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Structure–function relationship of the TRP channel superfamily

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Abstract Transient receptor potential (TRP) channels are involved in the perception of a wide range of physical and chemical stimuli, including temperature and osmolarity changes, light, pain, touch, taste and pheromones, and in the initiation of cellular responses thereupon. Since the last decade, rapid progress has been made in the identification and characterization of new members of the TRP superfamily. They constitute a large superfamily of cation channels that are expressed in almost all cell types in both invertebrates and vertebrates. This review summarizes and discusses the current knowledge on the TRP protein structure and its impact on the regulation of the channel function.

Introduction

Discovery of the first *Drosophila* transient receptor potential (TRP) channel involved in the response to light (Cosens and Manning 1969) led to the characterization of a large superfamily of cation channels that constitute important cation influx machinery in most vertebrate and invertebrate cell types. They are directly involved in thermo-, mechano-, chemo-, and nociception, responding to a wide variety of different physical and chemical stimuli (for recent reviews see Nilius and Voets 2005; Pedersen et al. 2005; Voets et al. 2005). Using structural homology as the criterion, the TRP channels have been classified into s127even subfamilies: TRPC (classical or canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ANKTM1 homologues) and TRPN (NOMP-C homologues) (Fig. 1) (Clapham 2003; Clapham et al. 2001, 2003; Vriens et al. 2004a).

TRP channels are intrinsic membrane proteins with six putative transmembrane spans (TM) and a cation-permeable pore region formed by a short hydrophobic stretch between TM5 and TM6 (Fig. 2). The length of the intracellular amino (N) and carboxy (C) termini and structural domains they encompass vary significantly between members of the TRP

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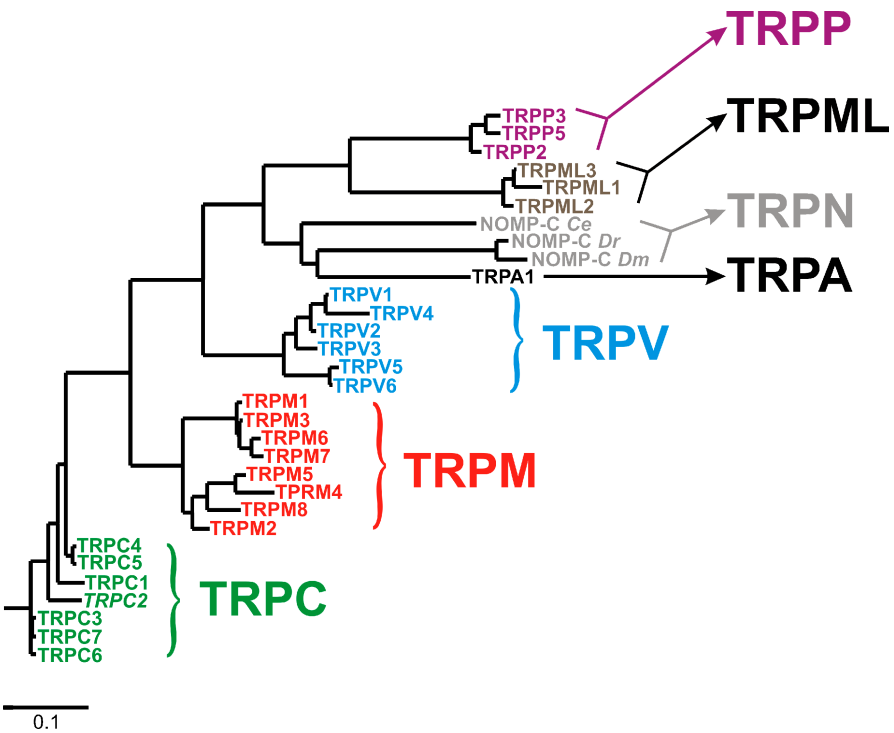

Fig. 1 Phylogenetic analysis of channels of the TRP superfamily; if not, annotated sequences of human channels were used for analysis. TRPC2 is a pseudogene in human and therefore the mouse channel sequence was used. The scale represents the evolutionary distance expressed in the number of substitutions per amino acid. *Dr*, *Danio rerio; Dm*, *Drosophila melanogaster; Ce, Caenorhabditis elegans*

channel subfamilies (Clapham 2003; Vriens et al. 2004a) (Table 1). These cytoplasmic parts play important roles in the regulation and modulation of channel function and trafficking. Functional TRP channels consist of four identical or similar TRP subunits (Garcia-Sanz et al. 2004; Hoenderop et al. 2003b; Kedei et al. 2001; Kuzhikandathil et al. 2001; Lintschinger et al. 2000; Strubing et al. 2001). In this review, we will discuss the current knowledge of the structure of TRP channels, with particular emphasis on structural elements involved in channel permeation and regulation. End Grabbed content

TRPs, a versatile superfamily of cation channels

The TRPC channel subfamily comprises the closest homologues of *Drosophila* TRP. There are seven TRPC channels in mammals. They are mainly phospholipase C (PLC) -dependent Ca^{2+} permeable cation channels formed by four either identical or different TRPC channel subunits (Clapham 2003; Clapham et al. 2003; Gudermann et al. 2004; Harteneck et al. 2000; Hofmann et al. 2002; Nilius 2003; Schilling and Goel 2004; Vazquez et al. 2004;). It is still controversial whether TRPCs are regulated by the depletion of intracellular Ca^{2+} stores (Grimaldi et al. 2003; Gudermann et al. 2004; Hofmann et al. 1999; Nilius 2003, 2004; Putney 2005; Strubing at al. 2001). Depending on the combination of TRPCs in the functional tetrameric channel, they play an important role in pheromone sensing (TRPC2;

Table 1 Putative motifs in the N- and C-terminal parts of human TRP channels

Channel	Potential motifs	Localization	Proposed function	References
TRPV1	Ankyrin (3 motifs)	201–230	*ND*	
Q8NER1[a]		248–277		
		333–362		
	N-terminal CaMBD	*189–222*[d]	Regulates capsaicin-activated currents	Rosenbaum et al. 2004
	PKC site (3 sites)	*502*	Regulates PMA enhancement of capsaicin-evoked currents	Bhave et al. 2003, Numazaki et al. 2002
		704	Regulates direct activation of TRPV1	Bhave et al. 2003
		800	Regulates PMA enhancement of capsaicin-evoked currents and direct activation of TRPV1	Bhave et al. 2003, Stowers et al. 2002
	PKA site	*116*	Interferes with the desensitization of capsaicin-evoked current	Bhave et al. 2002
	(3 sites)	*774*	Regulates channel desensitization	Mohapatra and Nau 2003
		820	Regulates channel desensitization	Mohapatra and Nau 2003
	TRP box	*684–721* or 697–702	Oligomerization	Garcia-Sanz et al. 2004
	C-terminal CaMBD	*768–802*	Regulates desensitization	Numazaki et al.2003
	PiP_2 binding site	*778–793*	Inhibition of the channel	Chuang et al. 2001, Prescott and Julius 2003
TRPV2	Ankyrin (3 motifs)	162–191	*ND*	
Q9Y5S1		208–237		
		293–323		
	TRP box	659–664	*ND*	
TRPV3	Ankyrin (3 motifs)	214–243	*ND*	
Q8NET8		261–291		
		340–369		
	TRP box	691–696	*ND*	
TRPV4	Ankyrin (3 motifs)	237–266	slight effect on hypotonic stimulation	Liedtke et al. 2000
Q9HBA0		284–313		
		369–398		
	PTK site	*253*[c]	Regulates channel function	Xu et al. 2003
			Phosphorylation of this residue has no effect	Vriens et al. 2004b
	TRP box	732–737	*ND*	
	CaMBD	*812–831*	Ca^{2+}-dependent potentiation	Strotmann et al. 2003
TRPV5	Ankyrin (5 motifs)	44–74	Regulates assembly, trafficking (residues 63–77)	Chuang et al. 2004
Q9NQA5		78–107		
		116–145		
		162–191		
		239–268		

