REVIEW

Barbara A. Niemeyer

Structure-function analysis of TRPV channels

Abstract In recent years many new members of the family of TRP ion channels have been identified. These channels are classified into several subgroups and participate in many sensory and physiological functions. TRPV channels are important for the perception of pain, temperature sensing, osmotic regulation, and maintenance of calcium homeostasis, and much recent research concerns the identification of protein domains involved in mediating specific channel functions. Recent literature on TRPV channel subunit composition, protein domains required for subunit assembly, trafficking, and regulation will be reviewed and discussed.

Keywords TRP channels · Ankyrin · Tetramerization · Subunits · Calmodulin · Protein kinase · Assembly · Trafficking

Introduction

TRP channels have evolved as a family of ion channels initially defined by their relatedness to their seminal member, the *Drosophila* Transient Receptor Potential (TRP) channel (Hardie and Minke 1992; Montell and Rubin 1989). Their 27 mammalian homologs are currently grouped into the subfamilies TRPC, TRPM, TRPV, TRPA, TRPP, TRPN (no mammalian homolog) and TRPML. Members of the TRP family are involved in many sensory processes such as invertebrate vision and hearing, mammalian temperature-, pain-, gustatory and auditory sensation. These senses are often restricted to very specialized cells, but many TRP family members are also involved in more ubiquitous processes such as sensing osmotic stress or regulating the intracellular magnesium concentration (for review see Clapham 2003; Montell 2001; Vriens et al. 2004a).

B. A. Niemeyer (✉)
Institut für Experimentelle und Klinische Pharmakologie und
Toxikologie, Universität des Saarlandes,
Gebäude 46,
66421 Homburg, Germany
e-mail: barbara.niemeyer@uniklinik-saarland.de
Tel.: +49-6841-1626405

With the rate of new publications defining pathways that involve TRP channel function, a current summary is soon outdated. Rather than trying to cover functions of TRP channels in general, this review will focus on a more detailed structure-function analyses mainly of TRPV channels and will only include some pertinent data from other TRP subfamily members. Reviews on the function of TRPC channels can be found in this issue (Dietrich et al. 2005; Gosling et al. 2005; Groschner and Rosker 2005; Plant and Schaefer 2005; Zufall 2005). So far almost all of the TRP channel proteins form cation conducting ion pores with a range of different selectivities, for reviews see (Clapham 2003; Montell 2001).

TRPC, TRPA, TRPN and TRPV channels contain multiple ankyrin (ANK) repeats within their intracellular N-termini. Although ANK repeats are common modular protein-interaction motifs, not much is known whether or not their specific sequence and/or number of repeats are required to form specialized interactions or about their role in the assembly of ion channels (see below). The general topology of a TRP subunit as shown in experimental detail for TRPC channels (Vannier et al. 1998) includes intracellular – and C-terminal regions of variable length and six transmembrane spanning domains with a pore loop between transmembrane domain 5 and 6. The localization of the pore loop has been confirmed by mutational analyses of residues within the pore for TRPV5 and TRPV6, TRPV1 and TRPV4 (Garcia-Martinez et al. 2000; Nilius et al. 2001; Voets et al. 2002, 2003). In analogy to voltage gated potassium channels it is thought that four subunits need to assemble to form a functional channel. Experimental evidence for tetramer formation exists for TRPV1, TRPV2, and TRPV5/6 (Hoenderop et al. 2003; Jahnel et al. 2001; Kedei et al. 2001). Of the three major subfamilies of TRP channels, TRPV, TRPC, and TRPM, it has been shown that some members within the TRPC and within the TRPV family can form heteromeric channels (Amiri et al. 2003; Goel et al. 2002; Hoenderop et al. 2003; Hofmann et al. 2002; Montell 1997; Strubing et al. 2001, 2003). In some cases, however, it remains to be seen whether the subunits forming these heteromeric channels in expression systems

are indeed coexpressed in the same native cells and results drawn from using concatameric constructs need to be critically evaluated in light of their propensity to form lower order byproducts (Nicke et al. 2003).

Ankyrin repeats

Ankyrin (ANK) repeats have originally been defined as a ~33 amino acid repeated motif in the sequence of Swi6p and CDC10p (yeast cell cycle regulators) as well as Notch and Lin-12 (Breeden and Nasmyth 1987). Their name stems from finding 24 of these motifs in the ankyrin protein (Lux et al. 1990). ANK repeats are rather variable in primary sequence, but show a high degree of conservation regarding their secondary and tertiary structure (for review see Mosavi et al. 2004; Sedgwick and Smerdon 1999). Mosavi et al. analyzed ~4,000 ANK repeat containing proteins and used the consensus information to design an idealized ANK repeat (Mosavi et al. 2002) and to define amino acids necessary for structure versus more variable surface residues that may confer specificity upon the interaction with other proteins.

