Cell Calcium 59 (2016) 271–279

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Review

# Mechanisms of lipid regulation and lipid gating in TRPC channels

Barbora Svobodova, Klaus Groschner*

*Institute of Biophysics, Medical University of Graz, A-8010 Graz, Austria*

ARTICLE INFO

*Article history:*
Received 1 February 2016
Received in revised form 24 March 2016
Accepted 25 March 2016
Available online 16 April 2016

*Keywords:*
TRPC channels
Lipid mediators
Lipid metabolism
Lipid rafts
Gating mechanism

ABSTRACT

TRPC proteins form cation channels that integrate and relay cellular signals by mechanisms involving lipid recognition and lipid-dependent gating. The lipohilic/amphiphilic molecules that function as cellular activators or modulators of TRPC proteins span a wide range of chemical structures. In this context, cellular redox balance is likely linked to the lipid recognition/gating features of TRPC channels. Both classical ligand-protein interactions as well as indirect and promiscuous sensory mechanisms have been proposed. Some of the recognition processes are suggested to involve ancillary lipid-binding scaffolds or regulators as well as dynamic protein–protein interactions determined by bilayer architecture. A complex interplay of protein–protein and protein-lipid interactions is likely to govern the gating and/or plasma membrane recruitment of TRPC channels, thereby providing a distinguished platform for signal integration and coincident signal detection. Both the primary molecular event(s) of lipid recognition by TRPC channels as well as the transformation of these events into distinct gating movements is poorly understood at the molecular level, and it remains elusive whether lipid sensing in TRPCs is conferred to a distinct sensor domain. Recent structural information on the molecular action of lipophilic activators in distantly related members of the TRP superfamily encourages speculations on TRPC gating mechanisms involved in lipid recognition/gating. This review aims to provide an update on the current understanding of the lipid–dependent control of TRPC channels with focus on the TRPC lipid sensing, signal-integration hub and a short discussion of potential links to redox signaling.



**Contents**



* Corresponding author at: Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, 8010 Graz, Austria.
*E-mail address:* klaus.groschner@medunigraz.at (K. Groschner).

http://dx.doi.org/10.1016/j.ceca.2016.03.012

## 1. Introduction

Protein-lipid interactions represent critical steps in a plethora of signal transduction processes, and lipid recognition motifs have been identified in a wide range of signaling molecules including hormone receptors [1], enzymes [2], plasma membrane transporters [3] as well as ion channels [4,5]. Regulatory lipids include low abundancy species such as phosphoinositides and diacylglycerols but also structural components such as cholesterol, which display dynamic turnover and spatial accumulation/segregation within membrane microdomains, an elaborate arrangement that sets the stage for efficient signal transduction. Control of plasma membrane ion channels by their lipid environment and spatiotemporal lipid signals has long been recognized to involve primary molecular recognition at variable levels of specificity. Both structurally well-defined lipid binding pockets with a significant ability to discriminate between divergent lipid species as well as positively charged domains that associate via electrostatic interactions in a rather nonspecific manner with negatively charged lipids have been suggested as regulatory domains in a wide variety of ion channels [6]. Discovery of the TRP cation channel superfamily led to the identification of molecules with remarkably promiscuous signal recognition and an exceptional ability for signal integration. Lipid sensitivity of some TRP channels is not limited to non-specific effects such as alterations in local surface charge or bilayer architecture but complies with a "lipid-ligand gating" concept as proposed earlier for phosphoinositide gating of $K^+$ channels [7,8]. Direct ligand gating of TRP channels in terms of lipid-agonists being required and sufficient for channel activation is considered for the prototypical *Drosophila* TRP (dTRP) channel and for some members of the TRPC subfamily. Specifically TRPC2/3/6/7 proteins received particular attention in terms of lipid-sensory features as they were found activated in a direct manner by the $PIP_2$ degradation product diacylglycerol (DAG) and thus gated in response to dynamic metabolism of low-abundancy membrane lipids [9]. Consequently this subset of TRPC channels has emerged as a paradigm for channels gated by a lipid second messenger. Although the feature of DAG-activation of TRPC2/3/6/7 largely fulfills criteria of a ligand-gated process, this concept was repeatedly challenged and the regulatory DAG binding pocket still awaits identification. Cellular function of other TRPC family members (TRPC1/4/5) is as well tightly controlled by membrane lipids, which appear to modulate channel activation by other non-lipid gating stimuli in a cofactor-like fashion [10,11]. In line with this concept, interaction of channel domains with membrane lipids has been suggested to shift channel sensitivity to other primary activation processes such as voltage changes [12] or G-protein association [10] and may represent a common principle of signal integration and coincidence detection by TRP proteins. Moreover, evidence for indirect control of TRPC channel function by lipids targeting the channel complexes to specific cellular locations, has repeatedly been reported [13–15]. TRPCs have early on been recognized as pivotal elements of spatially restricted signaling cycles involving linkage between lipid metabolism and lipid recognition by the channel [16]. With this review we summarize recent progress made in understanding the molecular communication between membrane lipids and TRPC channels with particular focus on phosphoinositides, diacylglycerols and cholesterol.

## 2. Regulatory lipid species and their recognition by TRPC channels

Regulation of TRPC channels by lipids, lipid-derived second messengers but also synthetic lipophilic activators may occur via different principles of molecular recognition:

1) By classical ligand gating in which availability of the lipid agonist at the channel is spatially and temporally well controlled and the lipid associates reversibly with a specific binding pocket to stabilize exclusively lipid-dependent conformational states and gating modes of the channel. In this sense reversible binding of the agonist is required and sufficient for channel activation. To characterize such interactions at the molecular level is challenging due to the obvious difficulty to determine affinities for the interaction of an insoluble, membrane resident molecule with a membrane-delimited ligand of ill-defined concentration at the binding pocket.
2) Alternatively, lipid binding to the protein may modulate activation of the channel by other (primary) gating stimuli such as membrane voltage, protein–protein interaction or mechanical force in terms of a cofactor, which may be required but not sufficient for activation. This concept might involve not only specific binding sites but also rather nonspecific protein-lipid interactions and multiple areas in the channel-lipid interface. Such indirect modulation may be crucial for integration of signals and coincident signal transduction.
3) Control of TRPC channels by lipid may occur in the absence of any direct lipid-channel interaction via a lipid-dependent secondary stimulus such as mechanical forces generated by altered lipid bilayer structure surrounding the channel or by targeting of the channel into a regulatory membrane domain via a lipid-guided adaptor molecule. These concepts will be discussed below for yet to be identified lipid activators/modulators of TRPC channels.

### 2.1. $PIP_2$ and phosphoinositides

The most comprehensively studied molecule in the context of lipid regulation of TRP channels is phosphatidylinositol-(4,5)-bisphosphate ($PIP_2$). The abundance of phosphoinositides (PIs) in the plasma membrane of mammalian cells is low and their dynamic metabolism complies well with the principle mechanism of lipid second messengers. $PIP_2$ represents only 1% of the lipids in the inner leaflet of the plasma membrane [17] and its (patho)physiological importance arises from its multifunctional character, being the source for secondary messengers ($IP_3$, DAG) and acting as a second messenger in its own right [18]. Among a range of cellular functions, $PIP_2$ controls endo- and exocytosis as well as plasma membrane-cytoskeleton assemblies and has been recognized as a key factor for proper operation of ion transporters and channels [19].

In the TRPC channel subfamily (TRPC1-7), the role of $PIP_2$ is highly ambiguous. Both promotion and suppression of channel activation by $PIP_2$ has been reported [10,20–22] and specifically in the TRPC4/5 channels multiple $PIP_2$ recognition sites are likely. Importantly, TRPC5 channels were observed to display sensitivity to activation by administration of $PIP_2$ to the cytoplasmic face of excised patches, while inhibitory $PIP_2$ modulation prevailed in whole cells recordings [23]. Similarly TRPC3/6/7 channels in excised patches were found activated by $PIP_2$, with most prominent sensitivity of TRPC7, and $PIP_2$ appears required for activation by DAG [20]. However, for native TRPC3/6/7 channels of vascular smooth muscle preparations inhibition of channel activity by $PIP_2$ was reported [24]. Large et al. suggested that in the channelsí resting state, $PIP_2$ is bound to TRPC6 channels and upon $PIP_2$ breakdown is replaced by DAG [25]. More recently concepts have emerged in which $PIP_2$ and DAG act independently as positive modulators and/or activators of the channel, respectively [26]. This concept was put forward to explain the common desensitization/inactivation feature observed for TRPC currents in many cell systems.

