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INVITED REVIEW

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From cardiac cation channels to the molecular dissection of the transient receptor potential channel TRPM4

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Abstract In 2006, we celebrate not only the milestone paper on the patch-clamp technique [14] but also the publication of the first single-channel measurements in cardiac cells revealing a Ca^{2+}-activated, nonselective cation channel [6]. Considerable effort has been undertaken since this time to identify molecular candidates for this class of cation channels that can be found in a variety of tissues. Recent work has shown that this channel is very likely TRPM4, a member of the TRPM ion channel family. The current review links the epochal Colquhoun et al. paper to the detailed molecular knowledge and structure function aspects of this TRP channel. It will be shown that TRPM4 is a Ca^{2+}- and voltage-activated channel, which is dramatically modulated by the phospholipid phosphatidyl inositol bisphosphate (PIP_2) and belongs to the heat-activated thermoTRPs. A functional hallmark of TRPM4, as for several TRP channels, is a dramatic shift of its voltage dependence towards negative, physiologically meaningful potentials.

Keywords Patch clamp · TRP channels · Non-selective cation channels · Calcium

The advent of a cardiac Ca^2+-activated nonselective cation channel

Colquhoun and colleagues performed in 1981, a few months after the publication of the groundbreaking Hamill et al. paper [14], the first single-channel measurements in cardiac cells and discovered a cation channel which was activated by Ca^{2+} (now frequently referred to as Ca^{2+}-activated nonselective channel, CAN [41]). This channel had a single-channel conductance of approximately 30 pS in inside-out patches, was activated by micromolar intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) but desensitized in the constant presence of Ca^{2+}, and showed a very weak voltage dependence of changes in open–close equilibrium, i.e., e-fold for ~200 mV. The molecular nature of this channel, its expression pattern, and its functional role, however, remained elusive for many years. A breakthrough was recently established when it was shown that in the super-family of TRP channels, which was considered to comprise only Ca^{2+}-permeable channels, a novel member, i.e. TRPM4, appeared to be a CAN channel [20]. Later work (described in [20, 31]) revealed several TRPM4 splice variants, whose significance is still not known. The majority of the functional characteristics described below relate to the full-length mouse and human cDNA, i.e., TRPM4b (later on referred to as TRPM4). It is interesting that some strikingly similar features have been unveiled between this channel and the Colquhoun channel [6]. Although it will be difficult to reconcile whether in the 1981 winter in Berne really TRPM4 was measured, this example nicely illustrates the development and the power of patch-clamp now combined with molecular biology.

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TRPM4 channel structure and permeation

The TRPM4 blue print

TRPM4 is a member of the TRP super-family of channel proteins, which can be subdivided into six mammalian ion channel subfamilies, TRPC (six members), TRPM (eight members), TRPV (six members), and the more distantly related TRPML (three members), TRPP (three members), and TRPA (one member) (for a review and more details, see [2, 37, 39]). The TRPM4 gene is located on human chromosome 19 and mouse chromosome 7. It consists of 25 exons, spanning 54 kb in the human and 31 kb in the mouse genome. Transmembrane regions are coded from exon 15 until 20. Within the TRPM subfamily, TRPM4 is most closely related to TRPM5, sharing approximately 50% homology. TRPM4 is widely expressed, e.g., in heart,

intestine, and vascular endothelium (see, for a review, [58]). It is interesting that, in the heart, it seems to be of higher expression in atrial than in ventricular myocardium (Flockerzi et al., personal communication). This may match the statement in the Colquhoun et al. classic that CAN is more "abundant" in the less differentiated neonatal rat cardiocytes [6]. As for other members of the TRP family, TRPM4 is likely to have six transmembrane domains (TM1-6) with a pore region between TM5 and TM6. Unlike other members of the TRP superfamily of membrane proteins, apparently no ankyrin repeats are present in the N terminus of TRPM4. In the N-terminal region, however, four stretches of moderate sequence homology to other members of the TRPM family are found (TRPM homology domain). A stretch of ~100 aa in the C terminus has a relatively high coiled-coil domain [40]. Several protein domains were identified in the TRPM4 protein sequence, including putative calmodulin binding sites (CAM, Fig. 1) in the N and C terminus as well as phosphorylation sites for PKA and PKC, four Walker B motifs (WB1-4, Fig. 1), two ABC transporter's like signature motifs (ABC, Fig. 1), and three arginine- and lysine-rich stretches (R/K) from which the most C-terminal one confers a putative PiP_2 binding site with homology to a pleckstrin homology domain and which includes a decavanadate binding site (see Fig. 1 and [20, 30, 33, 34, 40], for a review). Figure 1 gives an overview of the structure of TRPM4. The functional significance of these domains is becoming increasingly clarified and will be discussed in detail below.

