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Mechanisms and significance of Ca^{2+} entry through TRPC channels

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Abstract

The transient receptor potential (TRP) superfamily of plasma membrane cation channels has been recognized as a signaling hub in highly diverse settings of human physiopathology. In the past three decades of TRP research, attention was focused mainly on the channels Ca^{2+} signaling function, albeit additional cellular functions, aside of providing a Ca^{2+} entry pathway, have been identified. Our understanding of Ca^{2+} signaling by TRP proteins has recently been advanced by a gain in high-resolution structure information on these pore complexes, and by the development of novel tools to investigate their role in spatiotemporal Ca^{2+} handling. This review summarizes recent discoveries as well as remaining, unresolved aspects of the canonical subfamily of transient receptor potential channels (TRPC) research. We aim at a concise overview on current mechanistic concepts of Ca^{2+} entry through- and Ca^{2+} signaling by TRPC channels.

TRPC signaling mechanisms: a brief overview on Ca^{2+} handling and beyond

The gene underlying photosensory impairment of the *Drosophila trp* mutation was cloned in 1975 (Rubin and Montell), and its product identified as a molecule involved in phospholipid-controlled Ca^{2+} entry into photoreceptors of the fly (see review B Minke [1]). The proposed relationship that links this signaling molecule to the ubiquitous mechanism of store-operated or capacitative Ca^{2+} entry remains still controversial. Intensive research on gene products related to the *Drosophila* TRP channel led to our current picture of the TRP superfamily. As the *Drosophila* TRP protein was early on recognized to form a cation/Ca^{2+} permeable ion channel [2], the past three decades of TRP research were largely focused on concepts of Ca^{2+} permeation through a central pore structure within a homomeric or heteromeric TRP complexes. The corresponding Ca^{2+} flux across the plasma membrane generates spatiotemporal Ca^{2+} pattern, which are decoded by a wide range of downstream signaling targets [3]. Interestingly, a few TRP proteins were shown to lack appreciable Ca^{2+} permeability, while

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alternative signaling functions have been unveiled [4–7]. Molecular concepts, by which TRP molecules reportedly serve signal transduction in certain cellular settings range from enzymatic activities, to indirect modulation of local and global Ca^{2+} levels by physical or functional interaction with other Ca^{2+} handling elements (see below). Of note, some particular TRP functions appear even largely uncoupled from ion permeation. Such Ca^{2+} permeation independent function has also been described for the canonical TRP (TRPC) subfamily [7], which is most closely related to the invertebrate founding member and will be in focus of this review.

Our discussion of the Ca^{2+} signaling function of TRPC channels shall therefore start with the ability of TRPC isoforms to constitute a classical Ca^{2+} permeation pathway that comprises an ion selectivity filter and occluding gate structure(s). This feature was initially anticipated from sequence similarities to other voltage-gated channel structures, and a predicted membrane topology featuring six prominent transmembrane helical segments engulfing a short pore loop helix element [8]. The field of TRPC channels has advanced substantial by the recent elucidation of the structures of four members of the TRPC subfamily (TRPC3/4/5/6) by single particle cryogenic electron microscopy studies (cryoEM) [8]. Unfortunately, the conformation of an open TRPC channel has not been resolved yet and the structures of TRPC1 and TRPC7 are still missing. For recombinant TRPC1, the most enigmatic of the vertebrate proteins, generation of Ca^{2+} permeable, homomeric pore complexes by heterologous overexpression has not been documented. Homology models indicate functionally relevant differences in residues lining the selectivity filter when compared to all other TRPCs (Figure 1). TRPC1 has supposedly an important role in native heteromeric TRPC channels that feature appreciable Ca^{2+} permeability. This was shown in the brain for heteromeric channels in complex with TRPC4 and TRPC5 channels [9•, 10•]. For secretory cells, TRPC1 was identified as a Ca^{2+} permeable, nonse-lective cation channel, of which membrane presentation is governed by local Ca^{2+} signals generated by the classi-cal STIM/Orai pathway [11]. Notably, the Ca^{2+} permeability of native TRPC channels is essentially low when compared to voltage-gated and most Orai Ca^{2+} entry channels (see Table 1). The largely non-selective nature of the TRPC-mediated cation conductance implies that a significant part of downstream signaling is conferred by membrane depolarization and/or Na^{+} loading. Both have been reported to contribute to Ca^{2+}-mediated TRPC signal transduction [12] (for review see Ref. [13]). Hence, Ca^{2+} signaling by TRPC channels is complex in nature and engulfs: i) Ca^{2+} transport through the central pore of the TRPC complex, and/or ii) indirect modulation of cytoplasmic Ca^{2+} levels via Na^{+} entry as well as changes in membrane potential, and/or iii) physical and/or functional interaction with other Ca^{2+} transport proteins such as $IP^{3}R$ [14]; review [13]. Specificity of TRPC downstream signaling is achieved by distinct spatiotem-poral Ca^{2+} pattern, in particular on the Ca^{2+} amplitude generated within the channels vicinity and the localiza-tion of downstream target Ca^{2+} sensors such as calmodu-lin and calcineurin. The general concept of membrane delimited signaling processes being organized within specialized, laterally confined regions that are tailored for strategic positioning of upstream and downstream components of a pathway, is considered as the basis of TRPC signaling versatility. Specialized signaling regions cen-tered around Ca^{2+} entry channels in the plasma membrane, referred to as ‘micro- or nanodomains’, typically feature a highly structured cytoplasmic space formed by