#### 2.1.1. Direct and indirect recognition of PIs

Convincing evidence has been presented for both direct and indirect recognition of $PIP_2$ by TRPC proteins. The existence of

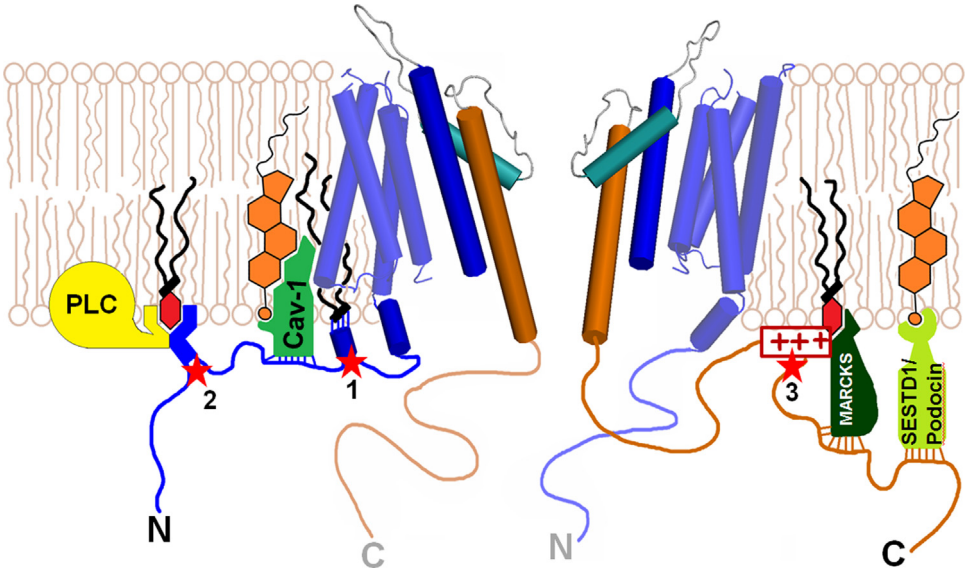


**Fig. 1.** Direct and indirect recognition of membrane lipids by TRPC channels.
Schematic localization of potential direct (1–3) and indirect channel-lipid interactions within the cytoplasmic domains of TRPC channels: (1) recognition of membrane lipids/DAG via the TRP_2 motif in TRPC3 [42]; (2) recognition of $PIP_2$ via an intermolecular pH domain formed by TRPC3 and PLCγ1 [43]; (3) binding to $PIP_2$ via positive charges in the C-terminus [29,35]. Recognition of $PIP_2$ and cholesterol via adaptor molecules: Sec14 and spectrin domain (SESTD1; [28]), podocin [15,44,45] and caveolin-1 (Cav-1; [46–48]).

$PIP_2$ binding structures was reported for cytoplasmic domains of TRPC4α (C-terminus,) and TRPC3 (N-terminus). The latter may involve PLCγ as an adaptor molecule that generates a composite/intermolecular lipid recognition (pH) domain [27]. Indirect linkage to $PIP_2$ was similarly proposed for TRPC4/5 via the $Ca^{2+}$-dependent scaffold SESTD1 [28]. The colocalization of TRPC1 channels with $PIP_2$ has been reported to involve myristoylated alanine-rich C kinase substrate (MARCKS) as a signaling partner in a mechanism that hypothetically engages $PIP_2$ recognition by both MARCKS and the channel [29]. These divergent molecular mechanisms by which TRPC complexes can be linked to membrane lipids are illustrated in Fig. 1. In view of the prominent existence of native TRPC channel as heteromeric complexes, multiple interaction sites with different affinities and specificities are expected in most native TRPC channels.

### 2.1.2. *Affinity and specificity of TRPC–$PIP_2$ interactions*

Determination of the affinity ($K_D$) for a specific lipid binding event to an ion channel in the plasma membrane is essentially difficult. Recent data from experiments using a FRET probe for plasma membrane $PIP_2$ provided a conceivable estimate of the affinity for $PIP_2$ binding recognition by TRPC3/6/7 channels [26]. These experiments suggested different affinities of homomeric channels with highest affinity for TRPC3 (1 μM) and lowest for TRPC7 (5 μM).

A general view of the architecture of such phosphoinositide binding sites was provided by Rosenhouse-Dantsker and Logothetis [30]. This study investigating the binding of 25 phosphoinositides to various proteins indicated that the phosphoinositide binding site comprises typically 60% of positively charged amino acids. These findings support the notion that the phosphoinositide binding to a protein is based primarily on electrostatic forces. The remaining 40% of amino acids most probably mediate an interaction with the acyl chains of phosphoinositides, and may confer a certain degree of specificity. Consitantly, Latorre and Rohacs et al. have hypothesised that positively charged amino acids within the TRP box may mediate the $PIP_2$ binding in TRPV1 [31,32]. Other studies suggest the $PIP_2$ or lipid-binding amino acids to be present within the S4-S5 linker or in the distal end of S6 and the TRP domain [33,34]. Kwon et al. suggested phosphoinositide binding to TRPC6 via positive charges in the C-terminal calmodulin binding site. Neutralizing a single charge (R853Q) within the calmodulin-binding region reduced PI binding as well as the channels response to agonist stimulation. On the contrary, a triple mutation R853Q/K860Q/R861Q showing impaired phosphoinositide binding resulted in a 2-fold increase of TPRC6 currents compared to the wild type protein [35]. A possible explanation for this unexpected finding may be disruption of the calmodulin binding site by the introducion of three consecutive glutamines and consequently disturbing other determinants of channel activity.

As an alternative to electrostatic interactions, $PIP_2$ may bind to a binding pocket in TRP proteins resembling the PH domain in $PLC\delta_1$ [36]. The observation of $PI(4,5)P_2$ being considerably more effective compared to $PI(3,4)P_2$ or $PI(3,5)P_2$ in TRPM4/8 channels despite all three compounds sharing the same electrostatic properties [34,37–39], suggests the existence of a specific binding pocket for $PIP_2$ rather than pure electrostatic interaction. Interestingly, TRPC6 showed PI specificity in terms of higher affinity to $PIP_3$ than to $PIP_2$ [35,40].

$PIP_2$ may impact on gating of the TRPC channel by divergent mechanisms: Either in a direct ligand gating processes [26] or in a modulatory co-factor like manner (gating by shifting the sensitivity to other stimuli) [10,12], or by a lipid-protein interaction-independent mechanism [41]. Notably, the evidence of $PIP_2$-metabolism causing rapid mechanogating of channels has convincingly been demonstrated for *Drosophila* photoreceptors. These gating principles will be further discussed below.

## 2.2. *Diacylglycerols*

An intimate linkage between PLC and TRPC channel activity with DAGs as primary activating second messengers is well accepted for a subset of TRP channels (dTRP, TRPC2/3/6/7). Groundbreaking, initial evidence came from Hofmann et al. who showed PKC-independent activation of TRPC3/6/7 but not TRPC1/4/5 by DAGs (1-oleoyl-2-acetyl-*sn*-glycerol, 1-stearoyl-2-arachidonoyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-sn-glycerol, 1-2-dioleoyl-*sn*-glycerol) [9]. Similarly, native TRPC2 channels respond to administration of DAGs [49]. The role of DAG as activating

species for *Drosophila* photoreceptor channels is more controversial as compared to the vertebrate relatives, most likely because of the particular difficulty to understand lipid changes at native photoreceptor channels. Nonetheless, quantitative estimates of lipid signaling in photoreceptors indicate rather large changes in local lipid composition [41,50], which may generate photomechanical effects potentially contributing to channel activation besides or alternatively to the direct ligand-activation by DAG [51].

Although the (patho)physiological significance of DAG gating of TRPC channels has repeatedly been demonstrated by pharmacological approaches and is commonly accepted for a wide range of tissues [52], the molecular basis of communication between these lipid messengers and TRP channels is still unclear.

#### 2.2.1. Direct and indirect recognition of DAGs

Similar to phosphoinositides, DAGs have been reported to control TRPC channels via direct association with domains in the channel protein as well as by secondary lipid sensing mechanisms not requiring any direct molecular recognition of the DAG [42,53]. Similar to recognition of PIs, the cytoplasmic parts of TRPC channel were in focus of the search for potential lipid binding domains. Early evidence provided by Van Rossum et al. identified a potential DAG-sensing TRP_2 domain in the N-terminus of TPRC3 channels [42] based on computational analysis of amino acid sequences and mutagenesis experiments. In TRPC3 the TRP_2 domain is proposed to enhance TRPC3 membrane currents by triggering the channels' insertion into the plasma membrane in a DAG-dependent manner [42]. This mechanism may not involve any classical channel gating process but merely an increase in the number of available channels. Similarly the characterization of a TRPC6 splice variant lacking part of the N-terminus was reported as lipid insensitive [54], an observation that was questioned later on [55]. Notably, TRPC7 channels in excised patches lacked direct activation by a synthetic DAG agonist [20], indicating a rather complex requirement on membrane lipid composition and membrane/cytoskeleton architecture for channel activation.

As the location of the DAG binding site in TRPCs is still elusive, also indirect interactions involving either PI recognition sites at PI-binding adaptor molecules (as discussed above) or additional unidentified cofactors may be considered. Consistently, one might speculate that the observed DAG-insensitivity of other isoforms (TRPC1/4/5) is based on such deficiency in cofactor regulation rather than lack of a principle inability to recognize DAG. Likewise, a critical involvement of a Src-dependent molecular complex has been suggested for DAG activation of TRPC3 [56]. It is of note that the dynamic formation of larger signalplexes in receptor/PLC-mediated activation of TRPC3 was shown to involve RACK1, a scaffold for Src tyrosine kinase and various lipid recognizing signaling proteins [57]. Evidence for other binding partners of RACK1 within TRP channel family has been obtained only for TRPP3 and TRPM6 [58,59]. On the whole, indirect mechanisms of DAG sensing via adaptor molecules cannot be excluded at present.