The pore

TRPM4 is a nonselective cation channel with a permeation profile for monovalent cations ($Na^+ > K^+ >> Cs^+ > Li^+$) which correlates to a modestly high field strength binding site, type Eisenman VII. The pore of TRPM4 is virtually impermeable to Ca^{2+} [20, 31]. These permeation properties of TRPM4 match the properties of the Colquhoun CAN channel remarkably well [6]. A sequence comparison of the TM5–TM6 loop of all TRPM members reveals a highly conserved structure [40]. This region consists of a hydrophobic stretch, which may correspond to the pore helix, followed by an invariant aspartate (site D), which is likely located in the selectivity filter [32, 40]. These highly homologous regions of TRPMs share also limited homology to KcsaA and TRPV channels (Fig. 2). At three sites, conserved negatively charged residues are present between the putative pore helix and the fully conserved aspartate (Fig. 2). Taking the invariant aspartate as a reference, they are localized at positions −7, −3, and −2 (Fig. 2). Substitution of the six amino acid stretch, EDMDVA, containing the conserved aspartate and the two proximal negative charges, with the selectivity filter of TRPV6, a highly Ca^{2+}-permeable channel, generated a weakly Ca^{2+}-permeable channel combining gating features of TRPM4 (activation by $[Ca^{2+}]_i$, voltage dependence) and TRPV6 (block by extracellular Ca^{2+} and Mg^{2+}). Neutralization of the second aspartate in the EDMDVA stretch resulted in a nonfunctional channel with a dominant negative phenotype when co-expressed with wild-type TRPM4. Furthermore, selected point mutations in this region altered the inactivation properties and monovalent permeability profile of TRPM4 [32]. Thus, the EDMDVA domain probably constitutes the selectivity filter of the TRPM4 channel pore. In addition, it also contains a binding motif for spermine, which determines its TRPM4 block [32, 35].

TRPM4 gating

TRPM4 functions as a Ca^{2+}-activated cation channel [15, 20, 31, 33–35, 55]. In the whole-cell mode in TRPM4

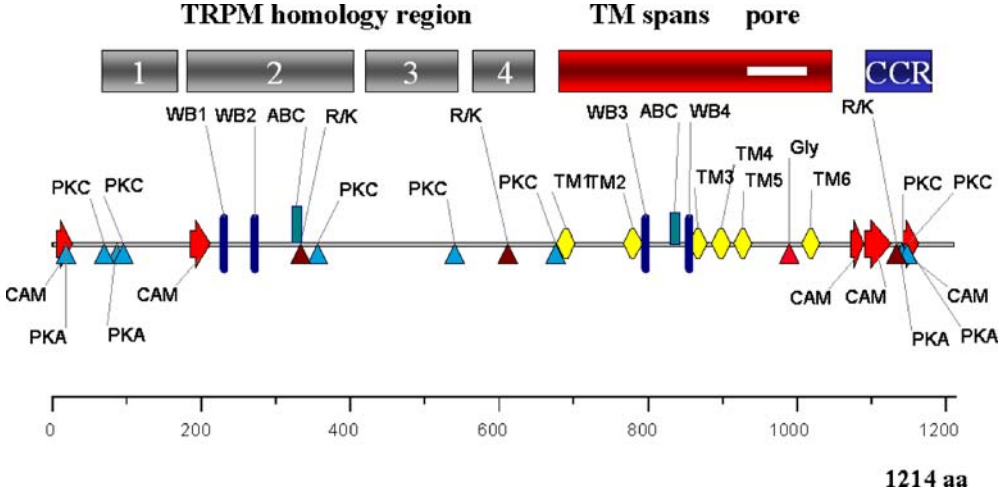

Fig. 1 A structural scheme of TRPM4 indicating putative functional domains. TRPM4 consists of an N terminus, which is conserved in the TRPM subfamily (TRPM homology domain), a membrane-spanning core, and a C terminus with a coiled-coil domain. Phosphorylation sites are marked as *blue triangles*, R/K refers to arg/lys-rich stretches. The second R/K stretch is part of the pleckstrin homology domain. *Blue bars* indicate Walker B ATP binding motifs, the *yellow symbols* mark the transmembarne domains (TM1-6), and the *red triangle* is a glycosylation site. The *thick red arrows* mark the putative calmodulin binding sites

