrate that the Ca²⁺ permeability of the light-sensitive current was profoundly reduced in trp mutants, leading to the proposal that it represented the major, Ca²⁺-selective component of the light-sensitive current, and that a second less Ca²⁺-permeable channel was responsible for the residual response in the trp mutant. At the same time, Phillips et al. (1992) reported the sequence of a TRP homologue (TRP-like, or TRPL), isolated as a calmodulin binding protein, and now believed to be responsible for this residual response (Niemeyer et al. 1996; Reuss et al. 1997). Phillips et al. (1992) found that TRPL shared ~40% identity with dTRP, and also recognized that the both sequences shared structural similarity with the voltage-gated Ca²⁺ channel family with six transmembrane helices.

Following the genetic demonstrations that TRP is required for the major Ca²⁺-selective component of the light response (Hardie & Minke, 1992), and TRPL for the residual response (Niemeyer et al. 1996; Reuss et al. 1997), both TRP and TRPL have been expressed in heterologous expression systems, indicating that they encode bona fide calcium- and cation-permeable channels (Vaca et al. 1994; Gillo et al. 1996; Hardie et al. 1997; Xu et al. 1997). The properties of the native TRP- and TRPL-dependent currents in vivo have been characterized by exploiting null mutants of both genes to isolate the respective currents providing characteristic biophysical ‘fingerprints’ (Reuss et al. 1997). TRP has a small single channel conductance (~8 pS) in physiological solutions due to a voltage-dependent divalent ion block, is highly Ca²⁺ selective (P_Ca : P_K ≈ 100 : 1), and sensitive to block by submicromolar La³⁺, whilst TRPL is a ~35 pS channel with modest Ca²⁺ permeability (P_Ca : P_K ≈ 4 : 1), and is insensitive to 100 μM La³⁺ (reviewed in Minke & Hardie, 2000).

dTRPL has been routinely expressed by many groups (Hu et al. 1994; Harteneck et al. 1995; Lan et al. 1996; Hardie et al. 1997; Xu et al. 1997), and its biophysical properties found to be indistinguishable from those of the endogenous TRPL-dependent current by a number of criteria, including single channel conductance and open times, rectification, ionic selectivity, and pharmacology of block (Hardie et al. 1997; Chyb et al. 1999). By contrast, it has proved difficult to express dTRP, and whilst the few published reports of its biophysical properties in heterologous expression studies indicated a Ca²⁺-permeable channel (Vaca et al. 1994; Gillo et al. 1996; Xu et al. 1997), none of these studies showed a convincing match with the properties of the in vivo TRP-dependent current (isolated in trpl mutants). Thus, whilst trp is indisputably required for the major light-sensitive conductance in Drosophila, and is the prototypical member of the TRP superfamily, a conclusive demonstration that it forms a pore-forming subunit in vivo is still lacking.

Trp of the iceberg. The light response in Drosophila is known to be mediated via a G protein-coupled phospholipase C (PLC) cascade (reviewed in Montell, 1999; Hardie & Raghu, 2001), and hence Hardie & Minke (1992) speculated that vertebrate homologues of dTRP might also underlie PLC-mediated Ca²⁺ influx phenomena, such as store-operated Ca²⁺ entry (SOCE) in vertebrates. The first vertebrate TRP homologues emerged in 1995, with PCR cloning of TRP fragments in mouse brain and Xenopus oocytes (Petersen et al. 1995), closely followed by reports of full length cDNAs of TRPC1, identified by homology to dTRP in human EST databases (Wes et al. 1995; Zhu et al. 1995). This first identification of a mammalian TRP homologue heralded an explosion of studies, now resulting in 29 vertebrate TRP isoforms, divided into initially 3 (TRPC, TRPV and TRPM) (Harteneck et al. 2000), and then a further 4 (TRPP, TRPML, TRPA and TRPN) distinct subfamilies (for recent comprehensive reviews see: Clapham, 2003; Montell, 2005b; Ramsey et al. 2006).

The remaining TRPCs were identified by bio-informatic approaches (i.e. by homology to dTRP and TRPC1), followed by heterologous expression studies. However, the other subfamilies were identified independently, either as disease genes (TRPP, TRPM, TRPML), by mutant screen (TRPN), or by expression cloning (TRPVs), and subsequently recognized as belonging to the same superfamily (Table 1). After the usual confusion that accompanies the discovery of any new gene family, a
dTRP (reviewed in Freichel which like the dTRPs, can all be activated downstream of homology to dTRP. There are seven isoforms (TRPC1–7), 40% identity) to dTRP and TRPL and were identified by be found (Table 1).

2002), and along with the four latecomers, this has become widely adopted, although the alternative names may still be found (Table 1).

3 and 4) are also heat activated, though all have multiple alternative modes of activation, most notably TRPV4 (Nilius et al. 2004). Two further members of this subfamily (TRPV5 (= ECAC) and TRPV6 (= CaT)) were identified independently by expression cloning in Xenopus oocytes, using mRNA from kidney cells to identify epithelial Ca²⁺ transporters with a C45 flux assay (Hoenderop et al. 1999; Peng et al. 1999). TRPV5 and 6 are the most highly Ca²⁺ selective of all the TRP channels.

TRPM (melastatin) was originally identified as a gene down-regulated in aggressive melanomas (Duncan et al. 1998). There are still no direct electrophysiological data to demonstrate that TRPM1 actually forms a channel, but substantial data on seven further TRPM homologues documents important roles for TRPM channels. These include TRPM4 and TRPM5, which are Ca²⁺-activated non-selective cation (CAN) channels; TRPM8, which is activated by cold and menthol; and the ‘chanzymes’ TRPM2, 6 and 7, which are unusual in including enzymatic domains in their structures in addition to a channel domain (reviewed in Fleig & Penner, 2004; Kraft & Harteneck, 2005).

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TRPPs, or polycystins, were first positionally cloned by linkage analysis of disease gene (Mochizuki et al. 1996). TRPPs (polycystin-2, PKD2 (3)) have many more subunits, most notably TRPV4, which is activated by cold and menthol; and the ‘chanzymes’ TRPM2, 6 and 7, which are unusual in including enzymatic domains in their structures in addition to a channel domain (reviewed in Fleig & Penner, 2004; Kraft & Harteneck, 2005).

unified nomenclature was proposed in 2002 for the first three subfamilies TRPC, TRPV and TRPM (Montell et al. 2002), and along with the four latecomers, this has become widely adopted, although the alternative names may still be found (Table 1).