Each individual ANK repeat represents a helix–turn–helix motif where the first helix (seven to eight residues) contains a mostly conserved TPLH motif and the second helix (nine to ten residues) usually two highly conserved leucine residues which might play an important structural role in stabilizing the turn of the L-shaped ANK repeat cross-section formed by antiparallel α-helices and the extended β-hairpin loop at an approximately right angle (Yuan et al. 2004). Multiple ANK repeats can create a hydrophobic core that creates a long-range communication: Mutation of a single residue within an individual repeat can thus affect the entire three dimensional core structure. For a review of the effects of mutations on the structure of the ANK repeats within the Notch protein see (Lubman et al. 2004). Because the sequence homology often is quite weak, it is difficult to unambiguously define the number of ANK repeats in a given protein, i.e., for TRPV6 the number of repeats given by different authors range from three repeats (Peng et al. 2000) to at least six (Wissenbach et al. 2001).

Although a number of studies have demonstrated interactions between ANK repeats and various interacting proteins, such as spectrin (Platt et al. 1993), erythroid anion exchanger (Michaely and Bennett 1995) and IP3 receptor (Bourguignon et al. 1993) among others, not much is known about a role for ANK repeats in the multimerization of proteins. For the chloroplast protein SRP43 it has been shown that its third and fourth ANK repeats are involved in dimerization of the protein (Jonas-Straube et al. 2001) and two recent studies demonstrate the importance of ANK repeats for the tetramerization of TRPV6 and TRPV5 ion channels (Chang et al. 2004; Erler et al. 2004). A very interesting hypothesis for the functional role for the 29 ANK repeat containing *Drosophila* NOMPC channel is that this repeat structure forms the gating spring of mechanoreceptors (Howard and Bechstedt 2004). Vertebrate TRPA1 channels that are candidates for the mechanosensitive transduction channel of hair cells only contain 17 ANK repeats, which may still confer sufficient gating spring stiffness (Corey et al. 2004).

Assembly signals of TRP channels

For the canonical (classic) members of the family (TRPC) channels several groups describe heteromeric combinations of TRPC subfamily members (Amiri et al. 2003; Goel et al. 2002; Hofmann et al. 2002; Montell 1997; Strubing et al. 2001, 2003), but only one group describes a potential N-terminal subunit multimerization domain within the murine TRPC1-β isoform (Engelke et al. 2002). Here, the authors find a self-interacting site using yeast-two hybrid analyses of TRPC1β cytosolic constructs. A potential interaction site is found between the last ankyrin repeat and the first transmembrane domain (Engelke et al. 2002) which presumably forms a coiled-coil domain. Deletion of this domain or of the ANK repeat containing domain leads to non functional channels. However, no data is presented on whether the deletion construct is unable to form protein tetramers as seen on sucrose density gradients or native gel electrophoreses. A corresponding and homologous domain within TRPC5 interacts with the growth-cone enriched protein stathmin-2 which appears to mediate vesicular trafficking of TRPC5 to the growth cone (Greka et al. 2003), which raises the question whether and how proteins compete for binding domains.

Several studies point to the role of the cytosolic N-terminal region in the assembly of functional homo- or heteromultimeric channels. Xu et al. show that in *Drosophila*, TRPL can physically coassemble with TRP-γ and that the TRPL mediated photoresponse is down-regulated after overexpression of an N-terminal TRP-γ construct (Xu et al. 2000). The same group demonstrated earlier that TRP and TRPL can also coassemble and that this coassembly signal also resides within the N-terminal domains (Xu et al. 1997). Again, N-terminal fragments function as dominant-negative regulators of TRP-mediated currents. In these studies the interaction site within the N-terminus is not further narrowed down.

An N-terminal dominant-negative approach is also used to investigate the role of TRPC3 in the oxidative stress-induced membrane conductances of porcine aortic endothelial cells (Balzer et al. 1999). Especially in light of the physical interaction seen by co-immunoprecipitation experiments, the dominant-negative data argues for an N-terminal coassembly signal, these N-terminal fragments could thus bind to full-length subunits and reduce the number of functional tetrameric channels in the plasma membrane.

In the subfamily of TRPV channels, assembly mechanisms have been investigated mainly for TRPV1 and TRPV5/TRPV6 ion channels. Within this family it has been shown that TRPV5 and TRPV6 are able to functional heteromultimeric channels (Hoenderop et al. 2003). Different ratios of TRPV5 or TRPV6 subunits tied in a concatameric tetramer result in calcium-dependent phenotypes

that lie in between the homomultimeric phenotypes. Two studies describe TRPV1 splice variants that lack N-terminal sequences (Schumacher et al. 2000; Xue et al. 2001). These splice variants result in non-functional channels, but whether or not they can exert dominant-negative effects on the full-length channel or if they affect subunit assembly has not been determined. A third TRPV1 splice variant that lacks only ten amino acids within the N-terminal cytoplasmatic domain points very clearly to a dominant-negative role of the N-terminal domain in TRPV1 (Wang et al. 2004a): This native splice variant can act as a dominant-negative regulator of TRPV1 currents. The deletion affects the protein region between the last predicted ANK repeat and the first transmembrane domain and appears to result in a less stable protein that, when coexpressed, can also affect the stability and function of wild-type subunits (therefore is likely to still assemble with the wild-type subunit). Although the affected region is not predicted as an ANK repeat amino acid sequence, its predicted secondary structure resembles the closely spaced α-helices of the prototypical ANK repeat. Very recently, two additional functional TRPV1 variants have been identified (Lu et al. 2005; Lyall et al. 2004).