Table 1 continued

Channel	Potential motifs	Localization	Proposed function	References
	TRP box	592–597	PiP_2 interaction site	Rohacs et al. 2005
	C terminus		Interaction with NHERF2, important for stabilization and targeting	Palmada et al. 2005
	C-terminal region	*596–601*	Regulates assembly, trafficking	Chuang et al. 2004
	C-terminal CaMBD	*650–729*	Regulates channel activity	Niemeyer et al. 2005
TRPV6	Ankyrin (5 motifs)	44–74	Tetramerization	Erler et al. 2004
Q9H1D0		78–107		
		116–145		
		162–191		
		239–268		
	N-terminal CaMBD	93–103	Regulates channel activity	Lambers et al. 2004
	TRP box	592–597	*ND*	
	Transmembrane CaMBD	*327–577*	Regulates channel activity	Lambers et al. 2004
	C-terminal CaMBD	691–711	Regulates channel activity	Niemeyer et al. 2005
				Lambers et al. 2005
TRPC1	Ankyrin (3 motifs)	46–75	Regulation of assembly, structure and channel function	Engelke et al. 2002
P48995		83–112		
		158–187		
	Coiled-coil domain	*212–267*[e]	Regulation of channel function	Engelke et al. 2002
	TRP box	659–664	*ND*	
	CaMBD1	*715–749*	Deletion has no effect	Singh et al. 2002
	CaMBD2	*758–793*	Affects SOCE, and Ca^{2+}-dependent inactivation	Singh et al. 2002
TRPC2[b]	Ankyrin (3 motifs)	46–75	*ND*	
Q9R244		83–112		
		158–187		
	TRP box	934–939	*ND*	
TRPC3	Ankyrin (4 motifs)	38–67	Regulates folding and trafficking	Wedel et al. 2003
Q13507		73–102		
		104–130		
		159–188		

Table 1 continued

Channel	Potential motifs	Localization	Proposed function	References
	PKG site (2 sites)	*11*	Effects TRPC3-mediated store-operated Ca^{2+} influx	Kwan et al. 2004
		263	Effects TRPC3-mediated store-operated Ca^{2+} influx	Kwan et al. 2004
	PKC site	*712*	Regulates channel activation	Trebak et al. 2005
	TRP box	684–689	*ND*	
	CIBR	777–797	Regulation of channel function	Wedel et al. 2003, Zhang et al. 2001
	C-terminal coiled-coil domain		Involved in trafficking	Wedel et al. 2003
TRPC4	Ankyrin (2 motifs)	69–98	*ND*	
Q9UBN4		141–170		
	TRP box	634–639	*ND*	
	CIBR	*688–759*	Interaction with NHERF and PLC-β1, important for allocation and regulation	Tang et al. 2000
		786–848		
	PDZ-binding motif	972–974	Regulates cell surface expression	Mery et al. 2002
TRPC5	Ankyrin (2 motifs)	69–98	*ND*	
Q9UL62		141–170		
	Coiled-coil domain	227–247	Interaction with stathmins	Greka et al. 2003
	TRP box	638–643	*ND*	
	PDZ-binding motif	*971–973*	Interaction with NHERF and PLC-β1, important for allocation and regulation	Tang et al. 2000
	PKC phosphorylation site	*972*	Phosphorylation regulates desensitization	Zhu et al. 2005
TRPC6	Ankyrin (4 motifs)	97–126	Interaction with MxA, regulates channel activity	Lussier et al. 2005
Q9Y210		132–161		
		163–189		
		218–247		
	TRP box	741–746	*ND*	
TRPC7	Ankyrin (4 motifs)	42–71	*ND*	
Q9HCX4		77–106		
		108–134		
		163–192		
	TRP box	686–691	*ND*	
TRPM1	TRP box	1019–1024	*ND*	
O75560				

Table 1 continued

Channel	Potential motifs	Localization	Proposed function	References
TRPM2	TRP box	1062–1067	*ND*	
O94759	Nudix hydrolase domain	1197–1503	ADPR pyrophosphatase	Perraud et al. 2001
TRPM3	TRP box	1051–1056	*ND*	
Q9HCF6				
TRPM4	TRP box	1057–1062	*ND*	
Q8TD43	C-terminal coiled-coil domain	*1136–1141*	Mediates the decavanadate effect	Nilius et al. 2004a
	PKC site (2 sites)	*1145* *1152*	Regulates Ca^{2+} sensitivity	Nilius et al. 2005b
	C-terminal CaMBD	*1076-1167*	Regulates Ca^{2+} sensitivity	Nilius et al. 2005b
TRPM5	TRP box	986–991	PiP_2 interaction site	Rohacs et al. 2005
Q9NY34				
TRPM6	TRP box	1083–1088	*ND*	
Q9BX84	PLIK	1708–1986	*ND*	
TRPM7	TRP box	1110–1115	*ND*	
Q96QT4	PLIK[d]	1554–1829	Regulation of channel activity	Runnels et al. 2001 Runnels et al. 2002
			Assembly and subcellular localization	Matsushita et al. 2005, Nadler et al. 2001 Runnels et al. 2001
TRPM8	TRP box	993–998	PiP_2 interaction site	Rohacs et al. 2005
Q7Z2W7				
TRPP2	Polycystin motif	316–328	*ND*	
Q13563	EF hand	750–785	*ND*	
	Coiled-coil domain	between *742-871*	Homodimerization and interaction with TRPP1	Tsiokas et al. 1997
	ER localization signal	*787–820*	ER retention	Cai et al. 1999
TRPP3	Polycystin motif	195–207	*ND*	
Q9POL9	EF hand	*637–665*	*ND*	Nomura et al.1998
	Coiled-coil domain	*656–687*	*ND*	
TRPP5	Polycystin motif	126–138	*ND*	
Q9NZM6				
TRPML1	Proline rich (2 domains)	*28–36*	*ND*	Sun et al. 2000
Q9GZU1		*197–205*		
	Nuclear localization motif	*43–60*	*ND*	
	Lipase serine active site	*104–114*	*ND*	
	C-terminal di-leucine motif	*563–566* *577–580*	Late endosomal/lysosomal targeting	

Table 1 continued

Channel	Potential motifs	Localization	Proposed function	References
TRPML2 **Q81ZK6** and TRPML3 **Q8TDD5**			Both channels remain to be fully characterized	
TRPA1 **O75762**	Ankyrin (15 motifs)	62–92 97–126 130–160 164–193 197–226 138–267 271–301 308–337 341–370 412–441 445–474 481–510 513–542 547–576 579–609	Formation of the gating spring	Corey et al. 2004

Numbers in italics correspond to the published data, while others are determined by Swiss-PROT. *ND*, not determined
[a] Accession number
[b] TRPC2 is pseudogene in human, therefore mouse TRPC2 sequence was used
[c] Function is still controversial
[d] Rat
[e] Mouse

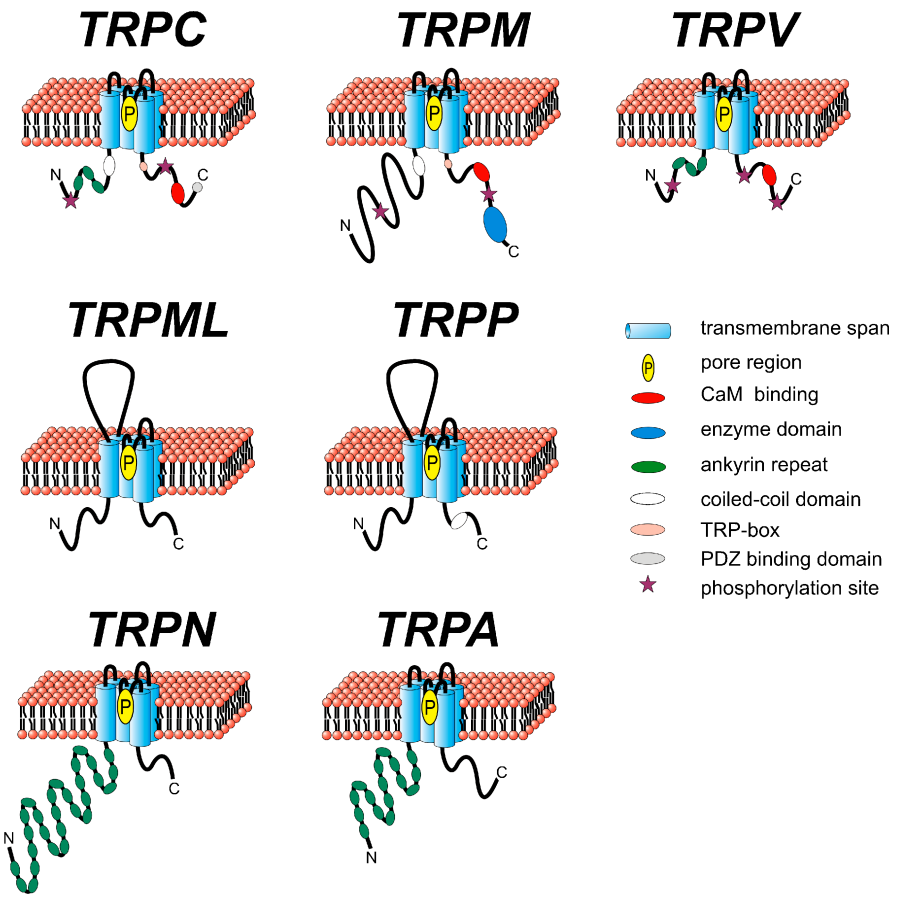

Fig. 2 Schematic representation of the structural topology of channels from the TRP-related subfamilies. The transmembrane segments are similar in all TRP channels. The putative pore region is localized between TM5 and TM6 and its length and amino acid composition are variable in different subfamily members. Only the most representative domains are annotated and lengths of the N- and C-termini are approximated

note that the human TRPC2 is a pseudogene), vasoregulation (TRPC3/4/5), signaling in the central nervous system (TRPC1/3/4), and functioning of smooth muscle cells (TRPC3/6/7) (Freichel et al. 2001; Kim et al. 2003; Lucas et al. 2003; Sakura and Ashcroft 1997; Stowers et al. 2002; Strubing et al. 2001; Tiruppathi et al. 2002). TRPC1 may also function as a stretch-activated channel involved in cellular mechanosensitivity (Maroto et al. 2005).