TRPCs (canonical TRPs) are most closely related (ca 40% identity) to dTRP and TRPL and were identified by homology to dTRP. There are seven isoforms (TRPC1–7), which like the dTRPs, can all be activated downstream of PLC (reviewed in Freichel et al. 2005).

TRPVs (vanilloid receptors) were identified independently using expression-cloning strategies. TRPV1 was identified using mRNA from sensory neurons to identify ion channels activated by capsaicin and by heat (Caterina et al. 1997). Three homologues (TRPV2, 3 and 4) are also heat activated, though all have multiple alternative modes of activation, most notably TRPV4 (Nilius et al. 2004). Two further members of this subfamily (TRPV5 (= ECAC) and TRPV6 (= CaT)) were identified independently by expression cloning in Xenopus oocytes, using mRNA from kidney cells to identify epithelial Ca²⁺ transporters with a C45 flux assay (Hoenderop et al. 1999; Peng et al. 1999). TRPV5 and 6 are the most highly Ca²⁺ selective of all the TRP channels.

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TRP channels all 6 transmembrane (6TM) proteins with homology to the voltage gated CaNa and K channel family. Like voltage-gated K⁺ channels, TRP genes encode only one 6TM peptide and the channels are assumed to be homo- or heterotetramers. dTRPs, TRPCs, TRPVs, TRPMs and TRPPs have all been shown capable of forming various heteromultimeric assemblies. References are given for discovery only, plus specialist reviews; other references in text.

Table 1. Summary of the 7 subfamilies of TRP proteins (number of vertebrate isoforms in brackets), history of discovery and brief notes as to the known (or in most cases, presumed) function and mode of gating

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Alternative names (no. of isoforms)</th>
<th>Discovery</th>
<th>Functions (isof orm number)</th>
<th>Gating (isof orm number)</th>
<th>Specialist reviews</th>
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<tbody>
<tr>
<td>TRPV</td>
<td>Vanilloid, ECAC, CAT (6)</td>
<td>Expression cloning (Caterina et al. 1997; Hoenderop et al. 1999; Peng et al. 1999)</td>
<td>Thermoreceptors (1,2,3,4); epithelial Ca²⁺ transport (5,6); taste (1,3)</td>
<td>Temperature (1,2,3,4); osmotic stress (4); PIP₂ (1,5); lipid ligands (1,3,4); other ligands incl . capsaicin, oregano, 2-APB, etc. (1,2,3,4)</td>
<td>(Gunthorpe et al. 2002; Patapoutian et al. 2003)</td>
</tr>
<tr>
<td>TRPM</td>
<td>Melastatin (8)</td>
<td>Differential cDNA display in aggressive melanoma line (Duncan et al. 1998)</td>
<td>CAN channels (4,5); Mg²⁺ transport (7); taste (5) cold/menthol receptor (8)</td>
<td>Ca²⁺ (4,5); PIP₂ (4,5,7,8); cADPR (2); Mg-ATP/Mg²⁺ (7); temperature (5,8); menthol,icilin (8)</td>
<td>(Fleig &amp; Penner, 2004; Kraft &amp; Harteneck, 2005)</td>
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</table>
two subgroups of polycystins, each with three members. Only the polycystin-2 group proteins (PKD2, PKD2L1 and PKD2L2 (= TRPP2, 3 and 5)) have six TM domains, and are generally recognized as TRP channels. The polycystin-1 (PKD1) group proteins have 11 TM domains and very little homology to the rest of the TRP superfamily, though they are believed to associate with polycystin-2s to form functional channels (Hanaoka et al. 2000). TRPPs were originally considered to be intracellular release channels, but recent evidence suggests they may function as plasma membrane channels, e.g. in epithelial monocilia, where it has been suggested they respond to cilia bending (reviewed in Delmas, 2005). Most recently, two polycystin isoforms (PKD1L3 and PKD2L1 (= TRPP3)) have been localized to a subset of taste receptors, and implicated as channels responsible for sour taste, possibly forming a heteromultimeric channel activated by protons (Huang et al. 2006; Ishimaru et al. 2006; Lopezjimenez et al. 2006).

The three mammalian TRPML channels were also first identified by linkage analysis of a disease gene, namely that mutated in mucolipidosis (Sun et al. 2000). They may represent intracellular channels on lysosomes (Venkatachalam et al. 2006).

Finally, there are two TRP channels with only a single isoform, which are both characterized by exceptionally long chains of ankyrin (ANK) repeats. These hint at roles in mechano-transduction, since such structures are potentially capable of forming molecular springs (Howard & Bechstedt, 2004; Lee et al. 2006). One of these, TRPN (nompC), with 29 ANK repeats, was identified in a Drosophila screen for mutants defective in mechanoreception, and is believed to represent a mechanosensitive channel (Walker et al. 2000). TRPN orthologues are found in lower vertebrates (Xenopus and zebrafish), where they may also represent mechanosensitive channels, but appear to be absent from mammalian genomes (Sidi et al. 2003). TRPA1 (= ANKTM1), with 14 ANK repeats, was identified in a bio-informatic search for 6TM channel-like proteins with ANK repeats (Story et al. 2003). Despite some controversy, it probably functions as a channel sensitive to noxious cold and is also activated by pungent chemicals found, for example, in wasabi and garlic (Bautista et al. 2005; Macpherson et al. 2005). It has been proposed as the mechanotransduction channel in vertebrate hair cells (Corey et al. 2004; Nagata et al. 2005). However, this has been seriously questioned by the recent finding that hearing in TRPA1−/− knockout mice is apparently unimpaired, though TRPA1 may play a mechanotransducer role in cutaneous afferents (Bautista et al. 2006; Kwan et al. 2006).

**The role of lipids in TRP channel gating**

Two issues, more than any other, have dominated the TRP channel field, and particularly that of the canonical TRPC family (which includes dTRP and dTRPL): firstly, the mechanism of gating, and secondly, the often closely related issue of identification of native channels and their functional roles in vivo. Gating mechanisms and functions of the TRP family are so diverse that they cannot all be reviewed here (but see Table 1); however, an emerging theme is that membrane lipids play important roles, and in many cases may represent the primary activating messengers or ligands.