Garcia-Sanz et al. (2004), Erler et al. (2004) and Chang et al. (2004) describe the identification of tetramerization domains of TRPV channels with partially divergent results. Garcia-Sanz et al. identify a tetramerization domain in the C-terminus of TRPV1. The authors use yeast-two hybrid analysis and pull-down assays to show self-interaction of ~23 C-terminal amino acids immediately adjacent to the last transmembrane domain, however, N-terminal regions are not investigated. This C-terminal domain is predicted to form a coiled-coil structure. Deletion of this domain yields non-functional channels with very reduced or unstable protein expression, underlining the importance of this region for the formation of functional and stable channels. However, coexpression of wild-type TRPV1 with constructs where the presumed association domain is deleted show—when coexpressed—a dominant-negative reduction of wild-type TRPV1 currents, which indicates that association is not completely abolished. Deletion of the "tetramerization" domain in a poreless mutant subunit, however, is able to suppress its dominant-negative effects.

For TRPV6 it has been shown that for dominant-negative effects on TRPV6 currents, the first 154 amino acids containing the first three ANK repeats are sufficient, whereas the N-terminal region of TRPC3 with four ANK repeats does not exert any dominant-negative effect (Kahr et al. 2004). As mentioned above, our group and (Chang et al. 2004; Erler et al. 2004) attempt to decipher the codes for intersubunit assembly sites within the closely related TRPV6 and TRPV5 proteins. We used both a bacterial-two-hybrid interaction screen as well as co-immunoprecipitation of tagged protein fragments with full-length TRPV6 from a stable cell line to narrow down interacting domains. Two N-terminal interactions sites are found that both reside in the ANK repeat domains. The primary site which includes the third ANK repeat appears to be the dominant site as deletions or point mutations within this domain abolish

interaction with full-length channel, destroy the ability to form tetramers as detected on sucrose density gradients and can furthermore relieve the dominant-negative effect of TRPV6 subunits with a mutated ionic pore. Corroborating the results by Kahr et al., no co-immunoprecipitation with the ANK domain containing TRPC3 is detected. Specificity for subunit assembly in proteins containing multiple ANK repeats may thus be conferred by the spacing of the ANK repeats, their three dimensional structure and exposition of variable amino acids either within the ANK repeat or between repeats. For correctly assembled and functional TRPV6 channels, we propose a molecular zippering process that starts at the first interaction site and proceeds past the second site, thus aligning the N-terminal region below the pore. Figure 1 shows a model of a TRPV6 tetramer with its N-terminal association domains located symmetrically below the ionic pore.

The second study investigates molecular determinants of TRPV5 (rabbit) ion channel assembly and concludes that there are two assembly domains, one located between amino acid position 64–77 (overlapping with ANK repeat 1) in the amino terminus, the other, much weaker site,
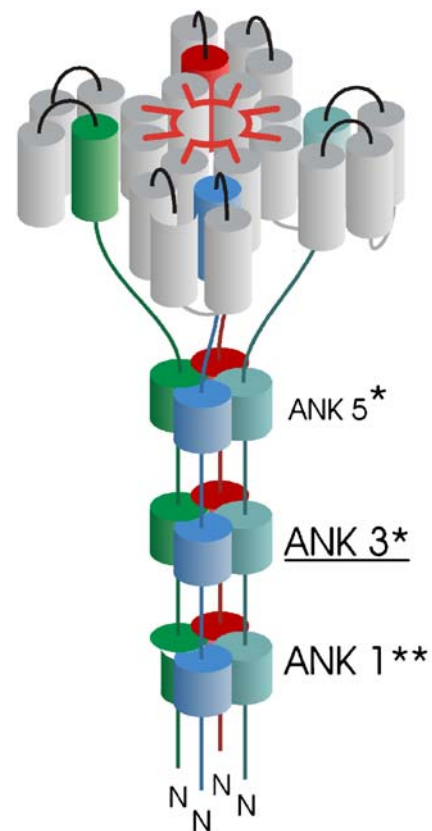