In the subfamily of TRPV channels, six mammalian homologues have been classified. TRPV1/2/3/4 are permeable to Ca^{2+} with a rather low selectivity for divalent and monovalent cations (Benham et al. 2002; Gunthorpe et al. 2002; Voets and Nilius 2003; Voets et al. 2002). The vanilloid receptor, TRPV1, mediates nociception and is involved in the detection and integration of thermal and diverse chemical stimuli (e.g., vanilloids, endovanilloids, and anandamide) (Caterina et al. 2000; Jordt and Julius 2002). TRPV2 and TRPV3 are activated in the noxious and warm heat range, respectively (Kanzaki et al. 1999; Peier et al. 2002b; Smith et al. 2002; Xu et al. 2002). TRPV4 contributes to nociception and osmo- and warmth sensation, and is activated by ligands such as α-phorbols or endogenous agonists such as epoxyeicosatrienoic acids (Liedtke et al. 2000; Liedtke and Friedman 2003; Nilius

et al. 2004b; Vriens et al. 2004b; Watanabe et al. 2002a, 2003). TRPV5 and TRPV6, the only highly Ca^{2+}-selective TRP channels, play an important role in Ca^{2+} reabsorption in kidney and intestine (den Dekker et al. 2003; Hoenderop et al. 2002a, 2002b, 2003a, 2003b; Nijenhuis et al. 2003; Vennekens et al. 2000, 2001a, 2001b).

A third subfamily of TRP-related channels includes close homologues of melastatin. Melastatin was originally identified based on its higher expression in nonmetastatic compared to highly metastatic melanoma cells (Duncan et al. 1998). The TRPM subfamily in mammals comprises eight members that are involved in processes as different as Mg^{2+} homeostasis (TRPM6, TRPM7 [Nadler et al. 2001; Schlingmann et al. 2002; Voets et al. 2004c; Walder et al. 2002]), taste detection (TRPM5 [Perez et al. 2002; Zhang et al. 2003]), cell proliferation (TRPM7 [Nadler et al. 2001]), and noxious cold sensing (TRPM8 [McKemy et al. 2002; Peier et al. 2002a; Voets et al. 2004a]). Except for TRPM1, the permeation properties of TRPMs are relatively well described. The Ca^{2+}-activated TRPM4/5 channels are the only Ca^{2+}-impermeable TRPs identified so far (Hofmann et al. 2003; Launay et al. 2002; Nilius et al. 2003a; Prawitt et al. 2003). TRPM2/3 and TRPM8 are Ca^{2+}-permeable with rather low Ca^{2+} selectivity (Grimm et al. 2003; Hara et al. 2002; Lee et al. 2003; McKemy et al. 2002; Peier et al. 2002a; Perraud et al. 2001; Sano et al. 2001), whereas TRPM6/7 are relatively highly permeable for divalent cations, especially for Mg^{2+} (Monteilh-Zoller et al. 2003; Nadler et al. 2001; Voets et al. 2003; Voets and Nilius 2003).

The polycystin subfamily, TRPP, is named after its founding member, PKD2, which was discovered as one of the genetic determinants of autosomal dominant polycystic kidney disease (ADPKD) (Mochizuki et al. 1996). There are three mammalian TRPP channels: TRPP2 (PKD2), TRPP3 (PKD2L1), and TRPP5 (PKD2L2) (Cai et al. 1999; Guo et al. 2000; Nomura et al. 1998; Veldhuisen et al. 1999; Wu et al. 1998). Functional expression of human TRPP2 channel in the plasma membrane depends on the interaction with PKD1, a large plasma membrane protein with 11 putative TMs (Hanaoka et al. 2000; Qian et al. 1997; Tsiokas et al. 1997). This interaction may occur via a putative coiled-coil domain in the C terminus of TRPP2. In the absence of PKD1, TRPP2 may function as an intracellular Ca^{2+} release channel (Koulen et al. 2002). There is evidence that TRPP channels may function as mechano-sensors in ciliated epithelial cells and might be important for organogenesis (Nauli et al. 2003; Stayner and Zhou 2001).

Mutations to TRPML1 (mucolipin-1; MCOLN1) lead to mucolipidosis type IV (MLIV), an autosomal recessive, neurodegenerative, lysosomal storage disorder characterized by psychomotor retardation and ophthalmological abnormalities, including corneal opacities, retinal degeneration, and strabismus (Bargal et al. 2000; Bassi et al. 2000; Berman et al. 1974). Human TRPML1 expressed in *Xenopus laevis* oocytes functions as a Ca^{2+}-permeable cation channel that is modulated by changes in Ca^{2+} concentrations (LaPlante et al. 2002). Mutations of mouse TRPML3 (MCOLN3) lead to deafness and defects of hair cell and pigmentation (the varitint-waddler mouse) (Di Palma et al. 2002). There are some indications that mutations in genes encoding TRPML2 (MCOLN2) and TRPML3 may be involved in hereditary and/or sporadic neurosensory disorders in humans (Di Palma et al. 2002; and for a general review see Nilius et al. 2005d).

TRPA and TRPN subfamilies are very closely related and poorly represented in vertebrates. ANKTM1 or TRPA1 is the only TRPA subfamily member characterized in vertebrates so far (Corey 2003; Story et al. 2003). Mammalian TRPA1, a Ca^{2+}-permeable, nonselective cation channel, is activated by noxious cold, bradykinin, cannabinoids, and several pungent compounds such as the isothiocyanates that are present in wasabi and mustard, cinnamaldehyde, and allicin (Bandell et al. 2004; Jordt et al. 2004; Macpherson et al. 2005; Story et al. 2003). It is highly expressed in hair bundles of sensory hair cells where it may

function as a mechanosensory transduction channel involved in the hearing process (Corey et al. 2004; Lin and Corey 2005).

The TRPN subfamily is named after the *n*o *m*echanoreceptor *p*otential *C* (NOMP-C) channel from *Drosophila*, which plays a crucial role in mechanosensation in processes such as hearing, balance, proprioception, and touch (Kernan et al. 1994; Walker et al. 2000). No obvious homologues of NOMP-C are present in the human genome. So far, the only vertebrate TRPN family member that has been identified is NOMP-C from zebrafish (Sidi et al. 2003). Mutations in NOMP-C of zebrafish larvae lead to impaired hair cell mechanotransduction and the loss of microphonic potentials (Sidi et al. 2003).

Transmembrane segments: a functional backbone of TRP channels

The transmembrane segments are the most conserved structures in all TRP channels. Although the number of hydrophobic regions can vary from one TRP channel to another, it is generally believed that only six α-helices are able to span membranes. By analogy to K^+ channels, TM5 and TM6 seem to play a central role and directly contribute to formation of the channel pore (see "Functional insights into the pore region of TRP channels"). In contrast to classical voltage-gated cation channels, TM4 contains only a few positively charged residues, which complicates determination of the residues responsible for voltage-dependent activation of TRP channels.