**Activation of dTRPs and TRPCs by DAG and PUFAs.** There is essentially unanimous agreement that, like Drosophila TRP and TRPL, all seven members of the TRPC family can be activated downstream of PLC, although there may be other routes for activation, and the exact mechanisms are still debated. Drosophila’s value as a genetic model is not restricted to initial discovery of the TRP channel family, but because of the extensive potential for gene discovery and genetic manipulation of the signalling cascade in vivo, Drosophila has also provided many insights into the mechanisms of TRP channel regulation. A long history of investigation has firmly established that the phototransduction cascade is mediated via a rhodopsin, G protein-coupled signalling cascades, whereby a Gq protein activates a PLCβ isoform (encoded by norpA), leading to activation of both TRP and TRPL channels. This cascade represents one of the fastest known G protein-coupled signalling cascades, partly due to the extreme compartmentalization of both the channels and the upstream elements of the cascade, which are localized in signalling complexes in tightly packed stacks of microvilli (∼1–2 μm long, 50 nm in diameter) (reviewed in Montell, 1999; Hardie & Raghu, 2001).

Initially it was widely assumed that the activation mechanism involved Ca2+ release from InsP3-sensitive stores – and possibly a SOCE mechanism. However, neither thapsigargin, Ca2+ or InsP3 were found capable of activating the channels (Ranganathan et al. 1994; Hardie, 1995, 1996; Hardie & Raghu, 1998), whilst phototransduction and light adaptation were completely unaffected by null mutants of the only InsP3R mutant in the Drosophila genome (Acharya et al. 1997; Raghu et al. 2000a). By contrast, genetic evidence, particularly involving mutations of the rdgA gene encoding diacylglycerol kinase (DGK), strongly implicated diacylglycerol (DAG) in the excitatory pathway. Specifically, both TRP and TRPL channels were found to be constitutively active in the dark in rdgA mutants, consistent with activation by the build-up of DAG by basal PLC activity (Raghu et al. 2000b). Recently, the requisite basal hydrolysis of PIP2 of PLC has been demonstrated in vivo using a genetically targeted PIP2 biosensor (Kir2.1 channel) in both WT and rdgA mutants. The basal PLC activity is in fact strikingly high, with turnover rates sufficient to deplete the entire cell of PIP2 within ca
5–10 min in the absence of PIP2 resynthesis (Fig. 2; Hardie et al. 2004). A second powerful argument is that rdgA mutations massively facilitate the response to light in severe PLC or Gq hypomorphs (Fig. 1). In such mutants, the response to light is normally reduced more than 100-fold due to the great reduction in light-induced PLC activity; however, when combined with the rdgA mutation, responses are restored to near WT levels (Hardie et al. 2002).

The Drosophila TRP channels can also be activated by metabolic inhibition, and activate spontaneously in whole-cell recordings without ATP in the electrode (Agam et al. 2000). Whilst there has been some debate as to the underlying mechanism, a recent study showed that spontaneous activation had an absolute requirement for PLC activity, and proposed that the primary, though not necessarily exclusive, mechanism was failure of DGK activity, resulting in build-up of DAG by basal PLC activity (Hardie et al. 2004). An apparent additional requirement of Ca2+ inferred from the ability of high concentrations of BAPTA to block the spontaneous activation (Agam et al. 2004) may have been due to a requirement of Ca2+ for PLC activity and a previously unrecognized action of BAPTA as an inhibitor of PLC activity (Hardie, 2005).

Whilst DAG has been clearly implicated by these and other studies, and is now also widely accepted as an activator of the vertebrate TRPC2, 3, 6 and 7 channels (see below), an obstacle remains – namely, exogenous application of DAG, either to photoreceptors or to heterologously expressed dTRP or TRPγ channels has little or no effect. (R. C. Hardie, unpublished observations: Estacion et al. 2001; Jors et al. 2006). The only potential agonists yet found to robustly activate the Drosophila channels are, in fact, polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA) or linolenic acid. PUFAs robustly activate TRPL (Chyb et al. 1999) and also TRPγ channels (Jors et al. 2006) in inside-out patches containing heterologously expressed channels, as well as the native TRP and TRPL channels in whole-cell recordings from photoreceptors, with an EC50 of ~10 µM (Chyb et al. 1999). PUFAs could in principle be released from DAG by DAG lipase; however, there is little evidence for the existence of such an enzyme in the transduction cascade in Drosophila. A mutation in a putative DAG lipase (rolling black out (rbo)) recently described by Huang et al. (2004) did, in fact, result in a severe block in phototransduction; however, details of its phenotype, namely a reported reduction in light-induced DAG production (interpreted as a reduction in PLC activity), and its use dependence (mutant flies only stopped responding to light after continuous bright illumination) were not consistent with such a proposed role.

One possible solution is suggested by recent quantitative estimates of the rate of PIP2 hydrolysis in the photoreceptors, which indicate that a single photon absorption results in PLC activity at rates sufficient to hydrolyse all the PIP2 (and probably also the PI and PIP reserve) in a single microvillus within less than 1 s (Fig. 2; Hardie et al. 2001, 2004). Given that a single microvillus contains several thousand PI molecules, a simple calculation suggests that locally, DAG concentrations within the microvillus can be expected to reach near millimolar levels. DAG is poorly

Figure 1. Genetic evidence for the excitatory role of DAG
Mutations in DAG kinase (DGK, rdgA gene) greatly facilitate responses in PLC (norpA gene) and Gwq hypomorphs. A, a bright flash in a severe PLC mutant (norpA P12) only elicits a few sporadic 1–2 pA ‘quantum bumps’, but in the double mutant norpA P12, rdgA, the response to the same intensity is enhanced ~100-fold. B, summary of averaged data (mean ± S.E.M.) for macroscopic response amplitude in two norpA alleles (P12 and P16) and also the Gwq mutant (left bar, single mutant; right bar, in double mutant combination with rdgA); note the logarithmic scale. Reference to the roles of PLC and DGK shows how the data can be readily interpreted if it is assumed that DAG is an excitatory messenger. For further details, see Hardie et al. (2002).
soluble and if the dTRP channels have evolved to respond to DAG levels in this concentration range, it may be experimentally impossible to apply exogenous DAG at such concentrations, whilst the more soluble PUFAs may act as surrogate, non-physiological agonists on the same channels.