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membrane elements of cellular organelle, such as endo-plasmic reticulum or mitochondria localized in close apposition to the plasma membrane. The complex membrane architectures of such junctional regions not only enables plasma membrane-organelle communication but also the spatial confinement and precise spatiotemporal tuning of Ca^{2+} signals. Within a Ca^{2+} microdomain, high Ca^{2+} levels, evoked by opening of a set of Ca^{2+} entry channels, allow the cell to specifically govern certain downstream Ca^{2+} sensors and orchestrate cellular processes [15]. TRPCs may function in itself as scaffolds to anchor or dynamically target downstream signaling molecules within its vicinity to achieve-specific control over rather low affinity Ca^{2+} sensors. It is important to note that TRPC function is tightly linked to membrane lipid handling and metabolism, which are in turn essential determinants for the architecture and function of signaling microdomains at the plasma membrane [16–18]. Lipid metabolism has long been recognized as a key input signal for the control of channel gating [19]. Recent structural information extended this concept by demonstrating multiple lipidation sites within TRPC complexes, not only for regulatory mediators but also for structural components of the lipid bilayer [8,19]. Hence, TRPC complexes are intimately linked to membrane lipid handling with highly effective lipid-protein interaction being involved in channel targeting, gating, permeation and downstream signaling, which in turn impacts on local lipid handling [20••, 21•].

Significance of TRPC-mediated Ca^{2+} signaling for physiopathology

TRPCs are reportedly expressed in almost all mammalian tissues, and a functional role of TRPC channels has been demonstrated for both excitable and nonexcitable cell types (for review see Refs. [3,22]). Most, albeit not all, physiologic and/or pathophysiologic functions assigned to TRPC proteins are mechanistically explained by an involvement in cellular Ca^{2+} homeostasis and contribution to pivotal signaling pathways [9•,11,23–27]. Consistently, TRPC channel mutations and malfunctions have been found associated with inherited diseases and a wide array of human pathologies including neurodegeneration, cancer and cardiac diseases [28,29]. Only a comprehensive understanding of the multifunctional role of TRPC in microdomain Ca^{2+} signaling will enable the development of strategies for their successful therapeutic targeting. Below we will provide an update on emerging concept regarding Ca^{2+} handling by TRPC proteins and the generation of spatiotemporal Ca^{2+} pattern that convey information specifically towards downstream targets.

The TRPC pore structure – focus on Ca^{2+} permeation

Essential for TRPCs function to generate a Ca^{2+} signal is the formation of a Ca^{2+} permeation pathway through channel tetramers. Though the pore structure of TRPCs is highly conserved (with TRPC1 as an exception), these channels exhibit varying degree of Ca^{2+} selectivity (Table 1).

Isoform specific Ca^{2+} permeation

A possible explanation for the difference in cation selectivity may be deduced from the residues lining the pore domain (Figure 1a). The structural basis of discrimination between Ca^{2+} and Na^{+} in voltage-gated Ca^{2+} (Ca_v) and Na^{+} (Na_v) channels was elaborated by Dudev and Lim [30]. The ion conducting pathway consists of a selectivity filter and two gates

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(upper and lower). Though the selectivity filter in both Ca_v and Na_v is fairly similar and consists of four glutamate residues, these channels exhibit high selectivity towards two different cations. The main difference in ion permeability is determined by the protein matrix surrounding the selectivity filter. The main mechanism of Ca^{2+} over Na^+ selectivity [30] was attributed to the ability to discriminate between minor differences in metal hydration structure and ion-water interaction energy. It is tempting to conclude that similar mechanism can define cation selectivity of TRPCs.