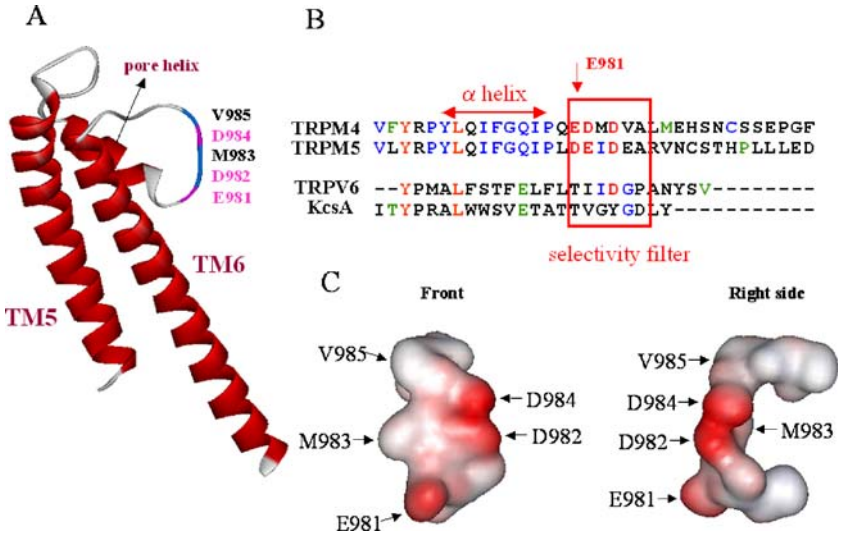

Fig. 2 Model of the TRPM4 pore. **a** Putative pore regions of human TRPM channels consisting of the selectivity filter and the pore helix. This model was calculated using the KcsA channel pore as a template [38]. **b** Multiple alignments of TRPM4 and TRPM5 and comparison with the highly Ca^{2+}-selective pore of the TRPV6 channel. Localizations of the putative pore helix and selectivity filter are annotated. **c** Distribution of electrostatic potentials on the surface of the putative selectivity filter of TRPM4. The negative and positive charges are in *red* and *blue*, respectively. Localization of the main residues is annotated

overexpressing HEK293 cells, currents can be activated by loading the cell via the patch pipette with Ca^{2+}, by application of extracellular agonist like ATP in HEK293 cells, by application of ionomycin, or by photo-uncaging of Ca^{2+} (see Fig. 3 and [31, 33, 34, 55], for a review). In inside-out patches, TRPM4 can be activated by adding Ca^{2+} to the inner side of the membrane, resulting mostly in very large currents up to 10 nA (+100 mV). The steady-state current–voltage relationship from whole cells or macroscopic currents in inside-out or outside-out patches revealed outwardly rectifying currents [31]. However, this rectification can be very variable as discussed later.

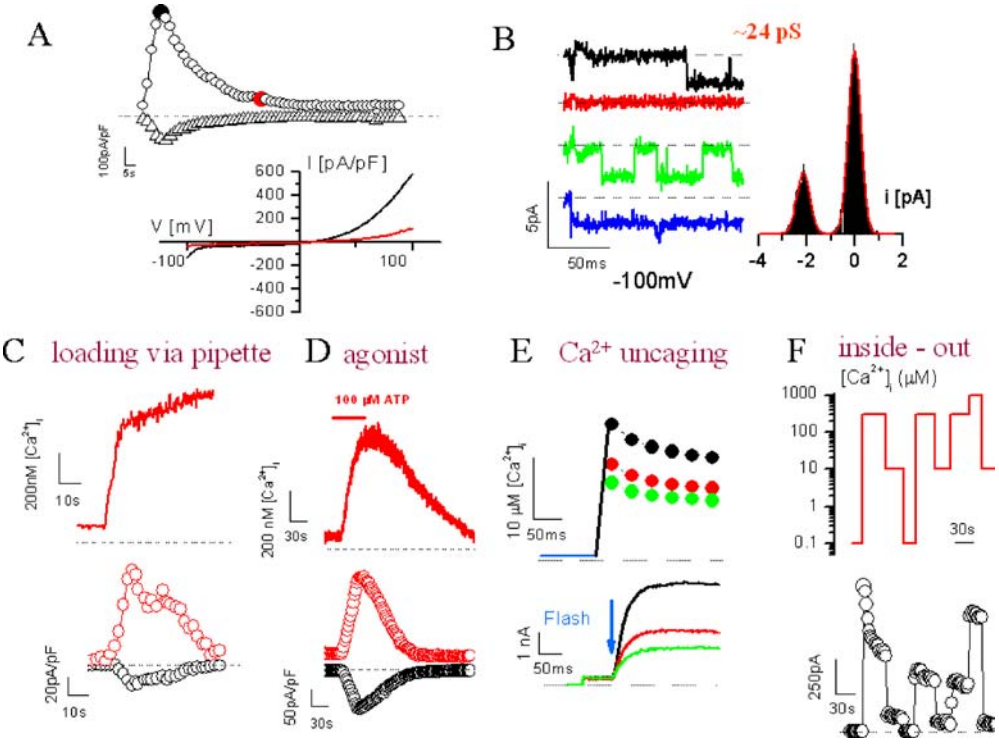

Fig. 3 Activation of TRPM4 by an increase of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. **a** Loading of HEK 293 cells transfected with human TRPM4 by dialysis with 100 μM Ca^{2+} activates TRPM4 currents. Note the transient activation and the complete desensitization. Shown are currents obtained from voltage ramps (from −100 to +100 mV; see, for a review, [31, 34]). **b** Single-channel recording obtained at −100 mV and 300 μM Ca^{2+} in inside-out patches. Note the sometimes very long opening interrupted by "nulls". This pattern is also shown in [6]. This pattern is more typical for "native" cells and under conditions of preventing PIP_2 depletion and PIP_2 addition (see supplement in [30]). **c** Simultaneous Ca^{2+} and current measurements. Cell is loaded with 500 μM $[Ca^{2+}]_i$. **d** Application of high concentrations of ATP in HEK293 cells induces a sufficiently large $[Ca^{2+}]_i$ increase to activate TRPM4. **e** Activation of TRPM4 by Ca^{2+} uncaging (see, for details, [55]). **f** Activation of TRPM4 by $[Ca^{2+}]_i$ variation in inside-out patches. Note the desensitization and the current increase at 1.0 mM Ca^{2+}, indicating that this desensitization is probably due to a shift in Ca^{2+} sensitivity