Activation of TRPCs by DAG. The first evidence for lipid regulation of vertebrate TRPs (Hofmann et al. 1999) appeared simultaneously with the report showing that lipids (PUFAs) could activate the dTRPs (Chyb et al. 1999). Both by homology and on functional criteria, vertebrate TRPCs can be further subdivided into four subgroups: TRPC3/6/7, TRPC4/5, TRPC1 and TRPC2. Of these, Hofmann et al. (1999) found that TRPC3/6/7 could be reliably activated by DAG in a PKC-independent and membrane-delimited fashion (i.e. in inside-out patches), with thresholds near 10 µM. However, the pharmacological profile of vertebrate TRPCs is distinct from the dTRPs, in that PUFAs were ineffective. Evidence for excitation by endogenously generated DAG was provided by the ability of a DAG lipase inhibitor (RHC80267) to activate the channels.

Since this original report, there have been numerous studies confirming that heterologously expressed TRPC3/6/7 can be activated by DAG, and their role as DAG-activated channels is widely accepted (reviewed

**Figure 2. PIP₂ in Drosophila photoreceptors monitored by PIP₂ biosensors (Kir2.1)**

A, dose–response function of Kir2.1R228Q channels expressed in Drosophila S2 cells to exogenous di-C₈ PIP₂, applied to inside-out patches (sample response in inset). The R228Q point mutation reduces the effective affinity for PIP₂ by a factor of ∼5 making the channels more sensitive to changes in physiological levels of PIP₂. Arrow indicates approximate effective resting (maximum) level of PIP₂ in the photoreceptors, implying that the current should be an approximately linear indicator of PIP₂ levels in vivo. B, currents mediated by Kir2.1R228Q channels genetically targeted to the microvillar membrane in trpl:trp double mutants lacking both light-sensitive channels. In the dark a large (∼1 nA) constitutive current is activated by the prevailing PIP₂ levels (extent indicated by double arrow). Calibrated light flashes (0.03, etc. expressed in effectively absorbed photons per microvillus) suppress the current due to hydrolysis of PIP₂ by PLC: flashes containing 1 photon per microvillus deplete the entire cell of the majority of detectable PIP₂ within ∼1 s. The Kir2.1 current is reactivated with a half-time of ∼60 s in the dark, representing the resynthesis of PIP₂. C, intensity response function of PLC activity, expressed as percentage total PIP₂-sensitive Kir2.1 current suppressed per second, measured from the maximum slope of individual flash responses as in B. D, basal PLC activity monitored in the dark in vivo as a function of time after establishing the whole-cell configuration: a control (WT) cell recorded in the dark with ATP in the patch pipette shows only very gradual rundown of the Kir2.1 current over 10 min. Without ATP, however, the current decays to near-zero within ∼6 min. A similar behaviour is seen in the rdgA (DG kinase) mutant demonstrating ongoing basal PLC activity in this mutant as well. Adapted from Hardie et al. 2004.
in Vazquez et al. 2004a; Dietrich et al. 2005a; Ramsey et al. 2006). However, a complication is that TRPC3, which is the most intensively studied of this subfamily, can behave in two very distinct fashions, apparently depending on level of expression. At low levels, TRPC3 appears to behave as a store/InsP3R-operated channel and is resistant to exogenous DAG, and only when it is highly expressed does it become reliably activated by DAG. Putney (2004) argues that with low levels of expression, TRPC3 may find appropriate endogenous partners (e.g. InsP3R and/or other channel subunits) to form a distinct, store-operated channel; however, when over-expressed it may be forced into a homomultimeric channel that is regulated by DAG. Certainly there is evidence that TRPC3 can heteromultimerize with TRPC6 and TRPC7 (reviewed in Dietrich et al. 2005a), and there is also a well-documented interaction of a C-terminal domain with the InsP3 receptor that can be disrupted by calmodulin (CaM) (Zhu, 2005). The physiological significance of this interaction remains to be clearly established, however, since TRPC3 channels can still be activated by store depletion in InsP3R knock-out DT40 cell lines (Vazquez et al. 2001). Furthermore, deletion of the TRPC3 CaM/InsP3R binding domain, proposed to interact with the InsP3R, indicated that this domain was required for targeting of the channels in a CaM- and InsP3R-independent fashion (Wedel et al. 2003).

Native TRPC channels activated by DAG. TRPC6. Such concerns over the effects of varying levels of expression also raise the question of whether native TRPC channels are regulated by DAG, or whether this is an artefact of over-expression. Native TRPC channels have been frustratingly difficult to identify, and it is debatable whether any native current can be unambiguously identified with a specific TRPC. Partly, this is because TRPCs are very widely expressed, with most cell types expressing several isoforms – potentially in heteromultimeric combinations. However, there is good evidence that TRPC6 channels are subunits of PLC-activated cation channels in a variety of vascular smooth muscle (reviewed in Albert & Large, 2006). In particular, the detailed biophysical and pharmacological properties of a noradrenaline-activated native current, characterized in rabbit portal vein (Helliwell & Large, 1997), closely match the properties of heterologously expressed TRPC6, and could be specifically down-regulated using TRPC6 antisense (Inoue et al. 2001). Importantly for the present argument, the native endogenous current was also robustly activated by the DAG analogue 1-oleoyl-2-acetyl-glycerol (OAG, 10 μM), and pharmacological investigations indicated, that like heterologously expressed TRPCs, it was activated downstream of PLC, and independently of PKC. Interestingly, although ineffective by itself, InsP3 was found to greatly potentiate the effect of DAG (Albert & Large, 2003). Whilst this action of InsP3 was heparin insensitive, it was probably not direct, as it could not be elicited in inside-out patches.