Another indirect mechanism that has repeatedly been discussed is the recognition of lipid signals such as conversion of $PIP_2$ to DAG inducing localized mechanical changes within the bilayer. This has been proposed for the impact of PI metabolism in *Drosophila* photoreceptors [60] and also for DAG activation of smooth muscle TRPC6 channels [53]. Indirect modulation of TRP channels by altered lipid architecture and/or lateral membrane tension does not involve either direct- or indirect molecular recognition but the existence of an as yet unidentified mechanosensor in TRPC channels. Two lines of evidence argue against mechanotransduction as the key process of DAG-TRPC interactions. Firstly, a certain degree of molecular specificity of DAG recognition has been reported and secondly, for native podocyte TRPC6 channels, a clear dissection between mechanosensitivity and DAG activation has been demonstrated [45]. Additionally, in certain tissues mechanosensitivity of TRPC6 may well involve lipid (DAG) gating, since G-protein coupled receptors serving as upstream signaling elements were identified as mechanosensors [61,62]. Hence, mechanosensitivity of native TRPCs may involve both lipid-dependent and independent pathways.

One of the current challenging tasks in the field certainly is to further test the concept of direct DAG-gating in TRPC channels. Such a direct lipid ligand-gating model for DAGs is well compatible with available functional data and recent quantitative estimates of plasma membrane DAG signals [26]. Alternative approaches in particular the reconstitution of purified TRPC complexes in artificial bilayers of defined lipid composition are awaited and expected to shed further light on this issue.

#### 2.2.2. Affinity and specificity of TRPC–DAG interactions

Similar to other channel-lipid interactions, the characterization of TRPC-DAG is complicated by lack of reliable methods to determine local concentrations of the lipid ligand and by difficulties in detecting its binding to integral membrane proteins. Valuable estimates have been provided by use of FRET fluorescence probes for measurements of membrane lipids and mathematical modeling of TRPC lipid gating processes [26]. The outcome of such an analysis approximated the $K_D$ of DAG binding in the range of 10–40 μM. Earlier estimates of local lipid changes associated with *Drosophila* phototransduction concluded DAG concentrations may rise locally close to the millimolar level. This is consistent with the observation that exogenous DAGs activate TRPC channels typically when applied at (nominal) concentrations in the high μmolar range. It is important to note that all such experimental data have to be considered cautiously since the actual lipid concentrations at the target are ill-defined during the process of channel activation and inactivation. A novel approach that may help to overcome this hurdle, is the use of caged or photochromic lipid ligands. These tools enable equilibration of the inactive precursor molecule at the channel and its rapid, quantitative conversion into the active ligand in a spatially and temporarily controlled way [63]. This approach is expected to substantially improve our understanding of lipid pharmacology and lipid gating of channel molecules. Recent functional studies with caged DAGs suggest significant structural specificity of the DAG effects at different targets including TRPC channels and support a direct lipid-ligand gating process [63].

### 2.3. Cholesterol

Substantial evidence has been accumulated for membrane cholesterol being a crucial determinant of TRPC channel function. Regulation of ion channels and transporters by the structural membrane component cholesterol has long been recognized. Cholesterol effects have been attributed to its binding to sterol recognition sites in proteins, to the ability of the sterol to alter membrane physical properties, or to its role in lateral compartmentation and dynamic anchoring/targeting of signaling molecules. Cholesterol-protein interactions have been extensively studied and specific sterol recognition elements such as the cholesterol recognition amino acid consensus (CRAC) domain has been defined [64]. Cholesterol recognition sites have been proposed for a variety of channels, including also TRP channels [4].

It is of note that direct association of cholesterol with ion channels was convincingly demonstrated for the nicotinic ACh receptor already in 1978 by Marsh and Barrantes [65]. Certain lipids were found associated and immobilized at the protein-lipid interface and to modulate channel properties. For a few proteins including the bacterial Kir channel (KirBac), evidence for the existence of a saturable sterol binding site has been provided by classical ligand binding assays, and more recently, cholesterol-channel interac-

tions have been demonstrated by life-cell FRET microscopy [66]. Saturable cholesterol binding sites have been shown to exist in ion channels, and the mechanisms by which cholesterol governs channel functions appear to involve either a ligand-gating/modulation mechanism, changes in bilayer physical properties or in coordination of cellular targeting of the channel complex.

The high pathophysiological significance of these mechanisms is evident from the established link between cholesterol metabolism and disease. For TRPC channels a functional significance of membrane cholesterol has been originally indicated by the pioneering work of Lockwich et al. demonstrating association of TRPC1 with the cholesterol-binding scaffold caveolin-1 (Cav-1) [46], which was later found to control the channel via an N-terminal Cav-1 binding motive and a C-terminal caveolin scaffolding consensus binding domain. In line with TRPC1 activity being governed by association with Cav-1, TRPC1-mediated currents were found abolished in cells lacking Cav-1 [67,68], which targets TRPC1 into the cholesterol-rich membrane environment of caveolae [46–48]. The significance of membrane cholesterol content for TRPC1 function has been demonstrated in different cell systems, and this effect was convincingly attributed to the requirement of cholesterol for proper targeting of the TRPC channel into caveolae [46,69]. Thus, cholesterol appears as a crucial factor for correct assembly of TRPC1 signalplexes, and inhibitory effects of cholesterol depletion on TRPC1 activity may mainly result from disturbance of lipid raft architecture and impaired local assembly of signaling molecules rather than disturbance of a direct lipid gating process. The impact of cholesterol on TRPC1 function was reported for several cell types—platelets, neutrophil and vascular smooth muscle cells [67,70–73].

Cholesterol sensitivity of TRPC3 and TRPC6 channels was demonstrated using pharmacological tools to modify membrane cholesterol content. Enhanced cholesterol levels were found to promote the activity of homomeric TRPC3 channels expressed in HEK293 cells [74]. Interestingly, inhibition of TPRC5 was observed by pregnenolone sulphate, pregnanolone, progesterone or dihydrotestosterone, and the existence of a rather specific and unique binding site has been proposed [4,75]. Impairment of TRPC6 function by cholesterol depletion was demonstrated to involve disruption of lipid raft structure and changes in membrane mechanical properties [76]. Moreover, the function of podocyte TRPC6 channels was found crucially dependent on targeting of the channel to cholesterol-rich domains via the cholesterol-binding adaptor podocin [45]. Experiments in human platelets suggested that cholesterol assists the heteromerization process between TRPC1/4/5 [71].

#### 2.3.1. Direct and indirect recognition of sterols

Cholesterol represents a membrane lipid component for which direct binding and regulation of ion channels has been unambiguously demonstrated [4]. For TRPC channels most available evidence points towards an indirect link between channel function and membrane cholesterol, based on the association with cholesterol-binding scaffolds (see Fig. 1), convincingly shown for TRPC1 [16] and TRPC6 [44]. Most TRPC channels show targeting to lipid rafts as a crucial determinant of their channel function [67,70–73,77–80].

Although the dynamic interaction of TRPCs with cholesterol-dependent scaffolds and their signaling partners is most likely the pivotal regulatory principle for these channels, additional effects of cholesterol on channel function cannot be excluded. The suggested reversible targeting of TRPCs into lipid rafts, and their exposure to environments of different lipid composition, specifically regarding PIs and cholesterol, may cause modulation of channel gating processes by protein lipidation, specific lipid binding events, or by altered membrane architecture and mechanics. Convincing evidence for such a mechanism comes from the observation that podocin-targeting of TRPC channels is essential for mechanosensitivity of the complex [15].

#### 2.3.2. Affinity and specificity of TRPC–cholesterol interactions

As direct regulatory cholesterol binding sites have not been identified within TRPC sequences and most of the functional changes observed with altered membrane cholesterol can be assigned to lipid interaction at an adaptor molecule, the features of potential cholesterol-TRPC interaction are subject to mere speculation. Based on published data on KirBac, such cholesterol-channels interactions may exhibit rather low affinity with $K_D$ values in the high μmolar range. Nonetheless, sterol-protein affinities were reported to vary considerably. TRPC5 channels exhibited recognition of sterols with appreciable specificity and affinity. More recently, cholesterol binding to domains of Orai1 channels have been characterized displaying $K_D$ values in the low μmolar range [66] Thus, further studies are required to clarify if specific TRPC-sterol interaction domains exist.

### 2.4. Other lipids and lipophilic activators

One of the early on identified potential lipophilic agonists of the *Drosophila* TRP channel(s) were free fatty acids, specifically PUFAS [41]. Nevertheless, binding sites for PUFAS have so far not been identified. Like cholesterol, sphingolipids tend to localize in liquid-ordered membrane microdomains [81]. Sphingosine-1-phosphate (S1P) generated from sphingomyelin was reported to activate TRPC1 and TRPC5 in various cell types [77,82–84]. TRPC activation via receptors binding sphingolipids seems to be physiologically important. Interestingly, Xu et al. reported that S1P is able to activate TRPC5 in smooth muscle cells via both intracellular and extracellular target sites. The authors observed that S1P was able to interfere with heteromerization between TRPC1 and TRPC5 in smooth muscle cells [84].