Currents through TRPM4 remarkably show run-down under whole-cell conditions within 30–120 s and also decrease to a steady-state level in the inside-out configuration. Other groups report a longer sustained activity [20]. The single-channel conductance of TRPM4 amounts ~25 pS (see Fig. 3 and [20, 31], for a review), which is similar to the classical CAN data, although the open probability is small (see later and also compare "...channels can also enter a long-lived closed state..." from [6]). The sensitivity of TRPM4 to intracellular calcium as determined by different research groups varies greatly. In whole-cell experiments, Launay et al. [20, 53] reported an EC_{50} value of channel activation of ~520 nM. Under our conditions, significant current activation occurs at ~1 μM $[Ca^{2+}]_i$ and higher [30, 31, 33, 34, 55]. In excised inside-out patches, an EC_{50} of between 150 and 300 μM was reported, which is at least 20 times higher than in whole-cell experiments [55]. However, we have shown that the Ca^{2+} sensitivity critically depends on a variety of factors: PKC-mediated phosphorylation, calmodulin, application of the negatively charged compound decavanadate, and PIP_2 all increase the Ca^{2+} sensitivity of TRPM4 [30, 33, 34, 36]. Thus, it is very likely that the Ca^{2+} sensitivity of TRPM4 is strongly regulated.

Although it should be clear that Ca^{2+} is essential for channel activation, TRPM4 is also a voltage-dependent channel. Figure 4a–d illustrate the fingerprint of the voltage-dependent TRPM4 channel activated under conditions of elevated $[Ca^{2+}]_i$ in an inside-out patch. In the presence of Ca^{2+}, currents deactivate at negative and activate at positive potentials (for a typical protocol and current responses, see Fig. 4a,b [31, 36]). From this, an outwardly rectifying steady-state current–voltage relationship is obtained (Fig. 4c, solid circles). The instantaneous *I–V* curve is approximately linear, indicative of an ohmic conductance of the open channel (Fig. 4c, open circles). Voltage-dependent channel activation can be described from the amplitude of the tail currents at each test potential and can be modeled by a classical two-state Boltzmann approach (Fig. 4d, solid squares). Current kinetics can be approximated adequately by a simple closed-open kinetic scheme (see discussion in [36]). The potential for half-maximal activation of TRPM4, $V_{1/2}$, varies greatly, between roughly −20 and +60 mV and depends strongly on a range of factors such as $[Ca^{2+}]_i$, presence of calmodulin, phosphorylation, temperature, and PIP_2 content. As discussed below, shifting of $V_{1/2}$ indeed seems to be a fundamental feature of channel activation under physiological conditions and is characteristic for a channel with a small gating charge (~−0.7 for TRPM4). Voltage-dependent characteristics similar to those of TRPM4 have been observed for other TRP channels such as TRPV1, TRPV3, TRPM5, TRPM8, and TRPA1 [36].