Recently TRPC3/4/5/6 structures were resolved by cryoEM analysis in the closed state. The selectivity filter features glycine residues within the narrowest region of the pore, only TRPC1 carries a serine in the same position. The cryoEM structures have shown that the gluta-mate residues of TRPC channels are not in the identical position as in the Na^+ (Na_v) channels. In TRPC channels their exposure to the central permeation pore is a critical determinant of divalent/monovalent permeability. These acidic residues form a negative electrostatic potential at the entrance to the selectivity filter and the outer pore region. Presumably, this potential provides the required attraction for divalent ions and appears essential for Ca^{2+} permeation and selectivity. It was shown that neutralization of acidic residues in TRPCs reduced or abolished Ca^{2+} permeation without any significant attenuation of Na^+ currents in TRPC3 and TRPC1 [38,39]. On contrary, mutation of E595Q/E598Q in TRPC5 (E591/E594 in hTRPC4) in specific TRPC4/5 EFTE sequence resulted in increased single channel currents and Ca^{2+} selectivity (P_{Ca}/P_{Na} from 1.79 to 4.28; [35]). Jung et al. hypothesized that neutralization of negative charges in that region removed binding of physiological cations from the 'gatekeeper' residues.

According to the cryoEM studies on TRPCs pore architecture, the closed lower gate is formed by Ile, Asn and Gln residues. Asparagines are found in the most constricted part of the lower gate in all analysed structures (hTRPC3 N659, [40] mTRPC4 and zTRPC4 N621, [41,42]; hTRPC5 N625, [43]; hTRPC6 N728, [40]. The only exception is hTRPC3 [44], where S6 shows a different conformation at the inner gate, locating I658 at the most restricted point of the inner gate instead of the asparagine. The difference in conformation of the cryoEM structures of the TRPC channels might be in part due to the experimental details such as the presence of antagonist versus agonist [40,45••]. The ion permeation path in the higher resolution TRPC6 structure (2.84 Å) appears more dilated with a shift in restricting residues by one helical turn. Unexpectedly, also the agonist bound hTRP6 structure revealed a closed inner gate [45••].

Sequence comparison of all human TRPC channels and homology modeling of TRPC7 (unpublished) based on the cryoEM structure of hTRPC3 (PDB ID: 6CUD) [44] revealed high structural and sequence similarity, including a selectivity filter that is conserved between TRPC3/ 6/7. In contrast, a homology model of hTRPC1 based on the closely related mTRPC4 cryoEM structure (PDB ID: 5Z96, [42]) revealed differences in residues forming the selectivity filter. According to sequence alignment, cryoEM structures and homology modeling, the residue at the most restricted position of the selectivity filter of TRPC3/4/5/6/7 channels is a glycine while TRPC1 carries a serine (S600) in the respective position. The larger side chain significantly narrows the ion permeation path and provides 4 additional hydroxyl group in the homotetramer (Figure 1a). S600 in TRPC1 structurally

resembles the bacterial voltage-gated Na^+ channel (Na_vRh), which also contains a serine in the respective position. Simulations on cation permeation through the Na_vRh channel [46] revealed that serine residues can easily reduce energy barrier for Na^+ ion, while Ca^{2+} permeation would be energetically disfavoured.

Most of the currently available information has been obtained from recombinant homomeric channels, while the biophysical features of native, frequently heteromeric channels are less well understood. A reported property of endogenous and recombinant TRPC1 is its ability to form heteromeric channels with variety of TRPCs and modulate the expression of other TRPs [47]. This interaction results in a significant reduction of Ca^{2+} selectivity, total ion currents and membrane targeting of other TRP channels. Interestingly, some reports doubt that TRPC1 homomeric channels have a channel function and instead suggest that TRPC1 could function as regulatory subunit [48]. Co-expression of recombinant TRPC1 and TRPC3 significantly suppressed carbachol-induced Ca^{2+} entry and abolished 1-oleoyl-2-acetyl-sn-glycerol induced currents through TRPC3 channels, when overexpressed in HEK293 cells [49]. Schindl *et al.* showed that TRPC1 cannot form a heteromeric channel with TRPV6 (highly Ca^{2+} selective), but was able to downregulate the surface expression of the TRPV6 and strongly reduce Ca^{2+} currents [50].