Basora et al. reported that a cytochrome P450-generated arachidonic acid metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE), is able to activate mouse TRPC6 channels expressed in HEK293 cells [85]. Among other arachidonic acid metabolites 5,6-epoxyeicosatrienoic acid (5,6-EET) is considered as a powerful modulator of TRPC1 activity [86]. Flemming et al. showed that native cation channels in vascular smooth muscle cells resembling TRPC5 respond to submicromolar concentrations of lysophospholipids independent of G protein signaling [87]. The lysophospholipid-induced effect in TRPC5 was head-group unspecific since the substitution of choline with inositol failed to show any changes in the TRPC5 activity, while the hydrophobic chain length of the lysophospholipids was of relevance. Structural specificity of lysophospholipid action was reported by Beech et al. in HEK293 cells overexpressing TRPC5 [88]. It is of note that some of the so far identified lipophilic activators of TRPC channels are generated or highly susceptible to oxidative metabolism and therefore represent a potential link between these channels and cellular redox processes as also discussed below.

## 3. The TRPC lipid-gating machinery

Lipid binding to sensor domains in TRPC channels or indirect lipid-dependent conformational changes determine the channel's gating behaviour. Structural information obtained recently for TRPV1 and TRPA1, using single particle cryo-electron microscopy, provided a reasonable basis to screen for regions involved in lipid ligand-induced gating of TRP channels [89,90]. Homology modeling, molecular dynamics approaches and functional characterization have recently shed light on molecular determinants of gating. Mutational analysis based on a homology model of TRPC3 helped to identify essential gating elements [91,92].

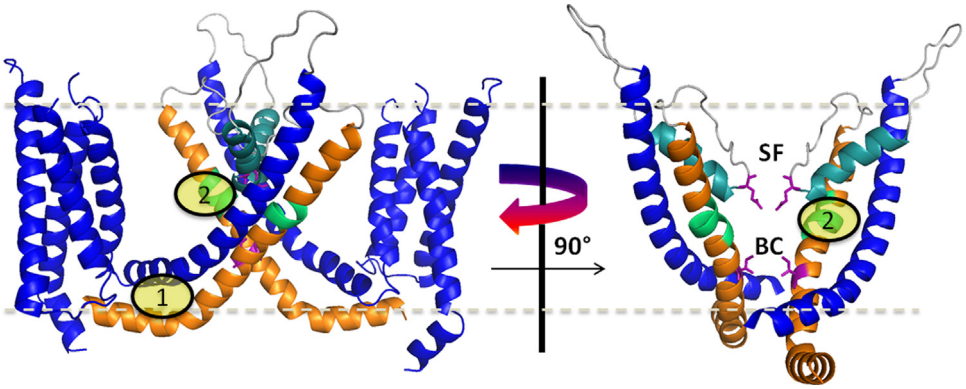


**Fig. 2.** Homology model of the TRPC3 structure based on TRPV1 as template, highlighting gating relevant structures deduced from computational analysis and functional characterization studies.
Structural elements potentially involved in lipid gating of TRPC. A homology model of TRPC3 was generated based on TRPV1 as a template structure. Domains potentially involved in lipid gating processes as identified by either mutagenesis or computational modeling analysis are highlighted. (1) membrane proximal region encompassing parts of the S4-S5 linker and residues within or close to the TRP box form gating-relevant H-bonds. (2) a structure of ill-defined helicity in the center part of the S6 helix, which is close to the selectivity filter, appears a crucial determinant of gating in TRPV1 and has been recognized also in homology models of TRPC3. These motives may relay structural changes in cytoplasmic domains initiated by lipid recognition (see Fig. 1). The open-closed transition of the channel is conferred by movements in the selectivity filter (SF) and/or the S6 bundle-crossing (BC) lower gate.

For TRPV1, a dual gating mechanism was reported, based on constriction/dilatation of the permeation pathway in both the selectivity filter (pore dilation, upper gate) and a lower occluding gate formed by hydrophobic residues in S6 corresponding to an S6 bundle crossing (BC) region. Similarly, a hydrophobic lower gate was identified in TRPC3, and mutational analysis revealed that movements in the lower gate are allosterically linked to the selectivity filter [91]. Conformational changes in lipid recognition sites may propagate to these gates via elements that allow conformational transitions in proximity of the permeation pathway. The perturbations, which trigger these conformational transitions may originate in distal cytosolic regions (Fig. 1), or in as yet unidentified lipid recognition sites at “annular” boundary regions in the channels transmembrane surface or at contact sites at central transmembrane segments, which may be accessible via fenestrations in the channel tetramer. Interestingly, the classical sensor domain S1-S4 appears rather static in TRP channels. Two domains are considered to confer potential conformational sensitivity to ligand binding in TRP channels. These structures are the membrane proximal region of S6 adjacent to the S4-S5 linker (Fig. 2(1)), which hosts potentially gating-relevant hydrogen bonding. The S4-S5 linker region has been identified as gating determinant in TRPC3/4 and 5 channels [93,94]. A geometric helical distortion located in the middle part of S6 could be potentially essential for gating transition in TRPV1 and TRPA1 [89,90,95] (Fig. 2(2)).

Collectively, our current understanding of lipid gating in TRPC channels combines the concept of multiple lipid recognition sites, most of which may reside in cytoplasmic domains allosterically connected to conformationally flexible domains in S6 and the S4-S5 linker. These critical domains in proximity to the permeation pathway may govern gating behaviour and represent potential target sites for lipids and lipophilic ligands.

## 4. The role of lipid-gating in redox sensing

Substantial evidence has been accumulated for a pivotal role of TRPC channels in cellular redox signaling. It appears important to note that TRPC proteins have been proposed as signaling molecules, linked to cellular redox balance, that acts both upstream and downstream of the generation of reactive oxygen species (ROS). TRPCs may on the one hand control ROS production [96] and on the other hand serve as ROS targets. The impact of excessive generation of ROS (oxidative stress) on TRPC signaling encompasses both changes (increases) in TRPC expression [96–98] as well as promotion of channel activity and TRPC-mediated $Ca^{2+}$ signals [99–101]. Collectively, the available evidence strongly indicates the existence of significant feed-forward cycles, in which mutual promotion of ROS generation and TRPC/$Ca^{2+}$ signaling leads to aggravation of diseased states in a variety of tissues and organs, including heart [96], lung [102–104], kidney [105,106], brain [107] and the immune system [97]. Importantly, the control of TRPC channel function by redox processes and by lipid mediators appears inseparably concatenated. The regulatory lipid species discussed above typically impact on key enzymes of ROS production, such as NADPH oxidases [8,45,49,62,106]. Thus, redox sensing may be considered as an indirect mechanism of lipid sensing by TRPC proteins [106]. In turn, evidence is available for indirect redox sensing by TRPC channels based on redox effects on the $PIP_2$/DAG pathway and redox-induced accumulation of lipid activators [103] or redox modification of the channels membrane lipid environment [99].

In essence, not only direct protein oxidation/modification, but also lipid recognition and lipid gating represents a potential interface between TRP channel function and cellular redox status. Regarding direct redox modification of TRPC complexes cysteine residues have been identified to mediate redox sensitivity in TRPC1 and TRPC5 [108]. Reactive oxygen- as well as nitrogen species have been reported to activate TRPC5. Likewise, oxidative modification of cysteine residues localized in proximity to the pore/selectivity filter region was found to activate the channel [109]. Moreover, cysteine residues in the N-terminus of TRPC5 have been reported to mediate channel activation by glutathionylation of homomeric neuronal channels [107]. Interestingly, reductive disruption of disulphide bridges has as well been implicated in TRPC5 activation [108]. Of note, redox sensitivity of TRPC containing channels, specifically TRPC3, was proposed to involve heteromerization with TRP proteins that function as direct redox sensor (TRPM2; [110,111]). It remains to be clarified how the formation of redox sensitive heteromeric channels may interfere with lipid sensing.

Lipid signaling and lipotoxicity are intimately linked to oxidative metabolism. Consequently lipid agonists/modulators of TRPC channels are subjects of oxidative modifications as are the TRPC-associated scaffolds. Thus, oxidants or disturbed cellular redox balance may impact on TRPC channel activity via interference with the processes outlined above. In principle, electrophilic species may generate active lipid mediators, interfere with lipid binding to the channel or modify lipid-binding scaffolds.