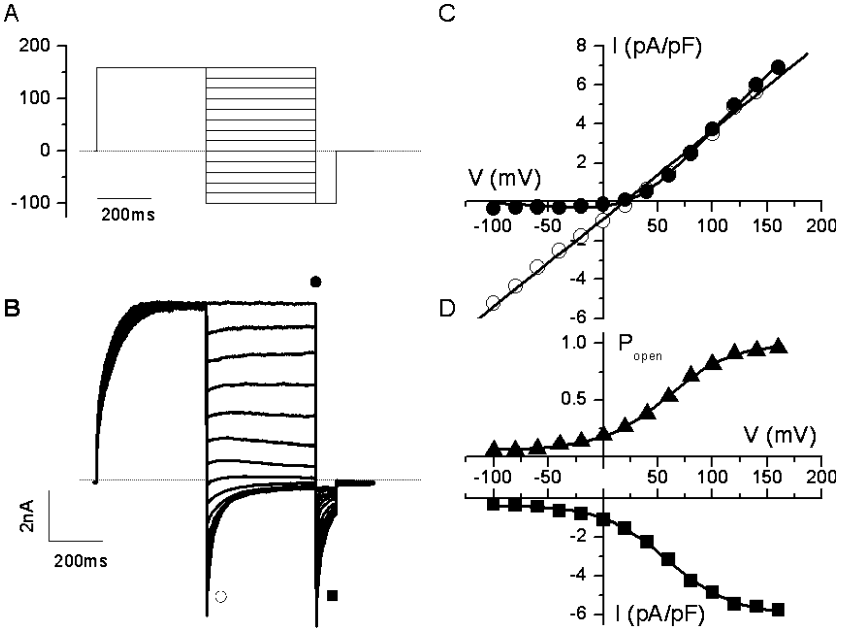

Fig. 4 Voltage dependence of TRPM4. **a** Voltage protocol to measure voltage-dependent features of TRPM4. Patches were held at 0 mV, stepped for 400 ms to +160 mV to fully activate the channels. After this pre-pulse, 400-ms steps from −100 mV to +160 mV for 400 ms were applied (decrement 20 mV). After 400 ms, a 75-ms back-step to −100 mV for measuring of tail current was applied. **b** Activation of TRPM4 channels [inside-out patch, 300 μM Ca^{2+} ($[Ca^{2+}]_i$) present at the inner side of the membrane] by depolarizing voltage steps. Note deactivation at negative potentials and activation at positive potential. **c** Current–voltage relationships (*I–V* curves) from the experiment shown in **b**: steady-state *I–V* curves (*solid circle*) show outward rectification This steady-state *I–V* curves can be fitted by a linear *I–V* curve with a Boltzmann activation term (parameters: conductance is 50.7 nA/mV pF, reversal potential 16 mV, $V_{1/2}$ 38 mV, and slope parameter *s* is 30 mV; e.g., an apparent gating charge of 0.67). Measurement of the linear instantaneous *I–V* curves is shown by the *open circles*. **d** *I–V* curves from the tail currents saturate at positive pre-potentials (*solid squares*). This curve was fitted by a Boltzmann function with the parameters of $V_{1/2}$=58 mV and *s*=20 mV. The activation curve was obtained by normalizing these current values with the maximal current for $V \rightarrow \infty$ (*solid squares*). *Solid line* represents a fit with the Boltzmann equation: $V_{1/2}$=58 mV, *s*=29.5 mV, e.g., *z*=0.81 (see also [36], for a review)

TRPM4 gating by shifting

In excitable cells, voltage-dependent channels are activated by a depolarization. Because the voltage sensor has a large charge, approximately 13e for the Shaker K^+ channel [1], this activation occurs in a relatively small voltage range. The moved charge for TRP channels is more than ten times smaller than for the classical voltage-dependent channels, causing a very flat activation curve, which is mostly shifted towards very positive potentials, i.e., these channels are almost closed in a physiological range of membrane potentials. A suitable mechanism for activation could be a leftward shift of this activation curve, which is a very efficient mechanism for channel gating as well. From a thermodynamic analysis, it can be derived that even small changes in the Gibbs free energy required for opening of the channel will induce large shifts in the potentials of half-maximal activation when the moved charge is small. This is indeed the case for TRP channels (for a detailed discussion, see [36]). There is overwhelming evidence that many TRP channels use such a mechanism as a gating tool [54, 59, 60]. For instance, we have recently shown that temperature sensitivity of at least TRPV1 and TRPM8 is modulated by the transmembrane voltage, and changes in ambient temperature result in graded shifts of the voltage dependence of channel activation [59]. For TRPM4, increasing of the intracellular Ca^{2+} concentration shifts the activation curve towards more negative potentials [33]. Activation by Ca^{2+} of TRPM4 is followed by a fast desensitization. This desensitization is accompanied or caused by a dramatic rightward shift of the activation curve, i.e., channels become unavailable [33]. It is important to note that all measures, which sensitize TRPM4 to Ca^{2+}, shift the activation curve towards negative potentials, e.g., phosphorylation by protein kinase C (see below, [36]). The vanadate decamer, decavanadate (DV), a charged compound with six negative charges, interferes dramatically with TRPM4 gating [33]. Application of DV to the inner side of excised patches results in a nearly complete disappearance of the voltage dependence of TRPM4. Tail currents deactivate rapidly and almost completely in the absence of DV but more slowly and incompletely in the presence of DV. Activation curves are dramatically shifted towards negative values. We identified a binding site in the C-terminal coiled-coil region as a cluster of positive charges, R^{1136}ARDKR^{1141} (R/K motif, see also Fig. 1). Deletion of this cluster or swap of the C terminus of TRPM5, which does not contain this motif, eliminates the activating effect of DV, e.g., the voltage shift of the activation of TRPM4 [33] strongly indicating that the site of action of DV resides in the C terminus of TRPM4. As discussed below, TRPM4-modulating factors such as heat, PIP_2, and PKC phosphorylation use a similar shifting mechanism to affect TRPM4 activity.

Structural determinants for Ca^{2+}- and voltage-dependent gating of TRPM4

When standard protein sequence analysis is performed, structural similarities between TRP channels and K_v channels can be detected. In K_v channels, the main voltage sensor consists of a cluster of positively charged residues located in S4 [22, 23]. Sequence alignment of TRPM4 with K_v1.2 revealed 37% similarity in a region corresponding to S4 and the S4–S5 linker. Only two of the four positively charged arginines that contribute to voltage sensing in *Shaker*-type K^+ channels are conserved in TRPM4. Eliminating of charges in this region induced a shift in the voltage-dependent activation [36]. The TRPM4 S4 region and probably the S4–S5 linker are likely involved in voltage sensing.