A similar (though not identical) current was also found to be activated by DAG in arterial myocytes from rabbit ear. This current showed a bell-shaped dose–response function with respect to DAG, larger doses inducing transient increases that declined to lower steady state level, with an EC50 of 2 μM and IC50 of 32 μM, respectively (Albert et al. 2005). Interestingly, pharmacological evidence indicated that the current was physiologically activated by DAG derived from a phospholipase D-dependent pathway. Assuming it does indeed represent a TRPC conductance, this would imply a novel mechanism of TRPC channel activation, otherwise generally regarded as being activated downstream of PLC.

The role of TRPC6 in vascular smooth muscle is further supported by analysis of TRPC6−/− knock-outs (Dietrich et al. 2005b). Surprisingly, these mice were hypertensive, and actually showed higher basal cation entry and enhanced agonist-induced contractility of arterial smooth muscle, as well as increased currents in response to application of exogenous DAG. This was explained by finding that these mice compensated for the loss of TRPC6 by up-regulation of TRPC3 channels, which have a higher level of basal activity. These findings may imply that the native channels are predominantly TRPC3/TRPC6 (17) heteromultimers, and that the inclusion of TRPC6 subunits suppresses the basal activity inherent in TRPC3. The partially redundant and overlapping roles of TRPC3 and TRPC6 revealed by this study, along with compensatory regulation of other TRPC isoforms also highlight the difficulty of uniquely identifying physiological roles for TRPC channels, even when knockout mice are available (of the TRPC family, only TRPC2, TRPC4 and TRPC6−/− knockouts have been reported).

TRPC2. Although it has thus far resisted attempts at heterologous expression, one of the clearest examples of a native DAG-sensitive channel is TRPC2. In humans, TRPC2 is a pseudogene; however, in mice it has a well-defined role in the vomeronasal organ (VNO) – an accessory olfactory structure responsible for responses to pheromones (Zufall et al. 2005). TRPC2 protein immuno-localizes to the dendritic tip of vomeronasal sensory neurons (VSN) and excised inside-out patch clamp recording from this region of the cells revealed a 42 pS non-selective cation channel that could be activated by exogenous DAG (10 μM), but not InsP3 or arachidonic acid (Lucas et al. 2003). The channels could also be activated by DGK inhibitors, suggesting that DAG, produced endogenously by basal PLC activity, was capable of activating the channels, reminiscent of the situation in Drosophila photoreceptors. Whilst the activation by DGK inhibitors as well as the physiological response of the VSNs to pheromones, was blocked by PLC inhibitors, channel
activation by DAG was unaffected by PLC or PKC inhibitors. The basic properties of the DAG-activated channel (including reversal potential, approximately linear $I-V$ relation and block by 2-APB) were indistinguishable from the transducer currents evoked by urine (a rich source of pheromones). Most importantly, the DAG-sensitive channel was completely eliminated in TRPC2$^{-/-}$ mice, which also show a profound loss of sensitivity to pheromone.

Whilst DAG is now widely accepted as an activator of a subset of TRPC channels, whether it interacts directly with the channel remains unclear. Its action appears to be membrane delimited in that DAG can activate channels in excised patches; DAG metabolites are also unlikely to be involved, because inhibitors of DGK or DAG lipase either have no effect or are excitatory themselves – again consistent with build-up of DAG due to basal PLC activity. However, Vazquez et al. (2004b) found that SRC kinase inhibitors completely blocked activation of TRPC3 by DAG. Since they found no evidence for SRC kinase phosphorylation of TRPC3 itself, they suggested that DAG action may not be direct but requires other (SRC-dependent) cofactors. Furthermore, in contrast to the effects of PIP$_2$ (see below) there is virtually no information on the possible location of a DAG binding site on the channel. Although a splice variant of rat TRPC6, lacking 54 amino acids from the N-terminal, was reported to be insensitive to DAG (Zhang & Saffen, 2001), a subsequent study failed to confirm this (Jung et al. 2003).

It should also be noted, that whilst activation of TRPCs by DAG has repeatedly been shown to be independent of PKC, TRPC channels can also be modulated by PKC. Thus, all three members of the TRPC3/6/7 group have been reported to be inhibited by PKC activators (phorbol esters), preventing their subsequent activation by DAG (Okada et al. 1999; Zhang & Saffen, 2001; Trebak et al. 2003). Subsequently, Trebak et al. (2005) showed that TRPC3 was phosphorylated by PKC at a specific, conserved serine residue (Ser$^{712}$), and suggested that DAG generated by PLC has dual roles: both as an activator of TRPCs, and also as a signal for negative feedback by PKC.

**Regulation of TRPs by PIP$_2$**

Whilst a subset of TRPCs are clearly directly or indirectly regulated by DAG, there are a growing number of reports that the activity of several TRP channels may also be negatively or positively regulated by its precursor, and PLC substrate, namely PIP$_2$. This reflects the growing realization that PIP$_2$ is an essential modulator of an increasing number of ion channels and membrane transporters (reviewed in Hilgemann et al. 2001; Suh & Hille, 2005).

**Drosophila TRP.** The first evidence for PIP$_2$ involvement in TRP channel regulation came from the *Drosophila* TRPL channel, which like many TRP channels is spontaneously active when heterologously expressed. Estacion et al. (2001) reported that PIP$_2$ could inhibit the activity of TRPL channels expressed in SF9 cells, but the channels could subsequently be re-activated by addition of PI-PLC. Subsequently, Hardie et al. (2001) found that conditions, which resulted in PIP$_2$ depletion in *vivo*, were often associated with the TRP channels (though not TRPL) entering a spontaneously active state. For example, in mutations of *rdgB* (a PI transport protein required for restoring PI to the microvillar membrane), the TRP channels remained indefinitely open following illumination sufficient to deplete PIP$_2$ in the membrane, both in whole-cell recordings (Hardie et al. 2001) and in electroretinogram recordings from intact animals (Milligan et al. 1997). In *trp* mutants, where the light response is mediated exclusively by TRPL channels, prolonged bright light also leads to rapid PIP$_2$ depletion, because the Ca$^{2+}$ influx via the TRP channels is normally required to inhibit PLC, and without this negative feedback PLC activity rapidly eliminates PIP$_2$ from the microvillar membrane (Hardie et al. 2001). Under these conditions, the TRP channels rapidly close and can no longer respond to illumination until PIP$_2$ is resynthesized (which occurs with a half-time of ~1 min in the dark, Fig. 2). It is this PIP$_2$-dependent decay of the light response that results in the transient receptor potential phenotype, which gave the TRP channels their name (Hardie et al. 2001). The response decay as PIP$_2$ is consumed is consistent with DAG (or a PUFA metabolite) being required for TRPL activation, but at odds with the original findings of TRPL inhibition by PIP$_2$ (Estacion et al. 2001). For TRP channels, the maintained activity often observed following conditions of PIP$_2$ depletion suggests that PIP$_2$ may be required to close the channels, and even that the channels may be synergistically gated by a simultaneous rise in DAG and fall in PIP$_2$ (Hardie, 2003).