For instance, oxidized phospholipids are generated by membrane phospholipid oxidation during oxidative stress. Recently, lipid factors like 1-palmitoyl-2-oxovaleroyl-phosphatidylcholine (POVPC) or 1-palmitoyl-2-glutaroyl-phosphatidylcholine (PGPC) were reported to affect TRPC5 or TRPC5-containing channels. Importantly, the effect of POVPC and PGPC on TRPC5 function was almost entirely dependent on G-protein signaling ($G_{i/o}$) [112], which might be taken as indication for a modulatory effect of the lipids on G-proteins. Comparable channel sensitivity to $G_{i/o}$ signaling and hence $PIP_2$ was shown in TRPC4 [10]. Examples for regulation by modification of lipid-binding scaffolds are TRPC3 and heteromeric TRPC3/TRPC4 channels. These channels were found sensitive to activation by oxidants with the molecular mechanism likely involving alterations in membrane microdomain structure. For TRPC3 oxidative modification of membrane cholesterol appears as the key event mediating redox regulation of the channel by interference with caveolae structure and function. Moreover, oxidative stress has been reported to result in downregulation of Cav-1 [113] and to inhibit diacylglycerol kinase [114]. Both effects may explain upregulation of channel activity.

In aggregate, membrane lipid metabolism and lipid channel interactions are an essential determinant and part of the (patho)physiologically important function of TRPC channels as cellular redox sensors. Consequently, it is expected that molecular understanding of lipid recognition in TRPC channels and development of pharmacological strategies to interfere with these processes will open new avenues for the therapy of diseases, which involve lipid and redox sensitive TRPCs.

## 5. Concluding remarks

Over the past decade TRPC channels have emerged as molecules that transduce and integrate not only neurohumoral stimuli but also critical information from cellular lipid and redox metabolism. One key feature of these proteins is the ability to recognize lipid species as well as lipid-dependent physical properties in their membrane environment and translate this information into distinct $Ca^{2+}$ signals. The molecular mechanisms of lipid recognition and gating in TRPC channels appear of substantial pathophysiological significance and are as yet incompletely understood. As most of our current understanding on these processes is based on information from heterologously expressed, homomeric complexes, further deciphering of these principles will be both rewarding and challenging for native TRPC complexes.

## Acknowledgements

We would like to thank Thomas Stockner and Michaela Lichtenegger for critical reading of our manuscript. This work was funded by the Medical University of Graz within the Doctoral College 'Metabolic and Cardiovascular Diseases' (FWF W1226-B18).

## References

[1] D. Sengupta, A. Chattopadhyay, 2015. Molecular dynamics simulations of GPCR-cholesterol interaction: an emerging paradigm, Biochim. Biophys. Acta. 1848 (2015) 1775–1782, http://dx.doi.org/10.1016/j.bbamem.2015.03.018.
[2] L.L. Gallegos, A.C. Newton, Spatiotemporal dynamics of lipid signaling: protein kinase C as a paradigm, IUBMB Life 60 (2009) 782–789, http://dx.doi.org/10.1002/iub.122.Spatiotemporal.
[3] G. Khelashvili, H. Weinstein, Functional mechanisms of neurotransmitter transporters regulated by lipid-protein interactions of their terminal loops, Biochim. Biophys. Acta 1848 (2015) 1765–1774, http://dx.doi.org/10.1016/j.bbamem.2015.03.025.
[4] I. Levitan, D.K. Singh, A. Rosenhouse-Dantsker, Cholesterol binding to ion channels, Front. Physiol. 5 (2014) 1–14, http://dx.doi.org/10.3389/fphys.2014.00065.
[5] B. Hille, E.J. Dickson, M. Kruse, O. Vivas, B.-C. Suh, Phosphoinositides regulate ion channels, Biochim. Biophys. Acta 1851 (2015) 844–856, http://dx.doi.org/10.1016/j.bbalip.2014.09.010.
[6] B.-C. Suh, B. Hille, PIP2 is a necessary cofactor for ion channel function: how and why? Annu. Rev. Biophys. 37 (2008) 175–195, http://dx.doi.org/10.1146/annurev.biophys.37.032807.125859.
[7] C.L. Huang, S. Feng, D.W. Hilgemann, Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by Gbetagamma, Nature 391 (1998) 803–806, http://dx.doi.org/10.1038/35882.
[8] S.B. Hansen, Lipid agonism: the PIP2 paradigm of ligand-gated ion channels, Biochim. Biophys. Acta 1851 (2015) 620–628, http://dx.doi.org/10.1016/j.bbalip.2015.01.011.
[9] T. Hofmann, A.G. Obukhov, M. Schaefer, C. Harteneck, T. Gudermann, G. Schultz, Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol, Nature 397 (1999) 259–263, http://dx.doi.org/10.1038/16711.
[10] D.P. Thakur, J.-B. Tian, J. Jeon, J. Xiong, Y. Huang, V. Flockerzi, et al., Critical roles of Gi/o proteins and phospholipase C-δ1 in the activation of receptor-operated TRPC4 channels, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 1092–1097, http://dx.doi.org/10.1073/pnas.1522294113.
[11] M. Freichel, V. Tsvilovskyy, J.E. Camacho-Londono, Handbook of experimental pharmacology, in: B. Nilius, V. Flockerzi (Eds.), Mamm. Transient Recept Potential Cation Channels, Springer, 2014, pp. 85–129.
[12] B. Nilius, F. Mahieu, Y. Karashima, T. Voets, Regulation of TRP channels: a voltage-lipid connection, Biochem. Soc. Trans. 35 (2007) 105–108, http://dx.doi.org/10.1042/BST0350105.
[13] I.S. Ambudkar, H.L. Ong, Organization and function of TRPC channelosomes, Pflügers Arch. Eur. J. Physiol. 455 (2007) 187–200, http://dx.doi.org/10.1007/s00424-007-0252-0.
[14] H.L. Ong, I.S. Ambudkar, Molecular determinants of TRPC1 regulation within ER-PM junctions, Cell Calcium 58 (2015) 376–386, http://dx.doi.org/10.1016/j.ceca.2015.03.008.
[15] T.B. Huber, B. Schermer, R.U. Müller, M. Höhne, M. Bartram, A. Calixto, et al., Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 17079–17086, http://dx.doi.org/10.1073/pnas.0607465103.
[16] I.S. Ambudkar, B.C. Bandyopadhyay, X. Liu, T.P. Lockwich, B. Paria, H.L. Ong, Functional organization of TRPC-Ca2+ channels and regulation of calcium microdomains, Cell Calcium 40 (2006) 495–504, http://dx.doi.org/10.1016/j.ceca.2006.08.011.
[17] S. McLaughlin, J. Wang, A. Gambhir, D. Murray, PIP(2) and proteins: interactions, organization, and information flow, Annu. Rev. Biophys. Biomol. Struct. 31 (2002) 151–175, http://dx.doi.org/10.1146/annurev.biophys.31.082901.134259.
[18] A. Rosenhouse-Dantsker, D. Mehta, I. Levitan, Regulation of ion channels by membrane lipids, Compr. Physiol. 2 (2012) 31–68, http://dx.doi.org/10.1002/cphy.c110001.
[19] D.E. Logothetis, V.I. Petrou, S.K. Adney, R. Mahajan, Channelopathies linked to plasma membrane phosphoinositides, Pflügers Arch. Eur. J. Physiol. 460 (2010) 321–341, http://dx.doi.org/10.1007/s00424-010-0828-y.
[20] L. Lemonnier, M. Trebak, J.W. Putney, Complex regulation of the TRPC3 6 and 7 channel subfamily by diacylglycerol and phosphatidylinositol-4,5-bisphosphate, Cell Calcium 43 (2008) 506–514, http://dx.doi.org/10.1016/j.ceca.2007.09.001.
[21] M. Trebak, L. Lemonnier, W.I. DeHaven, B.J. Wedel, G.S. Bird, J.W. Putney, Complex functions of phosphatidylinositol 4 5-bisphosphate in regulation of TRPC5 cation channels, Pflügers Arch. Eur. J. Physiol. 457 (2008) 757–769, http://dx.doi.org/10.1007/s00424-008-0550-1.
[22] K. Otsuguro, J. Tang, Y. Tang, R. Xiao, M. Freichel, V. Tsvilovskyy, et al., Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate, J. Biol. Chem. 283 (2008) 10026–10036, http://dx.doi.org/10.1074/jbc.M707306200.
[23] M. Trebak, L. Lemonnier, W.I. Dehaven, B.J. Wedel, S. Gary, J.W. Putner. Jr, NIH Public Access, 457 (2010) 757–769. 10.1007/s00424-008-0550-1.Complex.
[24] K. Itsuki, Y. Imai, Y. Okamura, K. Abe, R. Inoue, M.X. Mori, Voltage-sensing phosphatase reveals temporal regulation of TRPC3/C6/C7 channels by membrane phosphoinositides, Channels (Austin) 6 (2012) 206–209, http://dx.doi.org/10.4161/chan.20883.
[25] W.A. Large, S.N. Saleh, A.P. Albert, Role of phosphoinositol 4,5-bisphosphate and diacylglycerol in regulating native TRPC channel proteins in vascular smooth muscle, Cell Calcium 45 (2009) 574–582, http://dx.doi.org/10.1016/j.ceca.2009.02.007.
[26] K. Itsuki, Y. Imai, H. Hase, Y. Okamura, R. Inoue, M.X. Mori, PLC-mediated PI(4 5)P2 hydrolysis regulates activation and inactivation of TRPC6/7 channels, J. Gen. Physiol. 143 (2014) 183–201, http://dx.doi.org/10.1085/jgp.201311033.
[27] D.B. van Rossum, R.L. Patterson, S. Sharma, R.K. Barrow, M. Kornberg, D.L. Gill, et al., Phospholipase Cgamma1 controls surface expression of TRPC3 through an intermolecular pH domain, Nature 434 (2005) 99–104, http://dx.doi.org/10.1038/nature03340.
[28] S. Miehe, A. Bieberstein, I. Arnould, O. Ihdene, H. Rutten, C. Strubing, The phospholipid-binding protein SESTD1 is a novel regulator of the transient receptor potential channels TRPC4 and TRPC5, J. Biol. Chem. 285 (2010) 12426–12434, http://dx.doi.org/10.1074/jbc.M109.068304.