Fig. 1 Model of TRPV5/6 subunit assembly. *One asterisk* indicates that tetramerization requires ANK 3 as the assembly anchor and ANK 5 as one potential stabilizing region (from Erler et al. 2004). *Two asterisks* mark the region around ANK 1 as a critical assembly domain for TRPV5 identified by Chang et al. (2004). Intracellular C-terminal regions, which are not implicated in TRPV6 assembly (Erler et al. 2004), but appear important for TRPV5 assembly (Chang et al. 2004), have been omitted for clarity. Figure modified from Erler et al. (Copyright 2004, Journal of Biological Chemistry)

between amino acid position 596–601 in the intracellular C-terminal region (Chang et al. 2004). The methods used include GST pull-down experiments, immunoprecipitation experiments of N– and C-terminal fragments, surface expression of TRPV5 in oocytes and dominant-negative studies in HEK cells overexpressing wild-type TRPV5 alone or together with N– or C-terminal fragments and recording sodium currents in the absence of divalent cations. Both full-length N– and C-terminal fragments exert dominant-negative effects on wild-type surface expression and function.

How can the divergent results between Chang et al. for TRPV5 and Erler et al. for TRPV6 be explained? In light of their ability to form functional heteromultimeric channels (Hoenderop et al. 2003) one would expect similar association domains. Inherent problems associated with protein–protein interaction studies include difficulties to separate specificity of particular binding sites from the role these site may play for the three dimensional structure of the native protein. Deletion of the association domain within the first ANK repeat abolishes the N-term–N-term binding (Chang et al. 2004) but could affect the folding and representation of the later ANK repeats that are implicated in self-association by Erler et al. Conversely, deletion or alteration of the downstream ANK 3 domain (Erler et al. 2004) could affect the tertiary structure in such a way that the first interaction site is destroyed. Structural data on the folding of the intracellular protein domains could shed light on these questions.

Are chaperone/anchoring molecules necessary for surface expression of TRP channels?

Many ion channels require chaperone molecules for their efficient transport to the plasma membrane and/or anchoring molecules for their more permanent localization there. Examples include 14-3-3 proteins that are necessary for the exit of K(ATP) channels from the ER (see for example Yuan et al. 2003), KChAP for Kv2.1 and Kv4.3 (Abriel et al. 2000; Kuryshev et al. 2000) and potentially AKAPs (A kinase anchoring proteins), which might function to coordinate multiple components of signal-transduction pathways (for review see (Tasken and Aandahl 2004; Wong and Scott 2004)).

Let us first consider the founding member of the family of TRP channels, the *Drosophila melanogaster* transient receptor potential (TRP) channel, which, together with its close relative TRPL, constitutes the light activated conductance of the photoreceptors (Niemeyer et al. 1996). Genetic screens early on identified a mutant (*InaD*) with a phenotype that partially resembled the *trp* mutant fly. Shieh and Zhu (1996) first described a physical interaction between the TRP ion channels and the InaD protein and InaD was soon recognized as an important scaffold component that organizes the entire photoreceptor signaling complex (transducisome; Tsunoda et al. 1997). Absence of InaD protein leads to the mislocalization of TRP ion channels, PLC and eye-PKC. Although a mammalian homolog of InaD has been identified (Philipp and Flockerzi 1997), it

remains unknown whether or not this protein is required for localization or function of mammalian TRP homologs. However, a different PDZ domain containing protein (EBP50, NHERF) that binds PLC β 1,2 and TRPC4 (Tang et al. 2000) has been shown to be important for surface expression and localization of TRPC4 channels (Mery et al. 2002).

Within the family of TRPV channels, it has been shown that TRPV2 physically interacts with the RGA protein, a transmembrane domain containing protein residing on intracellular membranes (Barnhill et al. 2004). RGA however does not accompany TRPV2 to the plasma membrane and a possible function as a chaperone/targeting molecule during maturation remains speculative. Whether the interaction between TRPV2 and the potential AKAP protein ACBD3 (Stokes et al. 2004; see also regulation by Protein kinase A (PKA), below) has a role in trafficking has not yet been investigated.

A more detailed analyses of trafficking and targeting is conducted by van de Graaf et al. for TRPV5 and TRPV6 (van de Graaf et al. 2003). Here, a novel TRPV5 interacting protein, namely S100A10 (p11, calpactin light chain or annexin 2 light chain) is identified. Both TRPV5 and TRPV6 contain a conserved binding site for S100A10 binding localized to the intracellular C-terminal region shortly downstream of the last transmembrane domain (S6). Deletion of this PDZ binding motif "VATTV" or mutation of T601 within TRPV5 leads to greatly diminished surface expression and function (van de Graaf et al. 2003). The S100A10-annexin 2 heterodimer is also important for the localization of the voltage gated sodium channel NA 1.8 and the potassium channel TASK1 (Girard et al. 2002; Okuse et al. 2002). In the case of TASK1 the interaction signal localizes to the PDZ binding motif at the very C-terminus and, reminiscent of the trafficking of K(ATP) channels by 14-3-3 proteins, the interaction with S100A10 masks an ER retention signal.