Relatively few reports describe the functional impact of TM1–4 segments in regulation of the TRP channel function. The first insight in the functional role of TM2–4 came from a study on TRPV1 aiming at molecular determination of the specific sites that bind vanilloid compounds such as capsaicin or the endogenous TRPV1 agonist, anandamide (Gavva et al. 2004; Jordt and Julius 2002). Jordt and Julius (2002) showed that substitutions of a conserved tyrosine residue (Y^{511}), which is located within or adjacent to TM3, by phenylalanine, alanine or cysteine led to either a selective loss of capsaicin sensitivity or reduced capsaicin-activated currents. Similar effects were also induced by mutations to a neighboring seine (S^{512}) or R^{491} in the TM2. Thus, a proposed model of vanilloid binding pocket comprises an aromatic residue, Y^{511}, which interacts with the vanillyl-moiety of capsaicin on the cytosolic face of the membrane. The other residues, such as polar S^{512} or R^{491}, may interact with capsaicin via hydrogen bonds, whereas lipophilic residues in TM3 can be involved in stabilization via hydrophobic interactions with the aliphatic moiety of capsaicin within the plane of the membrane (Jordt and Julius 2002). This model was partially confirmed by Gavva et al. (2004), who investigated mechanisms of capsaicin insensitivity of rabbit TRPV1. Using either human/rabbit or rat/rabbit chimeras, they demonstrated that apart from Y^{511} additional residues in TM4, M^{547}, and T^{550} directly contribute to vanilloid binding. In contrast to Jordt and Julius, they propose that T^{550}, W^{549}, and M^{547} may be involved in interaction with the vanilloid moiety, whereas the aliphatic tail of capsaicin binds to Y^{511} (Gavva et al. 2004). This alternative model suggests that observed differences in affinity of ligands with longer (higher affinity) and shorter (lower affinity) aliphatic tails may be explained by their abilities to interact with Y^{511}. Both models are still preliminary and require additional biochemical and structural data for validation. Moreover, they do not explain why mutations of N- and C-terminal residues, R^{114} and D^{761}, in TRPV1 result in loss of capsaicin sensitivity (Jung et al. 2002; Vlachova et al. 2003).

Using sequence homology to TRPV1, a tyrosine important for ligand activation (Y^{555}) in TM3 has also been identified in TRPV4, a channel that is activated by a broad range

of stimuli such as osmotic cell swelling, heat, phorbol esters, and arachidonic acid (AA) (Vriens et al. 2004b; Watanabe et al. 2002a, 2002b, 2003). Mutation of Y^{555} to nonaromatic residues resulted in a strong decrease of TRPV4 activation by 4α-phorbol 12, 13-dideconoate (4αPDD) and heat, but does not affect activation by cell swelling or AA (Vriens et al. 2004b). This suggests that activation of TRPV4 by phorbol esters and heat both occur via a pathway that critically depends on an aromatic residue in TM3. Very likely other residues in TM2 or TM4, which remain to be identified, may also be involved in ligand binding in TRPV4.

Functional insights into the pore region of TRP channels

Ion channels are pore-forming transmembrane proteins that allow permeation of ions through biological membranes. The structure of the channel pore is crucial for determination of the ion permeation and selectivity properties of particular channels. In contrast to other families of ion channels, data concerning structure and localization of TRP channel pores are rather limited and only concerns TRPV, TRPC, and TRPM subfamilies (Fig. 3). Nevertheless, all available functional and theoretical data strongly support the general notion that the linker region between TM5 and TM6 is the pore-forming part in all channels of the TRP superfamily (Owsianik et al. 2006).

TRPVs

The structure–function analysis of TRPV channel pores is the most advanced among all TRP subfamilies. In all mammalian TRPVs, TM5–6 linker regions show significant sequence homology with the selectivity filter of the prokaryotic potassium channel KcsA, whose crystal structure has been determined at 2-Å resolution (Doyle et al. 1998; Zhou et al. 2001a). Mutations to negatively charged residues, D^{546} of TRPV1 and corresponding D^{682} of TRPV4, strongly reduce the permeability for Ca^{2+} and Mg^{2+} and decrease the affinity of the channels to the voltage-dependent pore blocker Ruthenium Red (Garcia-Martinez et al. 2000; Voets et al. 2002). Additionally in TRPV4, mutation of neighboring residue D^{672} further reduces the selectivity for divalent and also changes the relative permeability for monovalent cations, whereas the substitution of M^{680} with a negatively charged amino acid abolishes Ca^{2+} and Mg^{2+} permeability (for predicted localization of these residues see the scheme in Fig. 3B). Mutation of the only basic pore residue in TRPV4, Lys^{675}, did not significantly change the permeation properties of the channel (Voets et al. 2002). These results indicate that the putative TRPV1/2/3/4 pore motif, GM(L)GD, determines permeation properties of the channels and is functionally homologous to the GYGD signature sequence in the selectivity filter of K^+ channels.

Permeation properties of TRPV5/6 are also determined by the aspartate residues in the putative selectivity filter (Hoenderop et al. 2003b; Nilius et al. 2001; Voets et al. 2001, 2003). Aspartate-to-alanine mutations at position D^{542} of TRPV5 and D^{541} of TRPV6 result in the loss of Ca^{2+} permeation, Ca^{2+}-dependent current decay, and block by extracellular Mg^{2+} or Cd^{2+}, whereas permeation of monovalent cations remains unchanged. Other negatively charged residues in the pore region of TRPV5, E^{535} and D^{550}, have less impact on pore properties, whereas E^{522}, located N-terminal of the pore helix, functions as a putative pH sensor, regulating pH-dependent permeation properties of TRPV5/6 (Vennekens et al. 2001a; Yeh et

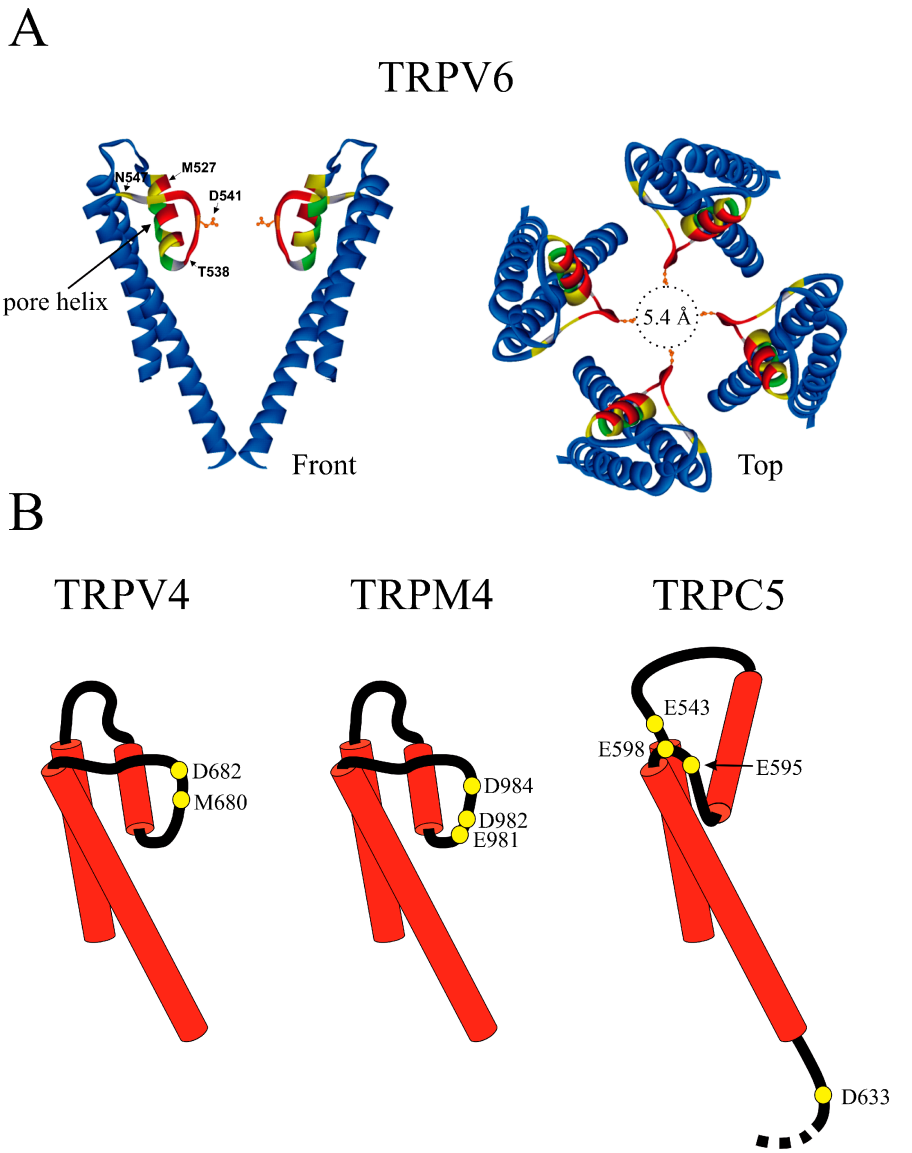

Fig. 3A, B Predicted topologies of the pore region of TRPV, TRPM and TRPC channels. **A** Structural model of the TRPV6 pore region, looking sideways at two opposite subunits (*left*) or looking down from the external solution to the complete homotetrameric channel. At the narrowest point, the pore is formed by the acidic side chain of Asp^{541} (*orange*) and has a diameter of 5.4 Å. *Blue* residues correspond to the residues in TM5 and TM6 and amino acids that were subjected to SCAM analysis (residues P^{526} to N^{547}) are colored in *green*, *yellow*, *red*, or *gray*. Residues in *red* reacted rapidly to Ag^+ (reaction rate $>5.10^6$ $M^{-1}s^{-1}$), residues in *yellow* reacted with Ag^+ at a rate $<5.10^6$ $M^{-1}s^{-1}$, and residues in *green* did not show significant reactivity to Ag^+. Residues where cysteine substitution resulted in nonfunctional channels are colored in *gray*. (Adapted from Voets et al. 2004b, with copyright permission from *The American Society for Biochemistry and Molecular Biology*). **B** Schematic representation of crucial residues in putative selectivity filters of TRPV4, TRPM4, and TRPC5 channels (see text for details)