Hence the key feature of Ca^{2+} permeability through the open TRPC pore seems to be critically dependent on multimeric assembly and subunit stoichiometry. Hetero-merisation is one aspect that determines the efficiency and specificity of downstream signaling in native cellular setting. Besides heteromeric pore assembly, also activating ligands as well as lipid coordination may impact on permeability features (Figure 2a).

Ligands may modify Ca^{2+} permeability

Ca^{2+} influx though TRP channels can be shaped by the nature of activating ligands. Direct activators of TRPC3/ 6/7 are more potent in inducting Ca^{2+} entry than classical DAG stimulation [51]. Englerin A was introduced as a direct activator that more efficiently triggered Ca^{2+} influx though TRPC4/5 channels compare to the receptor stimulation [52]. A possible explanation can be derived by a comparison to other TRP structures. For instance, ligands were shown to specifically shape the conformation of the pore in TRPV1 channels. CryoEM analysis, mutagenesis studies, combined with biochemical characterisation and computational modelling revealed that capsaicin and tarantula double-knot toxin (DkTx) induced different conformational changes in both upper and lower gates [53,54]. However, activation of TRPV1 by DkTx showed only moderate Ca^{2+} influx compare to stimulation by vanilloid [54]. Interestingly, the ligand-dependent selectivity of lysosomal two-pore channel 2 (TPC2) channel was noticed by group of Gerndt *et al.* [55]. In agreement with others, our group found that TRPC3 exhibited ligand-dependent Ca^{2+} selectivity. TRPC3 was found to have a carbachol (CCh)-induced P_{Ca}/P_{Cs} selectivity ratio of 3.7 [39], which was reduced when activated by a novel photoswitchable agonist OptoBI-1 (P_{Ca}/P_{Cs} = 3.06; [56]). Altogether, ligand-dependent Ca^{2+} selectivity is likely a general feature of the TRP channel family, which might be subunit stoichiometry dependent, and should be considered as an important factor for governing signaling functions.

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Spatiotemporal aspects of TRPC Ca^{2+} signaling as determinants of signaling efficacy and specificity

Besides divalent ion permeability of the TRPC tetramers (as discussed above), open probability and gating behavior underlying activation, inactivation and desensitization processes will essentially control downstream Ca^{2+} signaling. An important principle to achieve specificity of coupling between the TRPC channels and their downstream targets is the kinetics of channel activity and the frequency aspects of oscillatory Ca^{2+} changes. As shown for other Ca^{2+} channels, activation of downstream signaling targets in many cases requires a certain temporal Ca^{2+} pattern. The association of a given TRPC channel complex with other integral membrane proteins and scaffolds is essential to create functional Ca^{2+} microdomain of distinct signaling features [57].

The efficiency of control over downstream Ca^{2+} sensors by a TRPC channel complex is dependent on its capability to serve as a Ca^{2+} source. The amplitude of the Ca^{2+} signal over time generated at the downstream target will depend on i) the channel's Ca^{2+} permeability and ii) open probability as well as iii) the density (clustering) of channels within a functional microdomain and on iv) the distance between the channel cluster and the Ca^{2+} sensory target molecule.

Local control of TRPC gating

Precise control of TRPC channel activity is typically achieved by local recruitment of upstream signaling components such as PLC, or STIM1/Orai1 but also modulators such voltage gated Ca^{2+} channels (Figure 2). Importantly, TRP channel activity is in general highly sensitive to the lipid environment. Lipids can bind directly to the channel and act as obligatory activators, co-factors or allosteric inhibitors. There is some evidence that PIP2 may directly modulate TRPC activity [16], its depletion and concomitant production of DAG results in channels activation. TRPC1/3/4/6 were reported to accumulate within cholesterol-rich microdomains [19] and, consistently, sterol densities were resolved within TRPC structures (TRPC4 [42], TRPC5 [43], TRPC6 [40,45••]).

Generation of lipid mediators via receptor phospholipase C (PLC) pathway provides a key upstream event for the activation of TRPC channels. Cleavage of PIP2 produces inositol-1,4,5-trisphopshate (IP_3) and 1,2-diacyl-sn-glyc-erol (DAG) as second messengers. IP_{3}-triggered Ca^{2+} release by opening of IP_3R, which promotes activation of some TRPC channels linking them to store-operated calcium entry (SOCE) pathways [11]. The second product of PLC activity DAG remains with the plasma membrane where it is likely to interact directly with TRPC3/6/7 channels via recently identified lipidation sites [58•]. Interestingly, TRPC4/5 channels become sensitive to DAG only after dissociation of TRPC4/5-NHERF complex induced by depletion of PIP2 or inhibition of protein kinase C (PKC) [59•]. Of note PIP_2 itself has been found to impact significantly on the activity and trafficking of these channels [60].