Deletion or mutation of the chaperone interaction site prevents surface expression (see van de Graaf et al. 2003 for TRPV5). It is then also conceivable that overexpression of C-terminal fragments containing the chaperone interaction site could act as a dominant-negative regulator of wild-type channel expression by obstructing the transport pathway to the plasma membrane. This possibility is not considered by Chang et al. even though the TRPV5 chaperone (S100-A10) has already been identified by the same group. In the case of TRPV1, no chaperone has yet been identified; however, protein stability and perhaps surface expression are clearly affected by deletion of the "tetramerization domain" (Garcia-Sanz et al. 2004).

Another important mechanism of TRPV localization and targeting has been discovered in invertebrates: Here, it has been shown by an elegant study in *C. elegans* (Tobin et al. 2002), that one function of combinatorial TRPV (OSM9, OCR 1–4) expression is to specify subcellular localization. In order to be expressed in the sensory cilia of defined neurons, OSM9 for example requires the OCR2 protein and vice versa. Although one TRPV protein may require the other for localization, it is not yet clear whether they need

to form heteromultimeric channels. A very similar story emerged for the localization of the *Drosophila* TRPV homologs NAN and IAV. Hearing in *Drosophila* requires an anatomical structure, the antennal chordotonal organ, that transduces antennal vibrations into receptor potentials. Reminiscent of the hair bundles of vertebrate inner ear cells, ciliated neurons within the chordotonal organ are the primary sensory cells and they express and require the NAN protein in their sensory cilia to generate sound-evoked potentials (Kim et al. 2003). Interestingly, localization of NAN requires IAV and vice versa (Gong et al. 2004) and *iav* mutants also have severe hearing defects.

It will be very interesting to see if mammalian TRPV channels also show a related interdependency of localization and whether or not this requires physical interaction between channel subunits or whether it is a more indirect effect which is mediated by additional protein/chaperone components.

Translocation to the plasma membrane via regulated exocytosis

First evidence that TRP channels may be inserted upon demand into the plasma membrane stems from two reports describing the translocation of TRPV2 (formerly named GRC: growth-factor-regulated-channel) into the plasma membrane after stimulation of transfected CHO cells with insulin-like growth-factor (IGF-1; Kanzaki et al. 1999) or after stimulation of certain neuroendocrine, neuronal and transfected COS cells with the neuropeptide "Head activator" (Boels et al. 2001). In the latter study the authors also find that Head activator-induced activation and translocation of TRPV2 is inhibited by wortmannin and KN-93, blockers of the phosphatidylinositol 3-kinase (PI(3)K) and of the Ca(2+)/calmodulin-dependent kinase, respectively, which implies a role for both kinases in head-activator signaling to TRPV2.

More recently, Bezzerides et al. describe the rapid vesicular translocation and insertion of TRPC5 channels into the plasma membrane (Bezzerides et al. 2004). Here, the translocation can be induced by epidermal growth factor (EGF) and also requires the activity of PI(3)K as well as of the Rho GTPase Rac1 and of phosphatidylinositol 4-phosphate 5-kinase (PIP(5)K alpha). In contrast to Kanzaki et al., Bezzerides et al. cannot reproduce the translocation of TRPV2 after stimulation with IGF-1 but they do not mention whether or not they can shuttle TRPV2 via the head activator pathway. Another group identified binding partners of TRPV1 and showed that both the vesicular proteins snapin and synaptotagmin IX bind in vitro and in vivo to the N-terminal cytoplasmatic domain of TRPV1 (Morenilla-Palao et al. 2004). Results from their work indicate that PKC may promote SNARE dependent fusion of TRPV1 containing vesicles with the plasma membrane.

Regulation of TRP activity by vesicular fusion is a new and exciting possibility and raises many questions, i.e.: Which of the TRP channels are regulated in such a manner?

Is the mechanism dependent on the cell type and what exactly triggers the fusion events? Is fusion dependent on the classical SNARE proteins (see also Yao et al. 1999; Scott et al. 2003; Redondo et al. 2004; Singh et al. 2004 for regulation of SOC by vesicular fusion)? Most importantly, how can this process be distinguished from the normal vesicular transport pathways of transmembrane domain containing proteins targeted to the plasma membrane?

Regulation by calmodulin

Binding and regulation of TRP channels via calmodulin (CAM) appears to be a rather common modulatory mechanism that has already been described for the founding members of the family, the *Drosophila* TRP and TRPL channels (Phillips et al. 1992; Warr and Kelly 1996; Scott et al. 1997). Here, we will concentrate on the regulation of TRPV channels by calmodulin. TRPV1 binds calmodulin at an internal C-terminal domain and deletion of 35 amino acids within the C-terminal region abolishes CAM binding and reduces Ca2+ dependent desensitization (Numazaki et al. 2003). Recently, a different binding site for CAM was localized to the N-terminal region around ANK repeat 1 (Rosenbaum et al. 2004); the physiological role for CAM binding shown by utilizing a CAM mutant included feedback inhibition of the channel open probability. As shown in the more recent study different methods produced different results concerning the calcium dependency of CAM binding. Deletion of the N-terminal region containing a CAM binding site results in very reduced to no protein expression, where the expressed protein still retains some CAM binding capacity. This finding indicates that another binding site is still present.