al. 2003). Using the substituted cysteine accessibility method (SCAM), a more detailed pore structure of TRPV5/6 has been obtained (Dodier et al. 2004; Voets et al. 2004b). Cysteines introduced in residues that are N-terminal to $D^{542/541}$ show a cyclic pattern of reactivity, indicating that these residues form a pore helix similar to that in the KcsA crystal structure (Doyle et al. 1998; Zhou et al. 2001a). In TRPV6, the pore helix is followed by the selectivity filter with a diameter of approximately 5.4 Å at its narrowest point, as assessed by measurements of permeability to cations of increasing size (Voets et al. 2004b). The apparent pore diameter of TRPV6 increases significantly when D^{541} is substituted by amino acids with a shorter side chain, demonstrating that this aspartate residue shapes the narrowest part of the selectivity filter and contributes to the sieving properties of the pore (Voets et al. 2004b) (Fig. 3A). Thus, these results strongly indicate that selectivity and permeation properties of TRPV5/6 depend on a ring of four aspartate residues in the channel pore, similar to the ring of four aspartates and/or glutamate residues in the pore of voltage-gated Ca^{2+} channels (Heinemann et al. 1992; Talavera et al. 2001; Yang et al. 1993).

TRPCs

Unlike TRPV channels, the TM5–TM6 region of TRPCs does not share significant sequence homology with the pore region of K^+ channels. The most direct evidence for the location of the pore region of TRPC channels comes from functional identification of TRPC1 as a store-operated Ca^{2+} channel (SOCC) (Liu et al. 2003) and studies of La^{3+} potentiation of TRPC5 (Jung et al. 2003).

TRPC1 can potentially form eight hydrophobic α-helices but only six of them are believed to span the membrane (Dohke et al. 2004). One of the non-membrane-spanning α-helices is located in the region between TM5 and TM6 (note that the TMs are numbered differently than proposed in Dohke et al. 2004) and seems to form a pore helix similar to that in KcsA and TRPV5/6. Mutations to all seven negatively charged residues in the TM5–TM6 region of TRPC1 (D to N and E to Q) result in decreased Ca^{2+} but intact Na^+ currents through TRPC1, and induce shifts in the reversal potential (Liu et al. 2003). Interestingly, the crucial residues, E^{576}, D^{581}, and E^{615}, are located in the distal parts of the putative pore mouth, suggesting a different pore structure than that of the TRPV subfamily.

In analogy to TRPC1, neutralization of 3 of the 5 glutamates in the loop between TM5 and TM6 of TRPC5, E^{543}, E^{595}, and E^{598}, lead to a loss of La^{3+} potentiation (Jung et al. 2003). Moreover, the E^{595}/E^{598} double mutant shows altered single channel properties. Surprisingly, mutations of either E^{559} or E^{570} located in the central part of this loop do not affect the channel properties. More recently, Obukhov and Nowycky (2005) demonstrated that D^{633}, which is situated intracellularly between the end of TM6 and the TRP box, is a crucial residue for current block by intracellular Mg^{2+} in TRPC5 homotetrameric channels. Mutations of D^{633} to either noncharged or positively charged residues display markedly reduced inward currents and decreased voltage-dependent Mg^{2+} block (Obukhov and Nowycky 2005). In summary, all these results indicate that negatively charged residues that appear to be located close to but exterior of the pore region control permeation properties of TRPC1/5 channels (for predicted location of these residues see the scheme in Fig. 3B).

TRPMs

In all members of the TRPM subfamily, the TM5–TM6 region is highly conserved and shares limited homology to pore regions of KcsA and TRPVs channels. It consists of a putative hydrophobic pore helix, followed by an invariant aspartate, which seems to be located in the selectivity filter (Perraud et al. 2003). Other conserved negatively charged residues between the putative pore helix and the fully conserved aspartate may form a cluster of negative charges that contributes to the pore properties of TRPM channels. Recently, taking advantage of the theoretical prediction of the putative pore region of TRPM channels, Nilius et al. identified several residues responsible for the permeation properties of the TRPM4 pore and its blockade by intracellular spermine (Nilius et al. 2005a). Substitution of E^{981}DMDVA^{986} residues of TRPM4 with the selectivity filter of TRPV6 (T^{538}IIDGP^{543}) results in a functional channel that combines the gating hallmarks of TRPM4, such as activation by Ca^{2+} and voltage dependence, with TRPV6-like sensitivity to channel block by extracellular Ca^{2+} and Mg^{2+}. Furthermore, neutralization of E^{981} by alanine abolishes TRPM4 affinity to block by spermine, strongly indicating that E^{981} is placed in the inner part of the pore where it is exposed to intracellular spermine (Nilius et al. 2005a). Mutations of the neighboring aspartates, D^{982} and D^{984}, strongly affect the rundown and voltage dependence of the channel, whereas substitution of Gln^{977} by a glutamate, the site occupied by a negatively charged residue in divalent cation-permeable TRPMs, modifies monovalent cation permeability and leads to the channel with moderate Ca^{2+} permeability (for predicted location of these residues, see the scheme in Fig. 3B). These experiments provide, so far, the strongest direct proof that the TRPM4 selectivity filter is located between TM5 and TM6.

Additional information on the pore region of TRPM channels comes from functional analysis of TRPM3 splice variants. TRPM3α1–5 differ in the length of the putative pore region as one splice site is located in the TM5–TM6 loop (Oberwinkler et al. 2005). TRPM3α1, a variant that encompasses an optional stretch of 12 amino acids following the invariant aspartate, forms a channel with low permeability for divalent cations, whereas TRPM3α2, which lacks this stretch of amino acids, has more than tenfold higher permeability to Ca^{2+} and Mg^{2+} and is sensitive to block by extracellular monovalent cations. Again, these data indicate that TM5–TM6 region covers the pore region of the TRPM channels.

Intracellular determinants implicated in function of TRP channels

TRP domain

Discovery of mammalian members of the TRPC subfamily revealed the existence of a highly conserved structure localized in the C terminus close to TM6 (Bhave et al. 2003; Clapham et al. 2001; Minke and Cook 2002; Montell et al. 2002a; Prescott and Julius 2003). This so-called TRP domain consists of 25 amino acids, six of which are referred to as a TRP box. This TRP box has been postulated to serve as a putative signature of the TRP channel superfamily, but in view of the latest TRP channel classification (Clapham et al. 2003; Montell et al. 2002b), the use of the TRP box as a hallmark of the TRP superfamily has to be revised, as it is not conserved in TRPP, TRPML, TRPA, and TRPN subfamilies.

In TRPC channels, the TRP box is fully conserved and characterized by the specific amino acid sequence, EWKFAR. In TRPVs and TRPMs, the conservation of the TRP box is very low, going from IWxLQx (with x = K, R, or W) for TRPV1–4 and LWRAQx (with x =

V or I) for TRPV5–6, to xWKFQR (with x = I, V, or F) for TRPM1–3/5/7–8, YWKAQR for TRPM4, and LWKYNR for TRPM6. Surprisingly, nothing is known about functional role of the TRP box in TRPC channels. It has been recently shown that in TRPV1 the putative TRP-box motif (amino acids from D^{684} to R^{721}) may function as an association domain involved in oligomerization of the channel (Garcia-Sanz et al. 2004). Biochemical and immunological analysis indicate that self-association of recombinant C-termini of TRPV1 as well as of full-length TRPV1 monomers is blocked when the segment between D^{684} and R^{721} is deleted. Additionally, such deletion in a poreless TRPV1 mutant subunit suppressed its robust dominant-negative phenotype. These data suggest that the TRP-box region may act as a molecular determinant responsible for tetramerization of TRPV1 subunits into functional channels (Garcia-Sanz et al. 2004).

Another interesting insight in the function of the TRP box comes from the study of phosphatidylinositol 4,5-bisphosphate (PIP_2) -dependent activation of TRP channels (Rohacs et al. 2005). Mutations at conserved positively charged amino acid residues in the TRP-box, K^{995}, R^{998}, and R^{1008} of TRPM8 decrease the sensitivity of the channel to PIP_2 and enhanced channel inhibition by PIP_2 depletion. Similar results were obtained when analogue mutations were introduced into the TRP domain of TRPM5 (R^{1006}) and TRPV5 (R^{599}) (Rohacs et al. 2005). All these observations suggest that the positively charged residues in the TRP box are important determinants for interaction with PIP_2 and that regulation by PIP_2 is a common feature of members of the TRP channel family.