Another key aspect of local control of TRPC activity with a microdomain organization is that Ca^{2+} ions when transported by the central pore structure of TRPC complexes or by

adjacent transport molecules conveying a profound regulatory effect on the channel itself. In agreement with a different localization of potential Ca^{2+} binding acidic resides in the TRPC3/6/7 activity was found to be suppressed while TRPC4/5 was, on the opposite, potentiated by elevation of Ca^{2+} [34,35]. Modulatory effects of local Ca^{2+} has been reported to govern TRPC function within signaling domains harboring other Ca^{2+} entry channels such as CaV or Orai channels. These channels act as signaling partners that generate activating or modulatory signals (Figure 2).

One upstream, activating signal that might link TRPC1 to the ER filling state is the reported coupling to IP_3 receptors in a conformational coupling model [61]. It is important to note that SERCA and PMCA are also assembled within TRPC channelosomes to contribute to local, spatiotemporal Ca^{2+}signals and may shape these signals as required for regulation of specific physiological function [62].

Moreover, an even higher complexity of functional TRPC microdomains has been reported for many cell types. Coupling efficacy and target specificity will not only depend on upstream, TRPC-activating/modulating components such as microdomain-resident receptors, enzymes and their substrates but also require scaffolds and adaptor proteins, downstream targets as well as molecules that maintain adequate membrane lipid composition and bilayers architecture. This complexity was already recognized in early studies on the molecular physiology of the Drosophila TRP and TRPL channels, which were identified as part of higher-order protein complexes termed signalplexes [63]. Similar concepts have been proposed and experimentally supported for TRPC channels in various vertebrate tissues.

Scaffolds and local signal modifiers

TRPC channels are assembled into lipid (raft) microdomains via adaptor proteins. Scaffolding proteins play an essential role to target TRPC complexes into microdomain architectures suitable for efficient downstream signaling. Cholesterol-associated caveolins were found to interact with TRPC channels to regulate their plasma membrane expression and to control their activation. It was proposed that Caveolin-1 retains TRPC1 in ER-PM microdomains and hence governs activation of TRPC1-SOC by STIM1 [65]. Caveolin-1 was also found involved in association of TRPC1 channels with the calcium release channel (IP_3R) and thereby to mediate Ca^{2+} store release-induced Ca^{2+} entry in endothelial cells [23]. Moreover, the Homer-1 scaffolding protein was reportedly involved in the translocation of TRPC3-containing domains to the PM and the gating of TRPC3 by IP_3Rs [24]. Another group of important regulatory adaptor mole-cules are the Na^+/H^+ exchanger proteins (NHERFs), which have been reported to enable DAG-mediated activation of TRPC4 and TRPC5 channel in certain cellular settings [59•].

A peculiar feature of TRPC signaling is the potential of translating depolarizing Na^+ entry into Ca^{2+} signals by molecular signaling partners [66]. TRPCs have been identified to colocalize with molecules such as CaV1.2 or NCX1 that decode the accumulation of Na^+ ions in the cytoplasm along with membrane depolarization leading to a signaling competent Ca^{2+} elevation [25,64,67]. he importance of a tight coupling between TRPC6 and L-type (CaV) channels was corroborated by its impact on smooth muscle cell membrane potential

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and muscle contraction [68]. Furthermore, the TRPC3-NCX1 signaling is based on dynamic microdomain organization play a pivotal role in cardiac physiology or/and pathophysiology [12,69].

Positioning of downstream signaling targets

Downstream signaling by TRPC channels involves the generation of local and global cellular Ca^{2+} elevations in spatiotemporal manner to control cellular function. Cal-modulin (CaM) as a regulatory protein that binds Ca^{2+} ions via four EF-hand motifs, has been shown to interact with TRPC channels [26]. Besides a regulatory role of CaM on the TRPC channels function, CaM is also an essential component of downstream signaling. Evidence has been shown that TRPC channels are key players in Ca^{2+} transcription coupling in terms of a CaM/calcineurin (CaN)/NFAT-dependent gene transcription pathway. Specifically TRPC3/6 conductances have been demonstrated to control cardiac gene transcription in a spatio-temporal manner, leading to the upregulation of TRPC expression and function [70]. Specific control over NFAT activation through PKC-dependent modulation of TRPC-mediated Ca^{2+}influx is accomplished by spatial linkage of the membrane channel with downstream sig-naling elements including PKC, Ca^{2+} sensor CaM and the phosphatase CaN proteins within microdomains [71,72]. Most interestingly, a recent study revealed the importance of a TRPC5-PLSCR1 signaling complex in phos-phatidylserine (PS) externalization and apoptosis in neu-ronal systems as well as a pathological role of TRPC5 in cerebral-ischemia reperusion injury [21•] was unveiled. Hence, TRPCs may represent key players in plasma membrane lipid homeostasis by functioning as multi-modal lipid sensors that impact in turn on their membrane lipid environment.