**Vertebrate TRPs.** Although PIP$_2$ has yet to be directly implicated in vertebrate TRPC gating, there are several recent reports of PIP$_2$ regulating the activity of members of the TRPV and TRPM subfamilies. In most cases, PIP$_2$ has been found to promote TRP channel activation; however, the first example (TRPV1) was reported to be inhibited by PIP$_2$ (Chuang et al. 2001; Prescott & Julius, 2003).

**Negative regulation of TRPV1 by PIP$_2$?** Tissue injury releases pro-algesic agents such as nerve growth factor (NGF) and bradykinin (BK), which increase the response of nociceptors to pain and heat. Both agents can activate PLC, either via G protein-coupled signalling (BK$_2$ receptors) or via tyrosine kinases (NGF via TrkA). Whilst earlier studies had implied DAG and PKC-dependent...
Table 2. Putative PIP2 binding domains in TRP C termini

<table>
<thead>
<tr>
<th>TRP box/domain</th>
<th>TRP box</th>
<th>PH-domain</th>
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<tr>
<td>1. TRPM8 993-</td>
<td>VYKFCRYPFLVQEVYCRNIPPPFIVF</td>
<td></td>
</tr>
<tr>
<td>TRPM5 991-</td>
<td>FWKFCRNLIVEYHERPALAPPFILL</td>
<td></td>
</tr>
<tr>
<td>TRPV5 581-</td>
<td>LNRAQVVATTUMLKLPRCLWPRSG</td>
<td></td>
</tr>
<tr>
<td>But:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPM4 1057-</td>
<td>VYKACRYLHEFHSLAPALAPPFIYV</td>
<td></td>
</tr>
</tbody>
</table>

1. Alignment of TRP box/domain regions reported to affect PIP2 sensitivity in TRPM8, TRPM5 and TRPV5. Neutralization of R1008 in TRPM8, R1006 (TRPM5) and R599 (TRPV5) – all in bold were most effective in decreasing sensitivity to PIP2; neutralizing the other basic residues had minor effects (Rohacs et al. 2005). However, neutralization of K1059, R0162 and R1072 in TRPM4 (underlined) was reported to have little effect on sensitivity to PIP2 (Nilius et al. 2006).

2. Putative PH-domain in TRPM4; neutralizing all 4 residues, or substituting with C-terminal of TRPM5 blocked PIP2 recovery (Nilius et al. 2006).

3. Putative PIP2 interaction domain of TRPV1. Deletion of 777–792, or neutralization of R785 and K788 or K788 and R797 enhanced response and decreased potentiation by NGF (Prescott & Julius, 2003). However, Zhang et al. (2005a) suggest these effects may reflect an allosteric influence on tyrosine phosphorylation of a remote tyrosine residue (Y200).

Basic residues (K = lysine, R = arginine) highlighted. Residues in bold have been mutated, and reported to affect sensitivity to PIP2 (in some instances only indirectly).

Activation of TRPM channels by PIP2. Whilst the role of PIP2 inhibition of TRPV1 has been questioned, there is strong evidence suggesting that several members of the TRPM family, including TRPM4,5,7 and 8 are positively regulated by PIP2. Under experimental conditions where phosphorylation of TRPV1 in BK signalling (Cesare et al. 1999; Vellani et al. 2001), Chuang et al. (2001) reported that TRPV1 channel activity expressed in oocytes or HEK293 cells could be enhanced not only by PLC, but also by PIP2 antibodies applied to the cytosolic surface of excised patches. Although they did not test the effect of PIP2 itself, they proposed that PIP2 inhibited TRPV1 and that PIP2 depletion by PLC underlies the NGF- and bradykinin-mediated sensitization of pain responses in primary nociceptor afferents. Subsequently, Prescott & Julius (2003) identified a putative PIP2 binding domain in the C-terminus of TRPV1, characterized by clusters of positively charged residues interspersed with hydrophobic amino acids (Table 2). When this domain was deleted, or as few as two charged residues neutralized, the baseline currents were increased, whilst the ability of NGF to potentiate the currents was greatly reduced, effects apparently consistent with relief from PIP2 inhibition.

Although these results suggest that interaction of PIP2 with this domain inhibits channel activity, sensitivity to PIP2 itself was not directly tested, and the hypothesis that physiological changes in PIP2 underlie the pro-algesic sensitization has recently been questioned. Firstly, Zhang et al. (2005a) provided strong evidence that NGF could induce sensitization of TRPV1 by a completely different mechanism involving tyrosine phosphorylation (by SRC kinase) of TRPV1 at a unique tyrosine residue (Y200), which in turn promotes rapid insertion of new channels into the membrane. Zhang et al. (2005a) also showed that deletion of the putative PIP2 binding domain identified by Prescott & Julius (2003) promotes phosphorylation of Y200, thereby providing an alternative explanation for the effects of mutations in this site in reducing the potentiating effects of NGF. Most recently, and in direct contrast to the original results of Chuang et al. (2001), Stein et al. (2006) found that, rather than inhibiting TRPV1, exogenously applied PIP2 in fact strongly potentiated TRPV1 channel activity in excised patches, whilst a PIP2 scavenger (poly-L-lysine) suppressed activity. Consistent with the results of Zhang et al. (2005a), Stein et al. (2006) also found that sensitization of TRPV1 currents by NGF was associated with channel insertion by a PI-3 kinase-dependent mechanism, as monitored by real-time total internal reflection fluorescence (TIRF) microscopy of enhanced yellow fluorescent protein (EYFP)-tagged TRPV1.