[29] J. Shi, L. Birnbaumer, W.A. Large, A.P. Albert, Myristoylated alanine-rich C kinase substrate coordinates native TRPC1 channel activation by phosphatidylinositol 4,5-bisphosphate and protein kinase C in vascular smooth muscle, FASEB J. 28 (2014) 244–255, http://dx.doi.org/10.1096/fj.13-238022.
[30] A. Rosenhouse-Dantsker, D.E. Logothetis, Molecular characteristics of phosphoinositide binding, Pflügers Arch. Eur. J. Physiol. 455 (2007) 45–53, http://dx.doi.org/10.1007/s00424-007-0291-6.
[31] R. Latorre, C. Zaelzer, S. Brauchi, Structure-functional intimacies of transient receptor potential channels, Q. Rev. Biophys. 42 (2009) 201–246, http://dx.doi.org/10.1017/S0033583509990072.
[32] T. Rohacs, B. Thyagarajan, V. Lukacs, Phospholipase C mediated modulation of TRPV1 channels, Mol. Neurobiol. 37 (2016) 153–163, http://dx.doi.org/10.1007/s12035-008-8027-y.
[33] X. Steinberg, C. Lespay-Rebolledo, S. Brauchi, A structural view of ligand-dependent activation in thermoTRP channels, Front. Physiol. 5 (2014) 171, http://dx.doi.org/10.3389/fphys.2014.00171.
[34] T. Rohács, C.M.B. Lopes, I. Michailidis, D.E. Logothetis, PI(4 5)P2 regulates the activation and desensitization of TRPM8 channels through the TRP domain, Nat. Neurosci. 8 (2005) 626–634, http://dx.doi.org/10.1038/nn1451.
[35] Y. Kwon, T. Hofmann, C. Montell, Integration of phosphoinositide- and calmodulin-mediated regulation of TRPC6, Mol. Cell. 25 (2007) 491–503, http://dx.doi.org/10.1016/j.molcel.2007.01.021.
[36] K.M. Ferguson, M.A. Lemmon, J. Schlessinger, P.B. Sigler, Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain, Cell 83 (1995) 1037–1046, http://dx.doi.org/10.1016/0092-8674(95)90219-8.
[37] B. Nilius, F. Mahieu, J. Prenen, A. Janssens, G. Owsianik, R. Vennekens, et al., The Ca2+-activated cation channel TRPM4 is regulated by phosphatidylinositol 4 5-biphosphate, EMBO J. 25 (2006) 467–478, http://dx.doi.org/10.1038/sj.emboj.7600963.
[38] Z. Zhang, H. Okawa, Y. Wang, E.R. Liman, Phosphatidylinositol 4 5-bisphosphate rescues TRPM4 channels from desensitization, J. Biol. Chem. 280 (2005) 39185–39192, http://dx.doi.org/10.1074/jbc.M506965200.
[39] T. Rohacs, Regulation of TRP channels by PIP(2), Pflügers Arch. Eur. J. Physiol. 453 (2007) 753–762, http://dx.doi.org/10.1007/s00424-006-0153-7.
[40] P.-H. Tseng, H.-P. Lin, H. Hu, C. Wang, M.X. Zhu, C.-S. Chen, The canonical transient receptor potential 6 channel as a putative phosphatidylinositol 3 4,5-trisphosphate-sensitive calcium entry system, Biochemistry 43 (2004) 11701–11708, http://dx.doi.org/10.1021/bi049349f.
[41] R.C. Hardie, TRP channels and lipids: from *Drosophila* to mammalian physiology, J. Physiol. 578 (2007) 9–24, http://dx.doi.org/10.1113/jphysiol.2006.118372.
[42] D.B. van Rossum, D. Oberdick, Y. Rbaibi, G. Bhardwaj, R.K. Barrow, N. Nikolaidis, et al., TRP.2, a lipid/trafficking domain that mediates diacylglycerol-induced vesicle fusion, J. Biol. Chem. 283 (2008) 34384–34392, http://dx.doi.org/10.1074/jbc.M804707200.
[43] R.L. Patterson, D.B. van Rossum, D.L. Ford, K.J. Hurt, S.S. Bae, P.G. Suh, et al., Phospholipase C-gamma is required for agonist-induced Ca2+ entry, Cell 111 (2002) 529–541, S0092867402010450 [pii]).
[44] B. Schermer, T. Benzing, Lipid-protein interactions along the slit diaphragm of podocytes, J. Am. Soc. Nephrol. 20 (2009) 473–478, http://dx.doi.org/10.1681/ASN.2008070694.
[45] M. Anderson, E.Y. Kim, H. Hagmann, T. Benzing, S.E. Dryer, Opposing effects of podocin on the gating of podocyte TRPC6 channels evoked by membrane stretch or diacylglycerol, Am. J. Physiol. Cell Physiol. 305 (2013) 276–289, http://dx.doi.org/10.1152/ajpcell.00095.2013.
[46] T.P. Lockwich, Assembly of trp1 in a signaling complex associated with caveolin-Scaffolding lipid raft domains, J. Biol. Chem. 275 (2000) 11934–11942, http://dx.doi.org/10.1074/jbc.275.16.11934.
[47] A.M. Kwiatek, R.D. Minshall, D.R. Cool, R.A. Skidgel, A.B. Malik, C. Tiruppathi, Caveolin-1 regulates store-operated Ca2+ influx by binding of its scaffolding domain to transient receptor potential channel-1 in endothelial cells, Mol. Pharmacol. 70 (2006) 1174–1183, http://dx.doi.org/10.1124/mol.105.021741.
[48] S.-C.W. Brazer, B.B. Singh, X. Liu, W. Swaim, I.S. Ambudkar, Caveolin-1 contributes to assembly of store-operated Ca2+ influx channels by regulating plasma membrane localization of TRPC1, J. Biol. Chem. 278 (2003) 27208–27215, http://dx.doi.org/10.1074/jbc.M301118200.
[49] P. Lucas, K. Ukhanov, T. Leinders-Zufall, F. Zufall, A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impaired in TRPC2 mutant mice: mechanism of pheromone transduction, Neuron 40 (2003) 551–561.
[50] R.C. Hardie, Phototransduction in drosophila melanogaster, J. Exp. Biol. 204 (2001) 3403–3409.
[51] R. Delgado, Y. Munoz, H. Pena-Cortes, P. Giavalisco, J. Bacigalupo, Diacylglycerol activates the light-dependent channel TRP in the photosensitive microvilli of drosophila melanogaster photoreceptors, J. Neurosci. 34 (2014) 6679–6686, http://dx.doi.org/10.1523/JNEUROSCI.0513-14.2014.
[52] B. Nilius, V. Flockerzi, Handbook of Experimental Pharmacology/Mammalian Transient Receptor Potential (TRP) Cation Channels, Spriger, 2014.
[53] M.A. Spassova, T. Hewavitharana, W. Xu, J. Soboloff, D.L. Gill, 2006, A common mechanism underlies stretch activation and receptor activation of TRPC6 channels, Proc. Natl. Acad. Sci. U. S. A. 103 (2016) 16586–16591, http://dx.doi.org/10.1073/pnas.0606894103.