A similar somewhat confusing picture seems to emerge from the analyses of CAM binding to TRPV6 channels. Here, we have shown that human TRPV6 (CaT-L) binds CAM in a calcium-dependent manner at a distal C-terminal domain. Deletion of this domain or point mutations within the CAM binding helix abolished CAM binding and reduced the degree of calcium-dependent feedback regulation (Niemeyer et al. 2001). As mentioned above, the identified CAM binding site also contains a phosphorylation motif and phosphorylation by PKC prevents the binding of CAM to this site (Fig. 2). More recently, a different group investigated CAM binding sites within mouse TRPV6 and found several binding sites, within the N-terminal domain, the transmembrane domain and a different C-terminal domain (Lambers et al. 2004). Whereas the N-terminal domain is conserved in sequence between mouse and man, the C-terminal binding site is not conserved, which may partially explain the divergent results between mouse and human TRPV6. Compared to the CAM binding capacity of the C-terminal region, the N-terminal region only shows minimal CAM binding. No physiological effects of point mutants or deletions within the individual binding sites is presented, instead intracellular domains are swapped between TRPV5 and TRPV6 and the effects of mutant
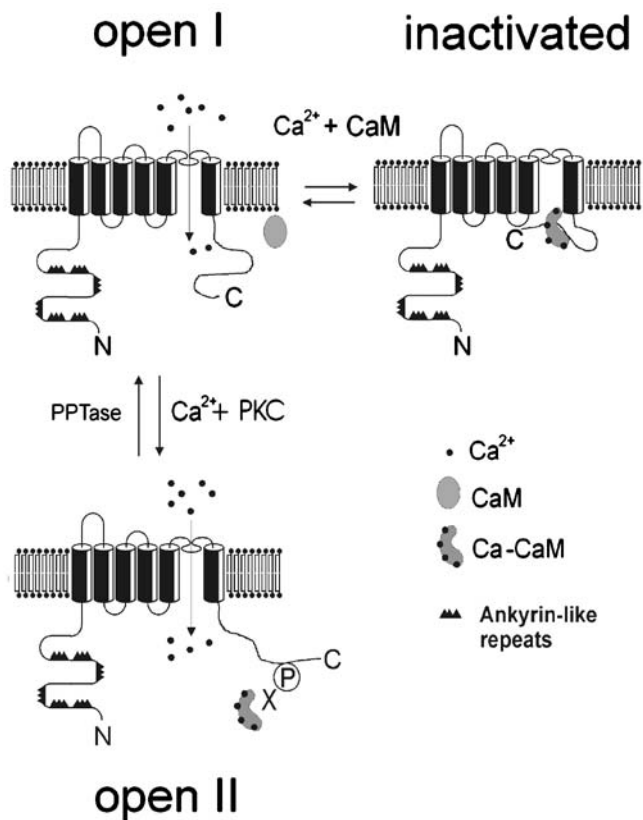
Fig. 2 Linear subunit model of hTRPV6 regulation by PKC and Ca^{2+}-CaM from Niemeyer et al. (2001). Copyright (2001) National Academy of Sciences, USA

CAM on the currents are investigated. Mutations or deletions within the N terminal region that overlaps with ANK repeat 2, may well affect the entire tertiary structure and stability of the channels as also seen with TRPV1.

A different regulatory mechanism seems to exist for the osmotically gated TRPV4 channels. Contrary to TRPV1 and TRPV 5/6, where CAM is involved in feedback inhibition, binding of Ca^{2+}/calmodulin to a C-terminal binding site potentiates the nonselective cation currents through TRPV4. Here, Ca^{2+} entry increases both the rate and extent of channel activation by a calmodulin-dependent mechanism (Strotmann et al. 2003), but very large increases in [Ca^{2+}]$_i$ via TRPV4 are still prevented by a Ca^{2+}-dependent negative feedback mechanism.

Regulation by protein kinases

A lot of recent literature describes the modulation of different TRPV proteins by protein kinases. Before discussing these effects in more detail, a few general problems that concern nearly all studies should be mentioned: Generally, protein kinases are stimulated by applying phorbol ester or derivatives thereof and/or DAG to cells. Besides their stimulatory role on protein kinases, they also act on a variety of other proteins containing C1 domains, such as Munc13s, RasGRPs, PKD, PKC mu and DAG kinases (for recent reviews see Geiger et al. 2003; Yang and Kazanietz 2003). Crosstalk between pathways involving other phorbol ester sensitive pathways may complicate matters. Actual protein phosphorylation of TRPV channels by biochemical methods is rarely shown and the potential effects of mutations within the phosphorylation sites on the three dimensional structure, stability and function of the channels are generally neglected.