To accomplish high specificity of information transfer by TRPC channels, not only spatial, but also temporal aspects of Ca^{2+} microdomain signaling should be taken into account. Association of signalplexes within ER/PM junctional domains enables direct and almost instantaneous communication between TRPCs and ER-resident ion channels. Therefore, TRPCs conduce to oscillatory Ca^{2+} signals and modulate their frequency and amplitude to selectively target downstream effectors [3].

Conclusion and outlook

As demonstrated for many other Ca^{2+} permeable channels, specificity and efficacy of TRPC downstream signaling requires dynamic molecular complexes within membrane regions of defined architecture and limited lateral mobility of the signaling molecules. A striking, recently emerging concept is the pivotal role of membrane lipids in almost all essential mechanistic aspects of TRPC Ca^{2+} signaling. This includes their impact on channel gating behavior, recruitment of modulating proteins, targeting of downstream elements and a role in decoding of the TRPC-mediated Ca^{2+} signal into cellular responses. Further in-depth delineation of tissue-, cell type- and phenotype-specific organization of TRPC signaling domains will lead to better understanding of the heterogeneity of TRPC signaling principles and pave the way towards exploiting these cation channels successfully as therapeutic targets.

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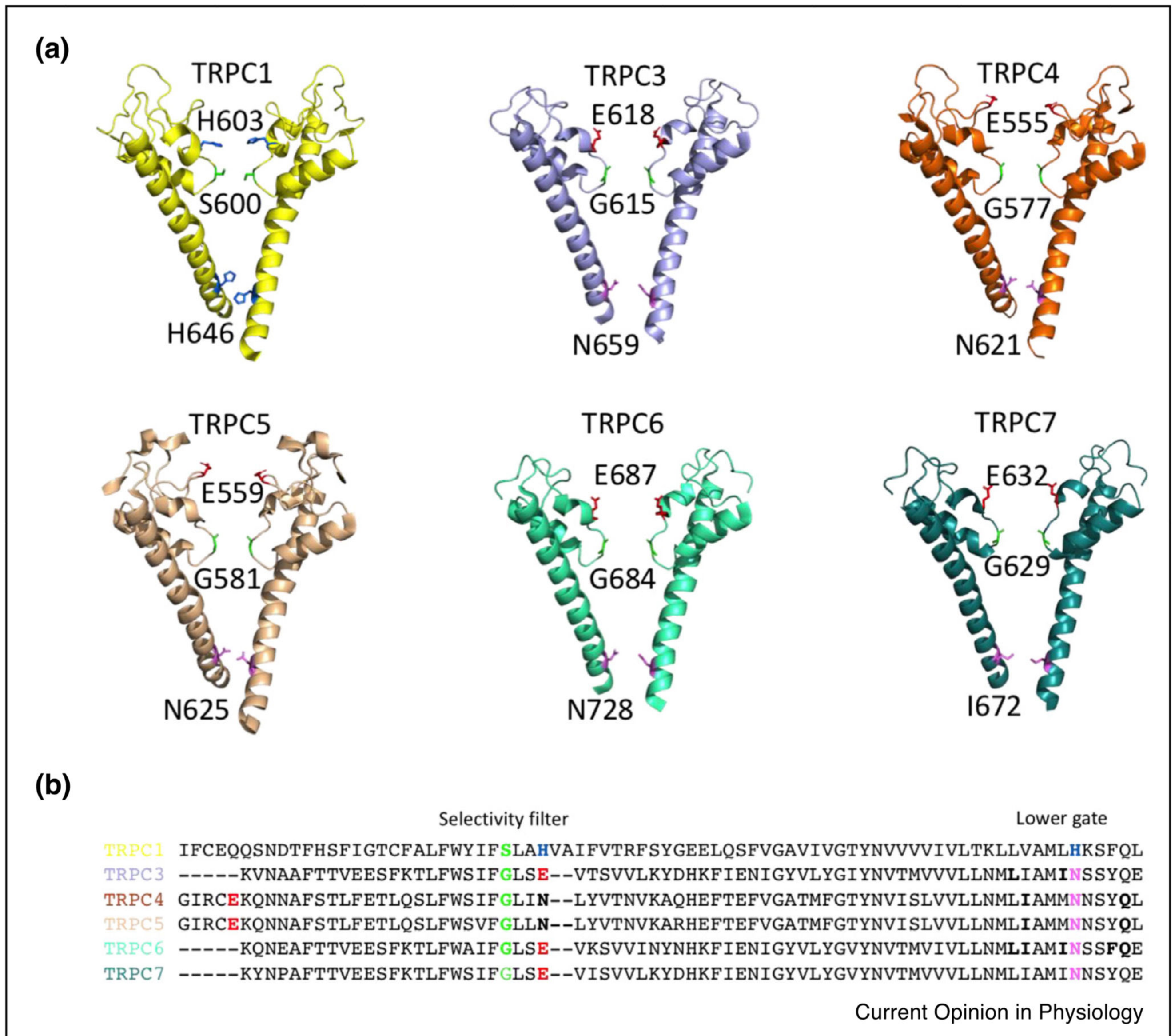