[54] L. Zhang, D. Saffen, Muscarinic acetylcholine receptor regulation of TRP6Ca2+ channel isoforms. Molecular structures and functional characterization, J. Biol. Chem. 276 (2001) 13331–13339, http://dx.doi.org/10.1074/jbc.M008914200.
[55] S. Jung, A. M??hle, M. Schaefer, R. Strotmann, G. Schultz, T.D. Plant, Lanthanides potentiate TRPC5 currents by an action at extracellular sites close to the pore mouth, J. Biol. Chem. 278 (2003) 3562–3571, http://dx.doi.org/10.1074/jbc.M211484200.
[56] G. Vazquez, B.J. Wedel, B.T. Kawasaki, G. St. John Bird, J.W. Putney, Obligatory role of Src kinase in the signaling mechanism for TRPC3 cation channels, J. Biol. Chem. 279 (2004) 40521–40528, http://dx.doi.org/10.1074/jbc.M405280200.
[57] B.C. Bandyopadhyay, H.L. Ong, T.P. Lockwich, X. Liu, B.C. Paria, B.B. Singh, et al., TRPC3 controls agonist-stimulated intracellular Ca2+ release by mediating the interaction between inositol 1,4,5-trisphosphate receptor and RACK, J. Biol. Chem. 283 (2008) 32821–32830, http://dx.doi.org/10.1074/jbc.M805382200.
[58] G. Cao, S. Thébault, J. van der Wijst, A. van der Kemp, E. Lasonder, R.J.M. Bindels, et al., RACK1 inhibits TRPM6 activity via phosphorylation of the fused α-kinase domain, Curr. Biol. 18 (2008) 168–176, http://dx.doi.org/10.1016/j.cub.2007.12.058.
[59] J. Yang, Q. Wang, W. Zheng, J. Tuli, Q. Li, Y. Wu, et al., Receptor for activated C kinase 1 (RACK1) inhibits function of transient receptor potential (TRP)-type channel Pkd2l1 through physical interaction, J. Biol. Chem. 287 (2012) 6551–6561, http://dx.doi.org/10.1074/jbc.M111.305854.
[60] R.C. Hardie, K. Franze, Photomechanical responses in drosophila photoreceptors, Science 338 (2012) 260–263 (80-.).
[61] M. Mederos y Schnitzler, U. Storch, S. Meibers, P. Nurwakagari, A. Breit, K. Essin, et al., Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction, EMBO J. 27 (2008) 3092–3103, http://dx.doi.org/10.1038/emboj.2008.233.
[62] U. Storch, M.M.Y. Schnitzler, T. Gudermann, G protein-mediated stretch reception, AJP Hear. Circ. Physiol. 302 (2012) H1241–H1249, http://dx.doi.org/10.1152/ajpheart.00818.2011.
[63] A. Nadler, G. Reither, S. Feng, F. Stein, S. Reither, R. Müller, et al., The fatty acid composition of diacylglycerols determines local signaling patterns, Angew. Chemie Int. Ed. 52 (2013) 6330–6334, http://dx.doi.org/10.1002/anie.201301716.
[64] R.M. Epand, Cholesterol and the interaction of proteins with membrane domains, Prog. Lipid Res. 45 (2006) 279–294, http://dx.doi.org/10.1016/j.plipres.2006.02.001.
[65] D. Marsh, F.J. Barrantes, Immobilized lipid in acetylcholine receptor-rich membranes from Torpedo marmorata, Proc. Natl. Acad. Sci. U. S. A. 75 (1978) 4329–4333.
[66] I. Derler, I. Jardin, P.B. Stathopulos, M. Muik, M. Fahrner, V. Zayats, et al., Cholesterol modulates Orai1 channel function, Sci. Signal. 9 (2016) ra10, http://dx.doi.org/10.1126/scisignal.aad7808.
[67] T. Murata, M.I. Lin, R.V. Stan, P.M. Bauer, J. Yu, W.C. Sessa, Genetic evidence supporting caveolae microdomain regulation of calcium entry in endothelial cells, J. Biol. Chem. 282 (2007) 16631–16643, http://dx.doi.org/10.1074/jbc.M607948200.
[68] C.G. Nichols, A.N. Lopatin, Inward rectifier potassium channels, Annu. Rev. Physiol. 59 (1997) 171–191, http://dx.doi.org/10.1146/annurev.physiol.59.1.171.
[69] B. Pani, L.O. Hwei, X. Liu, K. Rauser, I.S. Ambudkar, B.B. Singh, Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca2+ entry (SOCE), J. Biol. Chem. 283 (2008) 17333–17340, http://dx.doi.org/10.1074/jbc.M800107200.
[70] A. Bergdahl, Cholesterol depletion impairs vascular reactivity to endothelin-1 by reducing store-operated Ca2+ entry dependent on TRPC1, Circ. Res. 93 (2003) 839–847, http://dx.doi.org/10.1161/01.RES.0000100367.45446.A3.
[71] S.L. Brownlow, S.O. Sage, TRPC subunit assembly and membrane distribution in human platelets, Thromb. Haemost. 94 (2005) 839–845, http://dx.doi.org/10.1160/TH05-06-0391.
[72] K.B. Kannan, D. Barlos, C.J. Hauser, Free cholesterol alters lipid raft structure and function regulating neutrophil Ca2+ entry and respiratory burst: correlations with calcium channel raft trafficking, J. Immunol. 178 (2007) 5253–5261.
[73] S.H. Weerth, L.A. Holtzclaw, J.T. Russell, Signaling proteins in raft-like microdomains are essential for Ca2+ wave propagation in glial cells, Cell Calcium 41 (2007) 155–167, http://dx.doi.org/10.1016/j.ceca.2006.06.006.
[74] A. Graziani, C. Rosker, S.D. Kohlwein, M.X. Zhu, C. Romanin, W. Sattler, et al., Cellular cholesterol controls TRPC3 function: evidence from a novel dominant-negative knockdown strategy, Biochem. J. 396 (2006) 147–155, http://dx.doi.org/10.1042/BJ20051246.
[75] Y. Majeed, M.S. Amer, A.K. Agarwal, L. McKeown, K.E. Porter, D.J. O'Regan, et al., Stereo-selective inhibition of transient receptor potential TRPC5 cation channels by neuroactive steroids, Br. J. Pharmacol. 162 (2011) 1509–1520, http://dx.doi.org/10.1111/j.1476-5381.2010.01136.x.
[76] L. Lei, S. Lu, Y. Wang, T. Kim, D. Mehta, Y. Wang, The role of mechanical tension on lipid raft dependent PDGF-induced TRPC6 activation, Biomaterials 35 (2014) 2868–2877, http://dx.doi.org/10.1016/j.biomaterials.2013.12.030.
[77] L. Formigli, C. Sassoli, R. Squecco, F. Bini, M. Martinesi, F. Chellini, et al., Regulation of transient receptor potential canonical channel 1 (TRPC1) by