Concerning the Ca^{2+} dependence of TRPM4, we have described five calmodulin binding regions (see Fig. 1 and [34]). From a mutational approach, we could show that only two are functionally important, i.e., mutation of the putative calmodulin binding sites in the C terminus of TRPM4 strongly impaired current activation by reducing the calcium sensitivity of TRPM4 and shifting the voltage dependence of activation to very positive potentials. Co-expressing the dominant negative calmodulin mutant ($CaM_{1,2,3,4}$ with the four EF-hand Ca^{2+} bindings sites deleted) similarly shifts the channel activation towards positive potentials [34, 36]. Vice versa, application of calmodulin to the inner site of cell free patches induces a reduced desensitization of the channel, e.g., an increase in Ca^{2+} sensitivity.

TRPM4 modulation by PiP_2, PKC phosphorylation, ATP, and heat

Phosphatidyl inositol [4, 5] bisphosphate is a very effective modulator of several, if not all, TRP channels (Fig. 5a and see, for a summary, [30, 43, 49]). PIP_2, a substrate for phospholipase C, generates messengers such as inositol 1,4,5-trisphosphate, diacylglycerol, and phosphatidyl inositol [3–5] trisphosphate, regulates many ion channels and transporters, including voltage-gated K^+ and Ca^{2+} channels, and is involved in membrane anchoring of many proteins [27, 28, 49]. PIP_2 activates TRPM4 in the presence of an increased $[Ca^{2+}]_i$ by increasing its Ca^{2+} sensitivity to both Ca^{2+} and shifting the voltage range for activations dramatically to negative potentials [30, 61]. Depletion of PIP_2 from the cell via receptor stimulation, incubation of cells with wortmannin, an inhibitor of PI-4-kinase which delays PiP_2 replenishment, application of the PIP_2 scavenging agent poly-L-lysine, and overexpression of a PIP_2-depleting enzyme 5ptase IV lead to a reduction of TRPM4 [30]. Run-down (desensitization) can be prevented or reversed when PIP_2 was reapplied to the cytosolic side of excised patches. Recovery from desensitization can be achieved even in inside-out patches when Mg–ATP was added to the intracellular side during free perfusion, indicating that PIP_2 is probably replenished via activation

of lipid kinases. Inhibition of PLC activity by U73122 surprisingly induced similar effects as direct application of PIP_2. The effects of U73122 can be likely explained by activation of a Ca^{2+}-dependent PLC (e.g., PLCδ1, see supplemental data in [43]). Both PIP_2 and U73122 induce an apparent loss of voltage dependence, e.g., loss of time dependence, slowing of deactivation at negative potentials, because of the dramatic shift of the steady-state open probability towards more negative potentials. This is due to changing the membrane potential between already very high open probabilities. Two putative PIP_2 binding pleckstrin homology domains were identified in the C terminus of TRPM4 (Fig. 1; [30]). This stretch of positively charged amino acids also comprises the putative binding site for decavanadate (see above). The neutralization of all four positively charged amino acids in this stretch resulted in a channel exhibiting very rapid desensitization and highly reduced sensitivity to PIP_2. Because all the modulator effects could be also observed in excised inside-out patches, it is intriguing to speculate that PIP_2-modulating enzymes, such as lipid kinases (PIP4-K), a Ca^{2+}-dependent PLC, or PI phosphatases form a structural complex together with TRPM4.

TRPM4 activity is also modulated by protein kinase C activity and ATP. In an overexpression system, phorbol 12-myristate 13-acetate increased the Ca^{2+} sensitivity of TRPM4 fourfold and decreased desensitization. This effect was abolished when either of the two C-terminal Ser residues predicted to have the highest score for PKC phosphorylation was mutated (S1145 or S1152). The TRPM4 protein sequence contains several putative ATP binding motifs (see Fig. 1). Mg–ATP can indeed restore the Ca^{2+} sensitivity of TRPM4 after run-down. Mutations of the putative ATP binding sites all provoked attenuation of the ATP-induced recovery and drastically accelerated channel desensitization to Ca^{2+}. ATP obviously plays a crucial role in maintaining Ca^{2+} sensitivity of TRPM4, through direct binding to the channel protein and/or providing the substrate for lipid kinases which might be required to maintain a sufficient PIP_2 level which is necessary for channel activation (see below, [30, 34]). Figure 5 summarizes the actions of Ca^{2+}, PiP_2, and ATP in a comprehensive gating model.