Ankyrin repeats

Most of the TRP channels contain N-terminal ankyrin (ANK) repeats, which are 33-residue motifs consisting of pairs of antiparallel α-helices connected by β-hairpin motifs (for review, see Sedgwick and Smerdon 1999). ANK repeats are involved in specific protein–protein interactions and can interconnect membrane proteins with the spectrin-actin-based membrane skeleton (Denker and Barber 2002). The number of ANK repeats in the N terminus of TRP channels varies between different members of the same subfamily. TRPCs and TRPVs typically possess three or four ANK motifs, compared to 14–15 in TRPAs and approximately 29 (!) in TRPNs.

The role of ANK repeats in TRP channels is still unclear and controversial. In TRPC1, deletion of the region containing all three ANK repeats had no effect on dimerization of the channel, as shown by the yeast two-hybrid analysis (Engelke et al. 2002). Although such mutant channels are inserted correctly in the membrane, they do not form functional TRPC1 channels, suggesting that ANK repeats may interact with interaction partners that are needed for the correct assembly of the quaternary channel structure or regulation of channel function (Engelke et al. 2002). In contrast, a similar N-terminal deletion of the region comprising all three ANK repeats of TRPC3 results in retention of the truncated channel in intracellular compartments. Truncation of the N terminus up to the first ANK repeat does not influence channel function or targeting to the plasma membrane, indicating that these motifs may be involved in channel trafficking (Wedel et al. 2003). In TRPV6, which contains six ANK repeats (Peng et al. 2000), only the third ANK motif is a key determinant of tetramerization (Erler et al. 2004). It may serve as an initiator of the molecular zippering process that proceeds after the fifth ANK repeat, creating an intracellular anchor that is necessary for a functional assembly of TRPV6 subunits (Erler et al. 2004). Surprisingly, complete deletion of the TRPV4 N terminus including three ANK repeats had no effect on targeting the channel to the plasma membrane (Liedtke et al. 2000). Electrophysiological measurements

also show no significant differences in the responses of the TRPV4 N-terminal truncants to hypotonic stimulation, except that cells expressing the truncated protein respond less robustly than intact TRPV4 in the first 60 s after hypotonic stimulation (Liedtke et al. 2000).

More recently, an interesting hypothesis for a functional role of ANK repeats in TRPA and TRPN channels has been proposed. In mechanosensitive TRPA1 and TRPN1 channels, mechanical stress may be transduced to these channels via their cytosolic tails, which may be connected to cytoskeletal elements (Corey et al. 2004; Howard and Bechstedt 2004; Lin and Corey 2005; Sotomayor et al. 2005). Crystallographic studies have shown that multiple ankyrin repeats can form a helical structure, which may act as a gating spring. Theoretical calculations of the stiffness of such an ankyrin helix yield values of around 1–5 mN/m, which is on the same order of magnitude as the experimentally deduced stiffness of gating springs in vertebrate hair cells (Howard and Bechstedt 2004; Sotomayor et al. 2005).

Coiled-coil domain

Coiled-coil domains are protein oligomerization motifs that consist of two or more alpha helices that twist around one another to form a supercoil (Burkhard et al. 2001). Peptides with the capacity to form coiled coils are characterized by a heptad repeat pattern in which residues in the first and fourth position are hydrophobic, and residues in the fifth and seventh position are predominantly charged or polar. Analysis of TRP channel sequences reveals the presence of putative coiled-coil domains in TRPCs and TRPMs, but the function of these predicted motifs has not yet been extensively studied. Using yeast two-hybrid experiments, it has been shown that the N-terminal coiled-coil structure of TRPC1 facilitates a homomerization process (Engelke et al. 2002). Although the mutant lacking the coiled-coil region can be correctly inserted in the membrane, Ca^{2+} influx in cells expressing this mutant is significantly reduced compared to wild-type TRPC1. These data suggest that the N-terminal coiled-coil region is involved in regulation of the TRPC1 channel function via interaction with other proteins (Engelke et al. 2002).

More recently, Nilius et al. showed that deletion of the R^{1136}ARDKR^{1141} region in the putative C-terminal coiled-coil domain of TRPM4 eliminates the effect of decavanadate on TRPM4 activation (Nilius et al. 2004a). Interestingly, this site also shows some similarities with the pleckstrin domain of PLC (K-X_{3-11}-R/K-X-R-Hyd-Hyd; where Hyd corresponds to any hydrophobic amino acid), which mediates interaction with second messenger lipids such as PIP_2 (Harlan et al. 1994, 1995).

PDZ-binding domain

PDZ domains (named after the three proteins in which this motif was first described: the postsynaptic density protein PSD, disc-large tumor suppressor, and the tight junction protein ZO-1) are protein interaction domains that are often found in multidomain scaffolding proteins. PDZ-containing scaffolds assemble specific proteins into large molecular complexes at defined locations in the cell. They are specialized in binding to short peptide motifs, PDZ-binding motifs, at the extreme C-termini of other proteins (Kim and Sheng 2004). In the TRP channel superfamily, putative PDZ-binding domains have only been found in TRPC and TRPV subfamilies. In the *Drosophila* TRPCs, TRP and TRP-like (TRPL), the C terminus interacts with the PDZ domain-containing protein INAD (Tsunoda and Zuker 1999), which enables heteromultimerization of these channels in the signaling complex (Chevesich et al.

1997; Huber et al. 1998; Leung et al. 2000; Li and Montell 2000; Xu et al. 1998). The PDZ-binding motif of TRPC4 and TRPC5 is formed by a C-terminal stretch of five amino acids, VTTRL. The presence of the TRL sequence in this motif is essential for the interaction of TRPC4/5 with PDZ domain-containing proteins such as hydrogen exchanger regulating factor (NHERF) or ezrin/moesin/radixin-binding phosphoprotein 50 (EBP50) (Lee-Kwon et al. 2005; Mery et al. 2002; Tang et al. 2000). As shown by co-precipitation experiments, TRPC4 and TRPC5 are able to co-assemble with NHERF as well as with NHERF-interacting partner, PLC-β1, suggesting that this interaction forms an important mechanism for allocation and regulation of the channels (Tang et al. 2000). Furthermore, it has been also shown that deletion of the PDZ-binding motif in TRPC4 strongly reduces expression of the channel at the cell surface and also changes its general distribution in the cell membranes to a predominant expression in cell outgrowths (Mery et al. 2002).

No evident PDZ-binding domains have been identified in the C terminus of TRPVs, but coexpression studies in *Xenopus* oocytes revealed that TRPV5 conductance is activated by the scaffold protein NHERF2 by increasing the channel abundance at the plasma membrane. This stimulatory effect requires the presence of the serum and glucocorticoid inducible kinase SGK1 (Embark et al. 2004). The interaction of NHERF2 and TRPV5 is a Ca^{2+}-independent process that requires the second PDZ domain of NHERF2 and the C-tail of TRPV5. Deletion of the second but not the first PDZ domain in NHERF2 abrogates the stimulating effect of SGK1/NHERF2 on TRPV5 activity and abundance at the plasma membrane. Thus, these data indicate that the C-terminal tail of TRPV5 interacts with the second PDZ domain of NHERF2 and this interaction is required for TRPV5 stabilization at or TRPV5 targeting to the plasma membrane (Palmada et al. 2005).

Modulation by Ca^{2+} signaling and calmodulin binding

Calmodulin (CaM) controls many Ca^{2+}-dependent cellular processes and is an important modulator of various types of ion channels. Several studies have been conducted to dissect specific CaM-binding domains (CaMBD) and determine their function for regulation of TRP channel activity (for recent review, see Zhu 2005). The first indication of functional interaction between CaM and TRP channels comes from a study devoted to isolation of CaM-binding proteins in *Drosophila* (Phillips et al. 1992). This study resulted in isolation of TRPL, a close homologue of *Drosophila* TRP. CaM-binding assays showed that TRPL has two CaMBDs in the C terminus, CaMBD1 (anino acids 710–725) and CaMBD2 (amino acids 859–871) (Phillips et al. 1992). CaMBD1 binds calmodulin in a Ca^{2+}-dependent way, while binding of CaM to CaMBD2 is Ca^{2+}-independent. Interaction between CaMBD1 and CaM is regulated by phosphorylation of two serine residues, S^{721} and S^{722} (Trost et al. 1999; Warr and Kelly 1996). Phosporylation of S^{721} by protein kinase A (PKA) abolishes the CaM binding, whereas phosporylation of the adjacent S^{722} by PKC results in modulation of phosphorylation by PKA.