Figure 1. Molecular architecture of the TRPC Ca^{2+} permeation pathway.

(a) Pore structure of homology modelled TRPC1 (based on mTRPC4 with PDB ID: 5Z96 [42] and TRPC7 (using the hTRPC3 with the PDB ID: 6CUD as template [44] and cryoEM structures of TRPCs (hTRPC3 PDB ID: 5ZBG [40]; hTRPC4 PDB ID: 5Z96 [42]; mTRPC5 PDB ID: 6AEI; hTRPC6 PDB ID: 5YX9 [40]. Divalent ion recognition site (red; blue in TRPC1), the most constricted regions of selectivity filter (green) and lower gate (pink; blue in TRPC1) are highlighted. **(b)** Sequence alignment of TRPCs according to Fan et al. [44]. Divalent recognition site, most restricted region of the selectivity filter (green) and lower gate (pink; blue in TRPC1) are highlighted. Other important residues within the pore structure according to cryoEM and experimental data are highlighted bold.

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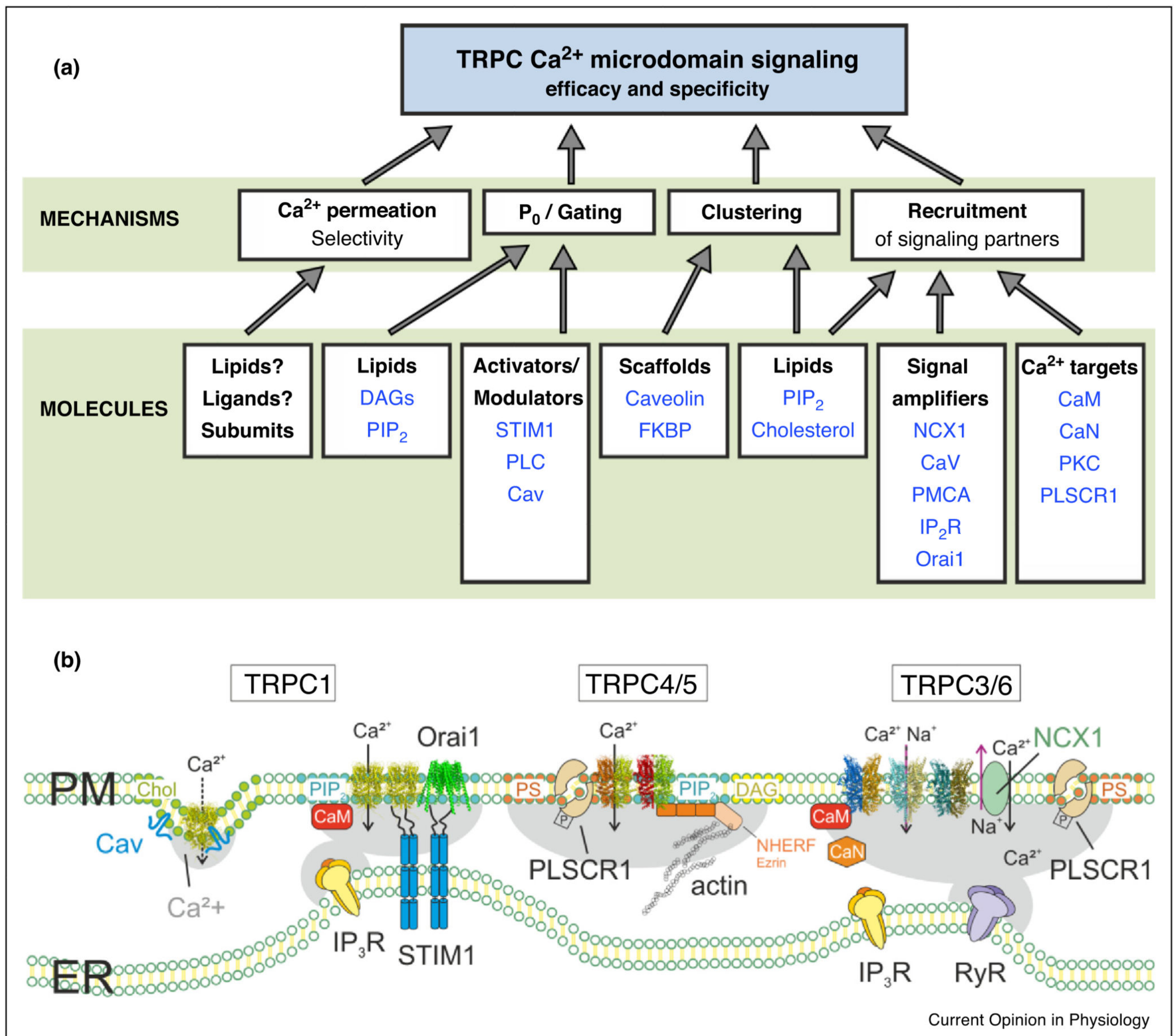