Furthermore, very recent data has shown that TRPM4 is a heat-activated channel. All ion channels are to some degree temperature dependent with a Q_{10} for gating of about 3–4. However, some TRP channels are characterized by very high Q_{10} values. TRPM4 shows at +25 mV a Q_{10} of 8.5±0.6 between 15 and 25°C. An increase of the temperature shifts the activation curve for voltage-depen-

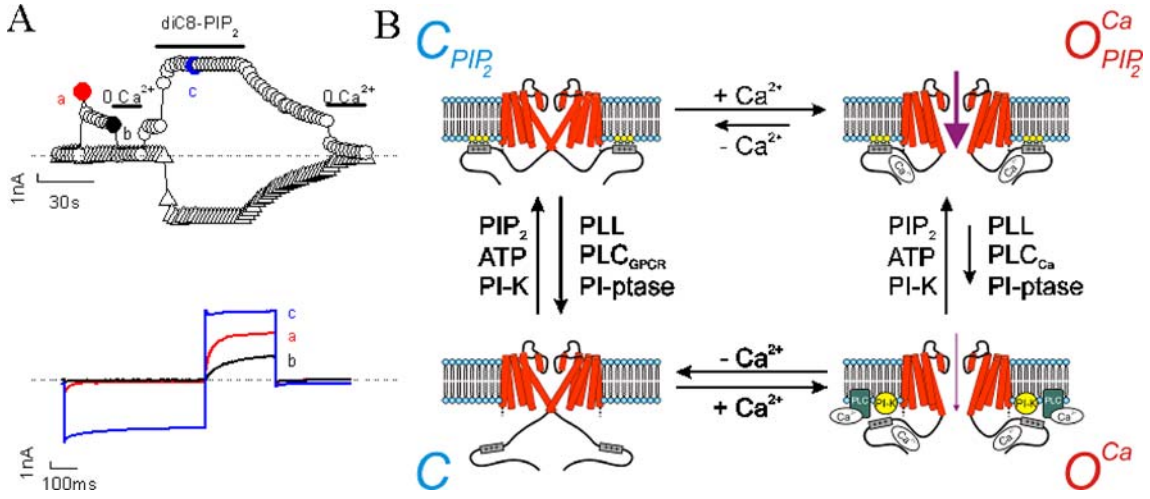

Fig. 5 Modulation of TRPM4 currents by PIP_2. **a** Activation of TRPM4 current in inside-out patches after application of 300 μM $[Ca^{2+}]_i$ (*circles* at +100 mV, *triangles* at −100 mV). The typical desensitization is shown. Currents immediately disappear in Ca^{2+}-free solution. Reapplication of Ca^{2+} restores the current level after desensitization. Application of 10 μM PIP_2 activates the current. Please note the near loss of voltage dependence of the current, in the presence of PIP_2. Washing out of PIP_2 results in a slow decline (desensitization; for details, see [30]). **b** Cartoon explaining the modulation of TRPM4 activity by PIP_2. Application of PIP_2 does not allow channel activation, i.e., the channel is in an unavailable mode, *C*. Current is activated by increase in Ca^{2+} if the channel is in an available configuration, e.g., with bound PIP_2, C_{PIP_2}. The first current activation represents channel activity in the high-Ca^{2+} affinity mode ($o^{Ca}_{PIP_2}$). Ca^{2+} withdrawal slowly closes the channel. During exposure to Ca^{2+}, TRPM4 desensitizes and goes into the low-Ca^{2+} affinity mode (O^{Ca}). Ca^{2+} withdrawal now rapidly closes the channel. Desensitization can be abolished by U73122, i.e., by inhibition of a Ca^{2+}-dependent PLC (PLC_{Ca}), which might have access to PIP_2 bound to the open channel, $o^{Ca}_{PIP_2}$. This also occurs in inside-out patches indicating a local interaction between TRPM4 and PLC. Poly-lysine (*PLL*) inhibits TRPM4 and closes the channel completely, but the first current is still large, indicating that PLL cannot scavenge PIP_2 if the channel is in the closed mode. Several mutations also close TRPM4 completely, including mutations of Walker B motifs, likely through preventing ATP binding to the channel [34]. Desensitization in inside-out patches can be reversed by application of PIP_2 and by perfusion with Mg–ATP, likely through activation of a phosphoinositol-kinase (*PI-K*), which may deliver PIP_2 to the channel. This interaction is again localized, i.e., itis effective in inside-out patches. Overexpression of a phosphinositol-phosphatase (*PI-ptase*) may decrease the fraction of available channels, C_{PIP_2}. Activation of G-protein-receptor-coupled PLC (PLC_{GPCR}) increases the fraction of unavailable channels (see text for more details)

dent channel opening towards negative potentials but had little effect on the Ca^{2+} dependence of channel activation. Thus, the heat dependence is not due to modulation of the calcium sensitivity of the channel but likely through shifting the voltage-dependent activation curves [54] and is identical to the mechanism of temperature sensing by TRPV1 [59].