Binding studies performed on TRPC3 revealed that the inositol 1,4,5-triphosphate (IP_3) receptor (IP_3R) and CaM interact directly with so-called CaM/IP_3R binding region (CIBR) at the C terminus of the channel (Boulay et al. 1999; Zhang et al. 2001). Interaction of CaM with TRPC3 has an inhibitory effect on the TRPC3 channel function. This inhibition is reversed in the presence of IP_3R, which competes for the binding to the CIRB region, resulting in displacement of CaM from the CIRB domain and activation of the channel (Zhang et al. 2001). Interestingly, TRPC3 mutants lacking the CIBR domain are predominantly localized in intracellular compartments, suggesting that CaM/ IP_3R binding can be involved

in trafficking of the channel to the plasma membrane (Wedel et al. 2003). By sequence homology analysis, the CIRB domain has been identified in all TRPC channels. However, the sensitivity and responses to CaM and IP_3Rs vary between different TRPC channels (Tang et al. 2001). In TRPC4, interaction with CaM also depends on two regions in the C terminus between residues 688–759 and 786–848, which bind CaM in Ca^{2+}-dependent manner (Trost et al. 2001). The C terminus of TRPC1 can interact with CaM in the two regions localized between animo acids 719–749 (CaMBD1) and 756–793 (CaMBD2) (Singh et al. 2002). Deletion of CaMBD1 region did not alter either thapsigargin-stimulated increase of the intracellular calcium level ($[Ca^{2+}]_i$) or Ca^{2+}-dependent feedback inhibition of the store operated calcium entry (SOCE). On the other hand, deletion of CaMBD2 of TRPC1 increases SOCE and decreases Ca^{2+}-dependent inactivation of the channel (Singh et al. 2002). Interestingly, it has been shown that the adaptor protein Homer facilitates a physical TRPC1–IP_3R association and is required for the TRP channel to respond to signals. The TRPC1-Homer–IP_3R complex is dynamic and its disassembly parallels TRPC1 channel activation (Yuan et al. 2003).

CaM-dependent regulation of the channel activity has also been assessed for several TRPV channels. TRPV1 exhibits two CaM binding sites. Disruption of CaMBD in the C terminus prevented TRPV1 desensitization (Numazaki et al. 2003), whereas binding of CaM to the N-terminal CaMBD decreases the capsaicin-activated currents (Rosenbaum et al. 2004). In TRPV4, CaM binds to a stretch of basic amino acids in the C terminus of the channel starting at position 814. Neutralization of positive charges in this region results in the loss of Ca^{2+}-dependent potentiation and of the spontaneous opening of TRPV4 in the absence of an agonist. The TRPV4 CaMBD also exhibits a consensus sequence for protein serine/threonine kinase phosphorylation, but mutations to these residues did not alter the Ca^{2+}-dependent potentiation (Strotmann et al. 2003).

In the case of TRPV5/6, two conserved CaMBDs have been identified in both the N and C termini (Lambers et al. 2004; Niemeyer et al. 2001). Interestingly, an additional CaM-binding site is present in the transmembrane region of TRPV6 (Lambers et al. 2004). The C-terminal CaMBD of TRPV6 overlaps with a consensus sequence for protein kinase C (PKC) phosphorylation. PKC-dependent phosphorylation of the site alters CaM binding and delays channel inactivation (Niemeyer et al. 2001). Co-expression of TRPV6 together with a CaM variant in which all four Ca^{2+}-binding sites (CaM_{1234}) are mutated significantly reduces inward Ca^{2+} currents upon hyperpolarization. No such effect can be observed for TRPV5-expressing cells (Lambers et al. 2004). Remarkably, Ca^{2+}-dependent inactivation of TRPV5 and TRPV6 are dramatically different; the initial inactivation of TRPV6 is much faster than that of TRPV5. Mutagenesis studies in TRPV6 show that residues L^{409}, V^{411}, and T^{412} in the intracellular loop located between TM2 and TM3 are responsible for the fast inactivation behavior of this channel (Nilius et al. 2002). In contrast, Ca^{2+}-dependent inactivation of TRPV5 is determined by two domains in the C terminus (Nilius et al. 2003b).

More recently, Nilius et al. found that overexpression of the CaM_{1234} mutant dramatically reduced TRPM4 activation (Nilius et al. 2005b). In vitro binding assays identified five short regions, two at the N terminus and three at the C terminus of TRPM4, which interact with CaM in a Ca^{2+}-dependent manner. Under Ca^{2+}-free conditions, four TRPM4 fragments display no binding and one shows weak binding to CaM. However, all CaM-binding fragments associate much more strongly with CaM in the presence than in the absence of Ca^{2+}. Interestingly, these CaM-binding sites appear to be multifunctional, as deletions of the C-terminal but not the N-terminal sites affected the Ca^{2+} sensitivity of TRPM4. Thus, all these data suggest that CaM binding to the C-terminal sites is vital for Ca^{2+} sensitivity of TRPM4 in the physiological range of intracellular Ca^{2+} concentrations (Nilius et al. 2005b).

Modulation by phosphorylation

Phosphorylation by protein kinases is a recurring and reversible post-translational modification that can regulate properties of ion channels. Studies of phorbolester- and Ca^{2+}-dependent protein phosphorylation in *Drosophila* demonstrate that, apart from the PDZ domain protein INAD, the TRP channel is a substrate of eye-specific PKC in isolated signaling complexes. This mechanism can be a part of a negative feedback loop that regulates Ca^{2+} influx through the channel (Huber et al. 1998).

TRPC3 can be phosphorylated by cGMP-dependent protein kinase G (PKG). Mutations at two consensus PKG phosphorylation sites, T^{11} and S^{263}, markedly reduce the inhibitory effect of cGMP on TRPC3-mediated store-operated Ca^{2+} influx. Treatment with PKG inhibitors had a similar effect (Kwan et al. 2004). More recently, it has been shown that TRPC3 is negatively regulated by PKC-dependent phosphorylation of S^{712}, a residue that is conserved in all mammalian TRPC channels. This mechanism is mediated by PLC-generated diacylglycerol, which serves both as a signal for TRPC3 activation and as a signal for negative feedback via PKC-mediated phosphorylation (Trebak et al. 2005).

Relatively limited data are available for other TRPCs. It has been shown that TRPC6 is directly phosphorylated by Src family protein-tyrosine kinases (PTKs) and this mechanism regulates TRPC6 channel activity (Hisatsune et al. 2004). TRPC5 can be phosphorylated by PKC. PKC inhibitors prevent TRPC5 desensitization after activation by G protein-coupled receptor, and the mutation of T^{972} to alanine dramatically slows this desensitization process. Thus, these results strongly suggest that desensitization of TRPC5 occurs via PKC-dependent phosphorylation of T^{972} (Zhu et al. 2005).

The functional role of phosphorylation on TRP channel function is probably best described in TRPV1. Early work on capsaicin and heat activation of TRPV1 revealed that this channel is a target for PKC-dependent phosphorylation (Chuang et al. 2001; Crandall et al. 2002; Hu et al. 2002; Numazaki et al. 2002; Premkumar and Ahern 2000; Tominaga et al. 2001; Vellani et al. 2001; Zhou et al. 2001b). Mutation of S^{800} to alanine significantly reduces phorbol 12-myristate 13-acetate (PMA)-induced enhancement of capsaicin-evoked currents and the direct activation of TRPV1 by PMA. In contrast, mutation of S^{502} to alanine reduces PMA enhancement of capsaicin-evoked currents with no effect on direct activation of TRPV1 by PMA, whereas mutation of T^{704} to alanine does not affect PMA enhancement of capsaicin-evoked currents but dramatically reduces direct activation of the channel by PMA. These results suggest that PKC-mediated phosphorylation modulates TRPV1 but does not directly gate the channel (Bhave et al. 2003; Numazaki et al. 2002; Vlachova et al. 2003). More recently, experiments conducted on a C-terminal truncated TRPV1 channel suggest that the distal C terminus of TRPV1 has an inhibitory effect on PKC phosphorylation-induced potentiation of the TRPV1 channel (Liu et al. 2004; Vlachova et al. 2003). TRPV1 can also be subjected for PKA-dependent phosphorylation. PKA-dependent phosphorylation of the N-terminal S^{116} interferes with the desensitization capsaicin-evoked whole cell currents (Bhave et al. 2002). Two other PKA phosphorylation sites in the C terminus, S^{774} and S^{820}, are also involved in regulation of TRPV1 channel desensitization (Mohapatra and Nau 2003). In contrast to TRPV1, a specific tyrosine residue localized in the first ankyrin motif of TRPV4 is phosphorylated upon hypotonic stress. This swelling-induced phosphorylation at Y^{253} is mediated via a member of Src family PTKs, the Lyn kinase, demonstrating that TRPV4 can be regulated by tyrosine phosphorylation (Xu et al. 2003). However, this mechanism of activation seems to be controversial since in a more recent study it has been shown that mutation of Y^{253} to phenylalanine does not affect hypotonic-induced activation of TRPV4 (Vriens et al. 2004b).