Figure 2.

(a) Molecular and mechanistic determinants of TRPC Ca^{2+} signaling: Efficient and specific signal transduction requires Ca^{2+} permeation as well as assembly of TRPC tetramers within signalplexes as well as membrane microdomains.

Ca^{2+} signaling involves control of amplitude but also kinetics of TRPC-mediated Ca^{2+} changes as well as positioning of downstream Ca^{2+} sensors within an adequate distance to the Ca^{2+} source. This requires a multitude of signaling partners including scaffolds and adaptor molecules, signal amplifiers and downstream targets.

(b) Heterogenity of the molecular organization in TRPC-centered Ca^{2+} signaling microdomains: TRPC channels interact with scaffolding proteins for plasma membrane targeting and for incorporation in signalplex components that determine channel activity, clustering, signal amplification and anchoring of downstream targets. TRPC1 of secretory

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cells interacts with Caveolin-1 (Cav-1) in cholesterol-rich vesicles and lipid rafts/caveolae [11,15] thereby rendering the channels rather inactive. Upon ER-store depletion, STIM1 translocates to ER-PM junctions activates Orai1 channels. Orai1-induced SOCE recruits TRPC1 into these junctional regions reducing the pool of TRPC1 associated with Cav-1 and allowing its activation by STIM1. TRPC1 proposedly interacts also with CaM/IP_3R in mutually exclusive manner within such ER-PM contact sites. TRPC4/5 channels assemble with NHERF, which anchors the channel via ezrin to the actin cytoskeleton, thereby imparting phosphorylation-dependent control over lipid gating, specifically on its interaction with DAG. TRPC4/5 channels as well as TRPC3/6 channels generate a Ca^{2+} microdomain, which impacts on the PM lipid environment via PLSCR1. This scramblase, like other downstream Ca^{2+} sensors such as calmodulin (CaM) and calcineurin (CaN), is reportedly activated within the channels vicinity, where it might physically be anchored to TRPCs [21 *] and in some cells is reportedly controlled by translation of Na^+ influx through TRPCs into local Ca^{2+} rises by the signal amplifier NCX1 [64].

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Table 1

Ion permeability properties of homomeric TRPC channels

Channel	Permeability ratio, P_{Ca}/P_{Na}	Reference
TRPC1	Non-selective	[31]
TRPC2	2.7	[32]
TRPC3	1.6	[33]
TRPC4	1.05	[34]
TRPC5	1.79-9.53[a]	[34–36]
TRPC6	5	[37]
TRPC7	5.9	[36]

[a]Difference in the reported permeability ratio for TRPC5 channels might be due to divergent experimental conditions and channel activators used in these studies.