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PIP2 resynthesis is prevented (e.g. by ATP removal or PI kinase inhibitors), all these channels rundown, or desensitize, presumably due to loss of PIP2 by basal lipid phosphatase or basal PLC activity; whilst under more physiological conditions several authors have reported that they can also be desensitized by agonist- or Ca2+-induced activation of PLC.

The requirement of PIP2 for channel activation was first reported for TRPM7 which is one of three TRPM ‘chanzymes’ (TRPM2, 6 and 7) that contain an enzymatic domain as well as a channel domain (Runnels et al. 2001). TRPM7, which contains an α-kinase domain, is permeable to a variety of divalent cations including Mg2+ and Ca2+ and is inhibited by physiological levels of intracellular Mg2+ and/or Mg-nucleotides. It is considered to represent an endogenous Mg2+-regulated current found, e.g. in lymphocytes, mast cells and smooth muscle and required for cell survival. There is a debate as to whether it is inhibited by free Mg2+ (Mg2+-inhibited current, or MIC: Kozak & Cahalan, 2003), or by Mg-ATP (Mg-nucleotide regulated metal ion current, or MagNuM: Monteilh-Zoller et al. 2003). In marked contrast to TRPC channels, Runnels et al. (2002) found that activation of PLC via a coexpressed muscarinic receptor inactivated TRPM7 in CHO cells with a time course that reflected the time course of PIP2 hydrolysis monitored using a fluorescent PIP2 reporter (PH-GFP). The channels could also be activated by PIP2 in inside-out patches, and inhibited by PIP2 antibodies. Activation by PIP2 may be related to the mechanism of Mg2+ inhibition, although this also remains controversial. Thus Kozak et al. (2005) proposed that free Mg2+ acts by screening the charges on PIP2 thereby interfering with the PIP2–channel interaction and found that a similar effect can be mediated by protons and other polyvalent cations. However, Demeuse et al. (2006) argue that the physiologically relevant mechanism is the binding of Mg-nucleotides (Mg-ATP) to the kinase domain.

TRPM4 (Launay et al. 2002) and the closely related TRPM5 (Hofmann et al. 2003; Liu & Liman, 2003; Prawitt et al. 2003) both represent calcium-activated non-selective cation channels (CAN channels), and are unique amongst the TRP channel family in having no detectable permeability to Ca2+. A clear physiological role in taste transduction has been demonstrated for TRPM5, with knock-out mice studies indicating that it is required for PLC-mediated sweet, bitter, and umami taste responses (Zhang et al. 2003). Initial reports of the Ca2+ dependence of heterologously expressed TRPM5 showed marked variations, with EC50 values ranging from ∼1 μM (Prawitt et al. 2003) to ∼30 μM (Hofmann et al. 2003). It has now been shown that the Ca2+ sensitivity of both TRPM5 (Liu & Liman, 2003) and TRPM4 (Zhang et al. 2005b; Nilius et al. 2006) is profoundly modulated by PIP2 levels, and that both channels become desensitized as PIP2 levels fall during rundown, or following activation of PLC. This has been most comprehensively studied in TRPM4, where the EC50 for Ca2+ in the desensitized state is ∼134 μM but shifts to ∼1 μM Ca2+ after resensitization by addition of 10 μM exogenous PIP2 (Nilius et al. 2006). Significantly, these authors were also able to identify a putative PIP2-binding pleckstrin homology (PH) domain in the C-terminus of TRPM4 (Table 2). Thus, they showed that neutralization of positively charged residues in the PH domain resulted in channels with much more rapid desensitization, and reduced sensitivity to exogenously applied PIP2. However, whilst a similar desensitization and PIP2-mediated recovery have been described for TRPM5 (Liu & Liman, 2003), this putative PH domain is not closely conserved between the two channels and a TRPM4–TRPM5 chimera containing the TRPM5 C-terminal had a reduced sensitivity to PIP2 (Nilius et al. 2006).

Like several TRP channels, TRPM4 shows intrinsic weak voltage dependence, being more active at depolarized potentials. With low free [Ca2+] and low [PIP2], the voltage for half activation (V50) is at very depolarized and unphysiological potentials (∼+75 mV); hence, the channels are essentially closed at resting potential. Nilius et al. (2006) found that the effect of PIP2 could be described as a dramatic left shift of the voltage activation curve (V50 ≈ −70 mV), resulting in channel activation at physiologically potentials. Nilius et al. (2005) have proposed that such shifts in the voltage dependence of TRP channels may be a widespread mechanism of TRP channel regulation, and have reported large voltage shifts for a range of modulators, including temperature, PIP2, decavanadate and Ca-calmodulin, in members of both the TRPM and TRPV subfamilies. The proposed mechanism exploits the weak voltage dependence, representing a small gating charge, which theoretically should allow small changes in the Gibb’s free energy of the channel to be translated into large shifts in voltage dependence (Voets et al. 2004; Nilius et al. 2005).

Finally, PIP2 has also been reported to be critical for activation of TRPM8 channels, which are normally activated by cold (temperatures below 25°C), or by micromolar concentrations of menthol (Peier et al. 2002). Rohacs et al. (2005) found that the channels could be directly activated by PIP2 in inside-out patches in the absence of menthol and at temperatures 10°C above their normal threshold, whilst in the absence of PIP2 (after scavenging inside-out patches with poly-L-lysine) menthol was ineffective even at concentrations as high as 500 μM. Dose–response functions for PIP2 shifted systematically to lower EC50 values either in the presence of menthol or as temperature was lowered. This suggests that PIP2 may be the primary activator of the channel, and that menthol and cold activate the channels by modulating their sensitivity to the prevailing PIP2 levels. Potentially, the most significant result of this study was finding that the PIP2 dose–response
functions were systematically shifted by point mutations neutralizing positively charged residues in and around the so-called ‘TRP box’, a conserved and defining feature of many TRP channels, which lies just C-terminal to the last transmembrane helix, TM6 (Table 2). The equivalent mutations in two other PIP2-regulated TRP channels, TRPV5 (Lee et al. 2005) and TRPM5 (see above), also appeared to suppress their sensitivity to PIP2, though this was assayed less directly by the effects of inhibiting of PI-kinases. Since the TRP box is the most highly conserved region of the TRP family, these findings raised the possibility that regulation by PIP2 binding to this region might be a general feature of the diverse TRP family. However, Nilius et al. (2006) found that an equivalent mutation in TRPM4 failed to seriously impact on their sensitivity to PIP2, and, as detailed above, identified an alternative putative PIP2 interaction site which is not well conserved in TRPM5.