sphingosine 1-phosphate in C2C12 myoblasts and its relevance for a role of mechanotransduction in skeletal muscle differentiation, J. Cell Sci. 122 (2009) 1322–1333, http://dx.doi.org/10.1242/jcs.035402.
[78] C. Ingueneau, U. Do Huynh, B. Marcheix, A. Athias, P. Gambert, A. Nègre-Salvayre, et al., TRPC1 is regulated by caveolin-1 and is involved in oxidized LDL-induced apoptosis of vascular smooth muscle cells, J. Cell. Mol. Med. 13 (2009) 1620–1631.
[79] S. Ye, L. Tan, J. Ma, Q. Shi, J. Li, Polyunsaturated docosahexaenoic acid suppresses oxidative stress induced endothelial cell calcium influx by altering lipid composition in membrane caveolar rafts, Prostaglandins Leukot. Essent. Fat. Acids 83 (2010) 37–43, http://dx.doi.org/10.1016/j.plefa.2010.02.002.
[80] B. Pani, H.L. Ong, S.-C.W. Brazer, X. Liu, K. Rauser, B.B. Singh, et al., Activation of TRPC1 by STIM1 in ER-PM microdomains involves release of the channel from its scaffold caveolin-1, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 20087–20092, http://dx.doi.org/10.1073/pnas.0905002106.
[81] P.J. Quinn, C. Wolf, The liquid-ordered phase in membranes, Biochim. Biophys. Acta 1788 (2009) 33–46, http://dx.doi.org/10.1016/j.bbamem.2008.08.005.
[82] S. Crousillac, J. Colonna, E. McMains, J.S. Dewey, E. Gleason, Sphingosine-1-phosphate elicits receptor-dependent calcium signaling in retinal amacrine cells, J. Neurophysiol. 102 (2009) 3295–3309, http://dx.doi.org/10.1152/jn.00119.2009.
[83] D. Mehta, M. Konstantoulaki, G.U. Ahmmed, A.B. Malik, Sphingosine 1-phosphate-induced mobilization of intracellular Ca2+ mediates rac activation and adherens junction assembly in endothelial cells, J. Biol. Chem. 280 (2005) 17320–17328, http://dx.doi.org/10.1074/jbc.M411674200.
[84] S.-Z. Xu, K. Muraki, F. Zeng, J. Li, P. Sukumar, S. Shah, et al., A sphingosine-1-phosphate-activated calcium channel controlling vascular smooth muscle cell motility, Circ. Res. 98 (2006) 1381–1389, http://dx.doi.org/10.1161/01.RES.0000225284.36490.a2.
[85] N. Basora, G. Boulay, L. Bilodeau, E. Rousseau, M.D. Payet, 20-hydroxyeicosatetraenoic acid (20-HETE) activates mouse TRPC6 channels expressed in HEK293 cells, J. Biol. Chem. 278 (2003) 31709–31716, http://dx.doi.org/10.1074/jbc.M304437200.
[86] N. Ben-Amor, P.C. Redondo, A. Bartegi, J.A. Pariente, G.M. Salido, J.A. Rosado, A role for 5 6-epoxyeicosatrienoic acid in calcium entry by de novo conformational coupling in human platelets, J. Physiol. 570 (2006) 309–323, http://dx.doi.org/10.1113/jphysiol.2005.100800.
[87] A.M. Flemming, S.-Z. Xu, J. Li, F. Zeng, J. Naylor, et al., Sensing of lysophospholipids by TRPC5 calcium channel, J. Biol. Chem. 281 (2006) 4977–4982, http://dx.doi.org/10.1074/jbc.M510301200.
[88] D.J. Beech, Y.M. Bahnasi, A.M. Dedman, E. Al-Shawaf, TRPC channel lipid specificity and mechanisms of lipid regulation, Cell Calcium 45 (2009) 583–588, http://dx.doi.org/10.1016/j.ceca.2009.02.006.
[89] C.E. Paulsen, J. Armache, Y. Gao, Y. Cheng, D. Julius, Structure of the TRPA1 ion channel suggests regulatory mechanisms, Nature 520 (2015) 511–517, http://dx.doi.org/10.1038/nature14367.
[90] E. Cao, M. Liao, Y. Cheng, D. Julius, TRPV1 structures in distinct conformations reveal activation mechanisms, Nature 504 (2013) 113–118, http://dx.doi.org/10.1038/nature12823.
[91] M. Lichtenegger, T. Stockner, M. Poteser, H. Schleifer, D. Platzer, C. Romanin, et al., A novel homology model of TRPC3 reveals allosteric coupling between gate and selectivity filter, Cell Calcium 54 (2013) 175–185, http://dx.doi.org/10.1016/j.ceca.2013.05.010.
[92] M. Poteser, H. Schleifer, M. Lichtenegger, M. Schernthaner, T. Stockner, C.O. Kappe, et al., PKC-dependent coupling of calcium permeation through transient receptor potential canonical 3 (TRPC3) to calcineurin signaling in HL-1 myocytes, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 10556–10561, http://dx.doi.org/10.1073/pnas.1106183108.
[93] A. Beck, T. Speicher, C. Stoerger, T. Sell, V. Dettmer, S.A. Jusoh, et al., Conserved gating elements in TRPC4 and TRPC5Channels, J. Biol. Chem. 288 (2013) 19471–19483, http://dx.doi.org/10.1074/jbc.M113.478305.
[94] S.M. Hanson, M.S.P. Sansom, E.B.E. Becker, Modeling suggests TRPC3 hydrogen bonding and not phosphorylation contributes to the ataxia phenotype of the moonwalker mouse, Biochemistry (2015), http://dx.doi.org/10.1021/acs.biochem.5b00235 (150626072742003).
[95] E. Palovcak, L. Delemotte, M.L. Klein, V. Carnevale, Comparative sequence analysis suggests a conserved gating mechanism for TRP channels, J. Gen. Physiol. 146 (2015) 37–50, http://dx.doi.org/10.1085/jgp.201411329.
[96] N. Kitajima, K. Watanabe, S. Morimoto, Y. Sato, S. Kiyonaka, M. Hoshijima, et al., TRPC3-mediated Ca2+ influx contributes to Rac1-mediated production of reactive oxygen species in MLP-deficient mouse hearts, Biochem. Biophys. Res. Commun. 409 (2011) 108–113, http://dx.doi.org/10.1016/j.bbrc.2011.04.124.
[97] X. Song, B.C. Liu, X.Y. Lu, L.L. Yang, Y.J. Zhai, A.F. Eaton, et al., Lovastatin inhibits human B lymphoma cell proliferation by reducing intracellular ROS and TRPC6 expression, Biochim. Biophys. Acta Mol. Cell Res. 1843 (2014) 894–901, http://dx.doi.org/10.1016/j.bbamcr.2014.02.002.
[98] Q. Jiang, X. Fu, L. Tian, Y. Chen, K. Yang, X. Chen, et al., NOX4 mediates BMP4-induced upregulation of TRPC1 and 6 protein expressions in distal pulmonary arterial smooth muscle cells, PLoS One 9 (2014) e107135, http://dx.doi.org/10.1371/journal.pone.0107135.
[99] M. Poteser, A. Graziani, C. Rosker, P. Eder, I. Derler, H. Kahr, et al., TRPC3 and TRPC4 associate to form a redox-sensitive cation channel: evidence for expression of native TRPC3-TRPC4 heteromeric channels in endothelial cells, J. Biol. Chem. 281 (2006) 13588–13595, http://dx.doi.org/10.1074/jbc.M512205200.
[100] N. Weissmann, A. Sydykov, H. Kalwa, U. Storch, B. Fuchs, M. Mederos y Schnitzler, et al., Activation of TRPC6 channels is essential for lung ischaemia-reperfusion induced oedema in mice, Nat. Commun. 3 (2012) 649, http://dx.doi.org/10.1038/ncomms1660.
[101] M. Balzer, B. Lintschinger, K. Groschner, Evidence for a role of Trp proteins in the oxidative stress-induced membrane conductances of porcine aortic endothelial cells, Cardiovasc. Res. 42 (1999) 543–549.
[102] F. Veit, O. Pak, R.P. Brandes, N. Weissmann, Hypoxia-dependent reactive oxygen species signaling in the pulmonary circulation: focus on ion channels, Antioxid. Redox Signal. 22 (2015) 537–552, http://dx.doi.org/10.1089/ars.2014.6234.
[103] M. Malczyk, C. Veith, R.T. Schermuly, T. Gudermann, A. Dietrich, N. Sommer, et al., NADpH oxidases—do they play a role in TRPC regulation under hypoxia? Pflugers Arch. Eur. J. Physiol. 468 (2016) 23–41, http://dx.doi.org/10.1007/s00424-015-1731-3.
[104] P.I. Aaronson, TRPC channel upregulation in chronically hypoxic pulmonary arteries: the HIF-1 bandwagon gathers steam, Circ. Res. 98 (2006) 1465–1467, http://dx.doi.org/10.1161/01.RES.0000231254.58548.b4.
[105] B. Zhao, H. Yang, R. Zhang, H. Sun, C. Liao, J. Xu, et al., The role of TRPC6 in oxidative stress-induced podocyte ischemic injury, Biochem. Biophys. Res. Commun. 461 (2015) 413–420, http://dx.doi.org/10.1016/j.bbrc.2015.04.054.
[106] E.Y. Kim, M. Anderson, C. Wilson, H. Hagmann, T. Benzing, S.E. Dryer, NOX2 interacts with podocyte TRPC6 channels and contributes to their activation by diacylglycerol: essential role of podocin in formation of this complex, Am. J. Physiol. Cell Physiol. 305 (2013) 960–971, http://dx.doi.org/10.1152/ajpcell.00191.2013.
[107] C. Hong, H. Seo, M. Kwak, J. Jeon, J. Jang, E.M. Jeong, et al., Increased TRPC5 glutathionylation contributes to striatal neuron loss in Huntington's disease, Brain 138 (2015) 3030–3047, http://dx.doi.org/10.1093/brain/awv188.
[108] S.-Z. Xu, P. Sukumar, F. Zeng, J. Li, A. Jairaman, A. English, et al., TRPC channel activation by extracellular thioredoxin, Nature 451 (2008) 69–72, http://dx.doi.org/10.1038/nature06414.
[109] T. Yoshida, R. Inoue, T. Morii, N. Takahashi, S. Yamamoto, Y. Hara, et al., Nitric oxide activates TRP channels by cysteine *S*-nitrosylation, Nat. Chem. Biol. 2 (2006) 596–607, http://dx.doi.org/10.1038/nchembio821.
[110] A.S. Roedding, S.Y. Tong, W. Au-Yeung, P.P. Li, J.J. Warsh, Chronic oxidative stress modulates TRPC3 and TRPM2 channel expression and function in rat primary cortical neurons: relevance to the pathophysiology of bipolar disorder, Brain Res. 1517 (2013) 16–27, http://dx.doi.org/10.1016/j.brainres.2013.04.025.
[111] A.S. Roedding, A.F. Gao, W. Au-Yeung, T. Scarcelli, P.P. Li, J.J. Warsh, Effect of oxidative stress on TRPM2 and TRPC3 channels in B lymphoblast cells in bipolar disorder, Bipolar Disord. 14 (2012) 151–161, http://dx.doi.org/10.1111/j.1399-5618.2012.01003.x.
[112] E. Al-Shawaf, J. Naylor, H. Taylor, K. Riches, C.J. Milligan, D. O'Regan, et al., Short-term stimulation of calcium-permeable transient receptor potential canonical 5-containing channels by oxidized phospholipids, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1453–1459, http://dx.doi.org/10.1161/ATVBAHA.110.205666.
[113] A. Mougeolle, S. Poussard, M. Decossas, C. Lamaze, O. Lambert, E. Dargelos, Oxidative stress induces caveolin 1 degradation and impairs caveolae functions in skeletal muscle cells, PLoS One 10 (2015) e0122654, http://dx.doi.org/10.1371/journal.pone.0122654.
[114] H. Atsumi, M. Kitada, K. Kanasaki, D. Koya, Reversal of redox-dependent inhibition of diacylglycerol kinase by antioxidants in mesangial cells exposed to high glucose, Mol. Med. Rep. 4 (2016) 923–927, http://dx.doi.org/10.3892/mmr.2011.524.