TRPM4, CAN, and cell functions

As discussed above, the first description of a TRPM4-like current at the single channel level in cardiac cells was the momentous [6] paper. Similar currents have been described in many tissues, e.g., cardiomyocytes [11–13] in neurons from entorhinal cortex [24, 25], dorsal root and vomeronasal neurons [21], neuroblastoma cells and astrocytes [9, 46], vascular and myometrial smooth muscle [10, 29], endocrine pancreas cells [48], in red blood cells [42], exocrine cells from the pancreas [52], brown adipocytes [17, 45], kidney epithelial cells [16], cochlear hair cells [56, 57], and vascular endothelial cells [7, 51]. From a functional point of view, it is widely believed that TRPM4 causes membrane depolarization and thus limits the driving force for Ca^{2+} entry. Furthermore, TRPM4 activity will also reduce the transmembrane gradient for Na^+ and K^+, which will cause a reduction of the driving force for all kinds of Na^+-linked substrate uptakes and will also induce an increase in the ATP expenditure on the Na^+/K^+ pump (see, for a review, [41, 58]). The large tail currents upon repolarization being observed, TRPM4 could also be involved in pacemaking or could cause irregular electrical activity under conditions of Ca^{2+} overload [11].

The direct approach towards a physiological role for TRPM4 has been done so far only through gene-knockdown studies, using siRNA or expression of a dominant negative TRPM4 splice variant [8, 19]. Endogenous CAN (TRPM4) currents in Jurkat cells were down-regulated, which caused prolonged calcium signals, sustained Ca^{2+} increase, and significantly higher interleukin-2 (IL-2) production upon stimulation of these cells by phytohemagglutinins [19]. This up-regulation is likely due to an increased driving force for calcium entry caused by the lack of the negative feedback induced by TRPM4-mediated cell depolarization. Thus, TRPM4 might be essentially required for regulating Ca^{2+} oscillations of T lymphocyte activation, which is required for NFAT-dependent IL-2 production [19]. Defective TRPM4 will likely result in immunological hyperresponsiveness, pro-inflammatory conditions, or allergy. Knockdown of TRPM4, using antisense oligonucleotides in vascular smooth muscle cells from cerebral artery, impairs pressure-induced depolarization, which causes myogenic constriction, i.e., the Bayliss effect. These data suggest that TRPM4 functions as a mechanosensitive channel [8]. Still, it should be noted that it is difficult to reconcile these data with the known problems of poor specificity in gene knockdown studies [4]. The most straightforward approach would certainly be to study the phenotype from TRPM4 knock-out mice, but so far no data is available from TRPM4-deficient animals.

From the available data in the literature, it is tempting to speculate further on the functional role of TRPM4 in mammalian tissues. For instance, TRPM4-like channels may induce a feedback inhibition in type-II pneumocytes, which are important for production, storage, and secretion of the pulmonary surfactant via exocytosis of lamellar bodies required [26]. A similar negative feedback might also be expected for agonist stimulation of endothelial cells, which activated a channel with strikingly similar features as TRPM4 [33, 35, 51]. It is interesting to note that this channel is regulated in endothelium by nitric oxide, NO, and also by ATP. NO donors such as *S*-nitroso-*N*-acetylpenicillamine and 3-morpholinosydnonimine inhibited TRPM4. In contrast, inhibitors of NO synthases potentiated TRPM4, whereas superoxide dismutase, which inhibits the breakdown of NO, inhibits TRPM4. This mechanism is again very similar to a feedback inhibition of NO production, indicating a role of TRPM4 in sensing the metabolic state of the cell and NO in endothelium [50, 51]. TRPM4-like channels are also present in macula densa cells in the kidney juxtaglomerular apparatus where they might be involved in a similar inhibitory manner in renin exocytosis [18]. Brown fat cells can activate a CAN channel with remarkably similar properties as TRPM4 [17]. It is interesting to speculate whether TRPM4 might play a role in non-shivering thermogenesis [5].

Some even more intriguing TRPM4 functions can be anticipated from experiments initiating spontaneous epileptic activity in neocortical slices. TRPM4-like channels obviously play an important role in triggering paroxysmal depolarization shift discharges followed by sustained after-depolarization waveforms. These effects depend on an increase in $[Ca^{2+}]_i$ and can be blocked by maneuvers which also inhibit TRPM4 [44]. Neuronal damage evoked by reduced blood supply to the brain ('vascular stroke'), which induces severe hypoxia and hypoglycemia, is very often accompanied by the phenomenon that susceptible neurons slowly lose their membrane potential and then suddenly enter a permanent state of complete depolarization, known as spreading depression-like hypoxic depolarization. This spreading depression is associated with an increase in $[Ca^{2+}]_i$. TRPM4 could intriguingly be a candidate for triggering these dramatic events [3, 47].

Conclusion

This review aims to bridge data from the seminal Colquhoun et al. paper [6] to structural and functional features of the recently cloned TRPM4 cation channel. It is shown how the development only in the last 5 years formed a quite clear and detailed picture of this CAN channel. However, we are still far away from understanding the detailed regulation of this channel in native cells, on the organ level or even the whole animal. The road ahead to necessarily answer these more integrative questions promises to be a similar endeavor as shown here for the

first CAN single-channel measurements towards the molecular description of TRPM4. Another anniversary to look forward to!

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