In summary, there is strong evidence that PIP2 can activate a variety of TRP channels (TRPM4,5,7,8 and TRPV5), and possibly also inhibit others (TRPV1, dTRPL/TRP). Although there have as yet been no direct demonstrations of PIP2 binding to the channels, a direct interaction of PIP2 with the channels is strongly indicated – though not proven – by the effects of charge-neutralizing mutations in putative, but disparate PIP2-binding domains (Table 2). Under experimental conditions, PIP2 has been shown to modulate the activity of these channels using a variety of approaches, including addition of exogenous PIP2, lowering PIP2 by PIP2 scavengers, activation of endogenous PLC (by both native and coexpressed receptors), or by inhibition of PI-kinases required for PIP2 synthesis. However, whilst it seems clear that PIP2 is required for the activity for many of these channels, the extent to which physiological changes in PIP2 levels are utilized to regulate channel activity in vivo remains controversial. For example, it seems that the clearest potential physiological mechanism, the pro-algesic effects of NGF on TRPV1 activation, may now be explained by an alternative mechanism (Zhang et al. 2005a; Stein et al. 2006). Despite the finding that PLC activation inactivated TRPM7 (Runnels et al. 2001), Takezawa et al. (2004) have questioned the physiological relevance of PIP2 for TRPM7 regulation and proposed that TRPM7 was instead primarily modulated by cAMP-dependent signalling via its kinase domain. Although PIP2 is hydrolysed by PLCβ during taste transduction, it is not known whether PIP2 levels change sufficiently to contribute to desensitization of TRPM5 under physiological conditions.

An embarrassment of lipids

Whilst PIP2 and DAG are the lipids most widely implicated in regulation of TRP channels, they are by no means the only ones. As described above the prototypical dTRP and dTRPL can be activated by PUFA's, although it is not clear whether it is these, or DAG, possibly in combination with PIP2, depletion, that represent the endogenous messenger. Curiously, the rather distantly related TRPV3 ion channel, first identified as one of the thermo-TRPs activated in the intermediate (31–39°C) range, shows an almost identical activation profile to dTRP/TRPL, being insensitive to 100 µM OAG, but potentiated by arachidonic acid (AA; 10 µM) as well as a range of cis- and trans-isomers of both mono- and polyunsaturated FAs and non-metabolizable AA analogues such as eicosatetraenoic acid (ETYA) and eicosatriynoic acid (ETI) (Hu et al. 2006).

The related TRPV4 can also be activated by arachidonic acid as well as the endocannabinoid anandamide, but Watanabe et al. (2003) found that the channels were in fact probably being directly activated by a cytochrome P450 epoxygenase-derived metabolite of AA and anandamide, namely 5′-6′-epoxyeicosatrienoic acid (5′-6′-EET), which was effective at submicromolar concentrations. TRPV4 is expressed in vascular endothelia and the authors speculate that this response may be important for the well-known vaso-relaxant effects of EETs and endocannabinoids, the latter also shown to activate TRPV1 (Ross, 2003) and TRPA1 (Jordt et al. 2004). Another cytochrome P450-derived AA metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE) has been reported to activate mouse TRPC6 channels expressed in HEK293 cells with an EC50 of 0.8 µM (Basora et al. 2003).

Arguably the best characterized arachidonic acid-activated current is ARC (arachidonic-regulated Ca2+–selective channel), which is an endogenous highly selective Ca2+ current found in several cell types, including native parotid and acinar cells. It is activated by PLC mobilizing agonists, but the AA is generated by cytosolic phospholipase (cPLA)2, rather than being derived from DAG (Mignen & Shuttleworth, 2000; Mignen et al. 2003, 2005). ARC is found together with a store-operated Ca2+ conductance (Icrac) in the same cells, but according to Shuttleworth et al. (2004) ARC is the physiologically dominant mechanism of Ca2+ entry. ARC has yet to be molecularly identified, but it has the same high selectivity to Ca2+ otherwise only found in TRPV5/6 (which, however, show no sensitivity to AA) or in Icrac itself, which has recently been identified as a novel 4TM protein, Orai (Feske et al. 2006).

Most recently sphingosine lipids have also been added to the list of active lipids. Specifically, the poorly characterized TRPM3 channel was found to respond directly to sphingosine (Grimm et al. 2005), and Xu et al. (2006) reported that TRPC5 could be activated by sphingosine-1-phosphate (S1P). Whilst extracellular S1P activated heterologously expressed TRPC5 via a G-protein- (and probably PLC)-coupled receptor, S1P also activated TRPC5 channels in inside-out patches suggesting a direct mechanism of activation as well.
Xu et al. (2006) also found that S1P could induce motile response in human smooth muscle cells, which probably express a TRPC1/5 heteromultimer; however, only the extracellular, G-protein-coupled response could be demonstrated in the native cells. TRPC5 has also recently been show to be activated by submicromolar concentrations of lysophospholipids, including those with commonly, naturally occurring chain lengths (C 16 : 0), in both inside-out and outside-out patches, with responses again being found in native TRPC5 like channels in vascular smooth muscle (Flemming et al. 2006).

Conclusion

This review has focused on the role of lipids in regulation of TRP channels, and these are certainly amongst the most widespread agents reported to activate this channel family. However, a hallmark of the TRP channel family is the extremely diverse mode of regulation, with even the same channel being capable of being regulated by diverse physical and chemical stimuli. The list of regulatory mechanisms is long and growing, and several have not even been mentioned here. They include mechanical force, temperature, voltage, lipid and water-soluble ligands (including both exogenous and endogenous compounds), protons, Ca2+ and calmodulin, serine and tyrosine phosphorylation, membrane insertion and retrieval as well as a range of protein–protein interactions. With the majority of studies still having been performed in heterologous expression systems, there will certainly be many more surprises along the way before we come to appreciate the full repertoire of gating and physiological function of this fascinating family of ion channels.

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