Protein kinase C

Several studies have documented effects of phorbol esters on enhanced pain perception. (Mizumura et al. 1997; Palecek et al. 1999) and an initial link to a direct effect on TRPV1 was found by (Premkumar and Ahern 2000). Phosphorylation by protein kinase C (PKC) directly modulates the function of the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). This modulation manifests itself as increased current when the channel is activated by capsaicin. Furthermore, addition of PKC-activating phorbol esters (i.e., PMA) induce TRPV1 currents in the absence of other ligands, which gave rise to the hypothesis that PKC may directly gate the channel (Vellani et al. 2001). By both mutating several potential phosphorylation sites, Numazaki et al. (2002) and Bhave et al. (2003), show which distinct sites mediate PKC mediated current enhancement and Bhave et al. furthermore show that the effect of PMA on channel gating is independent of its PKC activating properties as inactive phorbol ester derivatives also directly bind and modify TRPV1 gating. A similar potentiation via PKCmu utilizes a different serine than PKC (Wang et al. 2004b). For a recent review of regulatory mechanisms of TRPV1 see (Tominaga et al. 2004).

Not as much is known about regulation of TRPV4 by PKC, but activation of TRPV4 by phorbol derivatives that is independent of PKC activation has been described (Watanabe et al. 2002) and Gao et al. describe PKC dependent- and independent effects on TRPV4 function that also strongly depend on the temperature (Gao et al. 2003).

TRPV5 and TRPV6 also contain multiple consensus sites for phosphorylation by PKC. One potential phosphorylation site at the distal C-terminal region of human TRPV6 (CaT-L) was found be phosphorylated in vitro by PKC and overlaps with a calmodulin binding site. Phosphorylation at this site prevents the binding of calmodulin and mutation of the site leads to faster channel inactivation, giving rise to a model of competitive regulation of TRPV6 by protein kinase C and calmodulin (Niemeyer et al. 2001). Similar as for TRPV1, phosphorylation by PKC potentiates TRPV6 currents, here by blocking CAM dependent inactivation.

Protein kinase A

Similar to the regulation by PKC, it has been shown that phosphorylation by protein kinase A (PKA) potentiates capsaicin responses (De Petrocellis et al. 2001) by reducing TRPV1 desensitization (Bhave et al. 2002). The latter

group identified Serine 116 as the critical residue whose phosphorylation is responsible for PKA-dependent modulation of TRPV1. In contrast, Lee et al. report no effect of PKA on TRPV1 expressed in oocytes or Aplysia neurons (Lee et al. 2000) and Bonnington and McNaughton report no effect of pharmacological interference of PKA after sensitization of nociceptive neurons with NGF (Bonnington and McNaughton 2003). These controversial results emphasize again the need to control for caveats mentioned in the introductory paragraph for protein kinases. For TRPV2, a novel PKA signaling module for transduction of physical stimuli in mast cells has been proposed. This module, contains PKA and a putative A kinase adapter protein, Acyl CoA binding domain protein (ACBD)3 and interacts with TRPV2 in mast cells. The authors propose that regulated phosphorylation by PKA may be a common pathway for TRPV modulation (Stokes et al. 2004).

Ca^{2+}-calmodulin dependent kinase

Ca^{2+}-calmodulin dependent kinase (CAM kinase II) is coexpressed in most neurons that also express TRPV1 (Ichikawa et al. 2004). Interestingly, it appears that TRPV1 must be phosphorylated by CAM kinase II (CaMKII) before it can be activated by capsaicin. In contrast, dephosphorylation of TRPV1 by calcineurin leads to desensitization of the receptor. Moreover, point mutations in TRPV1 at two putative consensus sites for CaM KII failed to elicit capsaicin-sensitive currents and caused a concomitant reduction in TRPV1 phosphorylation in vivo. The dynamic balance between the phosphorylation and dephosphorylation of TRPV1 channels by CaMKII and calcineurin, respectively, may control the activation/desensitization states by regulating TRPV1 binding. Furthermore, because sensitization by protein kinase A and C converge at these sites, phosphorylation stress in the cell appears to control a wide range of excitabilities in response to various adverse stimuli (Jung et al. 2004). NGF induced rapid sensitization of nociceptive neurons can be blocked by pharmacological interference with CaM KII, while the blockage of PKA was without effect (Bonnington and McNaughton 2003).