In the TRPMs, the effects of channel phosphorylation have only been described for TRPM4 and TRPM7. The Ca^{2+} sensitivity of TRPM4 is modulated by PKC-dependent phosphorylation. In the presence of ATP, PMA sensitizes Ca^{2+}-dependent activation of TRPM4. This effect is abolished when either of the two C-terminal serines, S^{1152} and S^{1145}, which are predicted to have the highest probability for PKC phosphorylation, are mutated (Nilius et al. 2005b). Mutation of these two serines to glutamates to mimic the phosphorylated state of the channel results in a delayed deactivation of TRPM4 and shifts the activation curves toward more negative voltages (Nilius et al. 2005c).

An interesting feature of TRPM7 is the presence in its C terminus of an atypical protein-kinase domain, the so-called phospholipase C interacting kinase (PLIK) domain (Runnels et al. 2001). The crystal structure of this protein kinase domain has been determined. In its catalytic core, it shows unexpected similarity to eukaryotic α-kinases (Yamaguchi et al. 2001). It has been shown that TRPM7 activity can be up- and down-regulated via the PLIK domain in a cAMP- and PKA-dependent manner (Takezawa et al. 2004). However, the importance of the PLIK domain for the TRPM7 channel function is still controversial. Inactivation of PLIK kinase activity by site-directed mutagenesis and/or changes in intracellular ATP indicated that the endogenous kinase activity is essential for channel function (Runnels et al. 2001).

Deletion of the region that comprises the kinase domain resulted in a functional channel with increased sensitivity to Mg^{2+} and MgATP. These data suggest that the structural kinase domain alters the sensitivity of TRPM7 to extracellular Mg^{2+} (Schmitz et al. 2003). More recently, it was shown that the PLIK domain autophosphorylates TRPM7 at serine residues, S^{1511} and S^{1567}. Mutation of these two sites or of the catalytic site that abolished kinase activity (kinase-dead mutants) did not affect the channel function and inhibition by internal Mg^{2+} but abolished the kinase activity. Divalent cations such as Mg^{2+}, Zn^{2+}, and Ca^{2+} inhibit the channel activity. In contrast, the kinase activity is enhanced by Mg^{2+}, decreased by Zn^{2+} and in the case of Ca^{2+} no effects have been observed (Matsushita et al. 2005). In contrast to Schmitz et al. (2003), the authors of this latest study did not see functional expression of TRPM7 lacking the full kinase domain. Therefore, they suggested that neither current activity nor regulation by internal Mg^{2+} is affected by kinase activity or autophosphorylation, but that the kinase domain may play a structural role in channel assembly or subcellular localization (Matsushita et al. 2005).

Modulation by PIP_2 and possible PIP_2 binding sites

The first example of PIP_2-dependent modulation of a TRP channel was described for TRPV1, whose function is inhibited by PIP_2. Hydrolysis of PIP_2 by stimulation of PLC reverses the TRPV1 inhibition (Chuang et al. 2001). The molecular determinant for the PIP_2 interaction in TRPV1 channels is localized in the C terminus of TRPV1 between amino acids 777–820 (Prescott and Julius 2003). Similar effects are also observed for *Drosophila* TRPL channels. In excised inside-out patches, the spontaneous TRPL channel activity is strongly reduced upon application of PIP_2. Surprisingly, this effect is not observed in all patches. The reasons why PIP_2 is unable to inhibit TRPL in all patches is not known, but it is possible that there could be a state dependence of the TRPL channel necessary for the effect of PIP_2, or that some of the excised patches lack a specific protein (Estacion et al. 2001).

As already mentioned in the section entitled “Modulation by phosphorylation”, TRPM5, TRPV5, and TRPM8 are activated by interaction with PIP_2 to specific consensus residues in

the TRP domain (Liu et al. 2005; Liu and Liman 2003; Rohacs et al. 2005). Similar effects of PIP_2 have also been shown for activation of the TRPM7 channel, which becomes inactive upon stimulation of PLC activation and PIP_2 hydrolysis (Runnels et al. 2002). Recovery of carbachol induced TRPM7 current inhibition is accelerated after wash out in the presence of PIP_2. Furthermore, application of PIP_2 to inside-out patches after rundown results in a full restoration of TRPM7 single-channel activity (Pedersen et al. 2005; Runnels et al. 2002). For TRPM5, it has been shown that PIP_2 reverses the desensitization of the channel caused by a sustained exposure to Ca^{2+}, resulting in a partial recovery of the channel activity (Liu and Liman 2003). Interestingly, the closest TRPM5 homologue, TRPM4, contains a putative PIP_2-binding domain, which shares homology with pleckstrin domains (Nilius et al. 2006). Mutation of this putative PIP_2-binding domain of TRPM4 prevents activation of the channel by PIP_2 and decavandate, a compound with six negative charges, which may mimic the PIP_2 action (Nilius et al. 2004a, Nilius et al. 2006). In TRPM8, channel activation causes an influx of Ca^{2+}, which activates Ca^{2+}-sensitive PLC-dependent hydrolysis of PIP_2, resulting in closure or desensitization of the channel (Liu et al. 2005).

Endogenous enzymatic activities

The presence of a full enzyme in the C terminus is not only found in TRPM7 (see "Modulation by phosphorylation"). A similar α-kinase domain is also found in TRPM6, the closest homologue of TRPM7 (Chubanov et al. 2004; Schlingmann et al. 2002). Another TRPM member, TRPM2, contains a Nudix hydrolase domain in its C terminus, which functions as an ADP-ribose (ADPR) pyrophosphatase (Kuhn et al. 2005; Perraud et al. 2001; for review see Perraud et al. 2003). The TRPM2 Nudix domain shares 39% identity with NUDT9, a human ADPR pyrophosphatase. A characteristic feature of many members of the Nudix enzyme family is the presence of the conserved Nudix box, $GX_5EX_7REuXEEXu$ (X any amino acid residue and *u* a large hydrophobic residue). In TRPM2, some of the key positions in Nudix box are altered. Introduction of these different amino acids into the NUDT9 causes a strong decrease in the ADPR activity, similar to that obtained for TRPM2. The crystallographic data show that unlike its closest functional homologue, homodimeric *Escherichia coli* ADPRase, NUDT9 is active as a monomer with the substrate binding site located in a cleft between the N-terminal and the C-terminal catalytic domain (Shen et al. 2003).

It has been shown that ADPR activates the TRPM2 channel, suggesting that the enzymatic activity of the Nudix domain is an essential component of the gating mechanism of the channel (Perraud et al. 2001). The C-terminal splice variant of TRPM2, which contains a deletion of 34 amino acids in the region (between amino acids 1292–1325) distant from the Nudix box (DeltaC-strech), can still be activated by H_2O_2 but does not respond to ADPR (Wehage et al. 2002). Mutants lacking 19, 25, and 29 amino acid residues in the N-terminal part or having substitutions of amino acid residues in the remaining C-terminal part of the DeltaC stretch displays typical ADPR-induced currents. However, deletion or substitution of the amino acid residue N^{1326} immediately downstream of the DeltaC stretch abrogates ADPR gating. These data suggest that amino acid residues in the DeltaC stretch are not directly involved in ADPR gating but may act as a spacer segment stabilizing a conformation necessary for the essential N^{1326} residue to interact with other channel regions. Interestingly, prolonged binding rather than degradation of ADPR is required for the modulation of TRPM2 function, since enhancement of Nudix box activity abolishes the ADPR gating of the channel (Kuhn and Luckhoff 2004).

More recently, it has been shown by structure-guided mutagenesis that TRPM2 gating by ADPR and both oxidative and nitrosative stresses requires an intact ADPR binding cleft in the C-terminal NUDT7 domain (for a recent review, see Kuhn et al. 2005). The oxidative/nitrosative stress-induced TRPM2 gating can be inhibited by blocking ADPR production and by suppressing ADPR accumulation by cytosolic or mitochondrial overexpression of an enzyme that specifically hydrolyzes ADPR (Perraud et al. 2005).

Concluding remarks

In this review we have given an overview of the most recent data on the structure–function relationship of TRP channels. A broad range of thus far identified structural domains and motifs strongly emphasizes the diversity of functions and regulatory mechanisms in the TRP superfamily. However, it has to be stressed that despite the rapid progress made in the last few years, a detailed view on the role of particular domains in regulation of the channel function is still elusive. Continuation of scientific efforts will be required to further clarify the structural basis for the functioning of the fascinating superfamily of TRP channels.

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