Tyrosine kinases

Direct regulation of a transient receptor potential (TRP) channel by tyrosine phosphorylation has been shown for TRPV4. Here, SRC family kinase-dependent tyrosine phosphorylation of TRPV4 on tyrosine 253 mediates its response to hypotonic stress (Xu et al. 2003). However, these finding cannot be reproduced by (Vriens et al. 2004b) and an alternative pathway for the hypotonicity induced activation of TRPV4 is proposed that involves activation of phospholipase A2 (PLA2) with the subsequent release of arachidonic acid (AA) and activation of the cytochrome P450 epoxygenase pathway.

More indirect evidence comes from a study of the pro-algesic agents bradykinin and nerve growth factor (NGF).

Here both substances can release the capsaicin receptor from PtdIns(4,5)P$_2$-mediated inhibition (Chuang et al. 2001) and biochemical evidence is presented that TRPV1 forms a complex with the TrkA receptor and PLC-γ. But no data is presented whether TrkA directly phosphorylates TRPV1. As described above, signaling through tyrosine-kinase receptors could also modulate function by affecting translocation of TRP channels to the plasma membrane.

Concluding remarks

Since the birth of TRP channel research with the identification of the protein responsible for the *Drosophila melanogaster* eye phenotype of *trp* mutants, hundreds of publications have identified and characterized additional TRP proteins in vertebrates and invertebrates. Subfamily classification is largely based on linear sequence homology and with the progression of knowledge toward more structural data, it will be interesting to see whether structural components such as tetramerization domains and mechanisms of surface expression or regulatory mechanisms are conserved or related within a subfamily or whether they follow rules exquisitely tailored to their individual function.

References

Abriel H, Motoike H, Kass RS (2000) KChAP: a novel chaperone for specific K(+) channels key to repolarization of the cardiac action potential. Focus on "KChAP as a chaperone for specific K(+) channels". Am J Physiol Cell Physiol 278:C863–C864

Amiri H, Schultz G, Schaefer M (2003) FRET-based analysis of TRPC subunit stoichiometry. Cell Calcium 33:463–470

Balzer M, Lintschinger B, Groschner K (1999) Evidence for a role of Trp proteins in the oxidative stress-induced membrane conductances of porcine aortic endothelial cells. Cardiovasc Res 42:543–549

Barnhill JC, Stokes AJ, Koblan-Huberson M, Shimoda LM, Muraguchi A, Adra CN, Turner H (2004) RGA protein associates with a TRPV ion channel during biosynthesis and trafficking. J Cell Biochem 91:808–820

Bezzerides VJ, Ramsey IS, Kotecha S, Greka A, Clapham DE (2004) Rapid vesicular translocation and insertion of TRP channels. Nat Cell Biol 6:709–720

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35:721–731

Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW IV (2003) Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1).Proc Natl Acad Sci USA 100:12480–12485

Boels K, Glassmeier G, Herrmann D, Riedel IB, Hampe W, Kojima I, Schwarz JR, Schaller HC (2001) The neuropeptide head activator induces activation and translocation of the growth-factor-regulated Ca(2+)-permeable channel GRC. J Cell Sci 114:3599–3606

Bonnington JK, McNaughton PA (2003) Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol 551:433–446

Bourguignon LY, Jin H, Iida N, Brandt NR, Zhang SH (1993) The involvement of ankyrin in the regulation of inositol 1,4,5-trisphosphate receptor-mediated internal Ca2+ release from Ca2+ storage vesicles in mouse T-lymphoma cells. J Biol Chem 268:7290–7297

Breeden L, Nasmyth K (1987) Similarity between cell-cycle genes of budding yeast and fission yeast and the Notch gene of *Drosophila*. Nature 329:651–654

Chang Q, Gyftogianni E, van de Graaf SF, Hoefs S, Weidema FA, Bindels RJ, Hoenderop JG (2004) Molecular determinants in TRPV5 channel assembly. J Biol Chem 279:54304–54311

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962

Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517–524

Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung EL, Derfler BH, Duggan A, Geleoc GS, Gray PA, Hoffman MP, Rehm HL, Tamasauskas D, Zhang DS (2004) TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432:723–730

De Petrocellis L, Harrison S, Bisogno T, Tognetto M, Brandi I, Smith GD, Creminon C, Davis JB, Geppetti P, Di Marzo V (2001) The vanilloid receptor (VR1)-mediated effects of anandamide are potently enhanced by the cAMP-dependent protein kinase. J Neurochem 77:1660–1663

Dietrich A, Mederos y Schnitzler M, Kalwa H, Storch U, Gudermann T (2005) Functional characterization and physiological relevance of the TRPC3/6/7 subfamily of ion channels. Naunyn-Schmiedebergs Arch Pharmacol (in press)

Engelke M, Friedrich O, Budde P, Schafer C, Niemann U, Zitt C, Jungling E, Rocks O, Luckhoff A, Frey J (2002) Structural domains required for channel function of the mouse transient receptor potential protein homologue TRP1beta. FEBS Lett 523:193–199

Erler I, Hirnet D, Wissenbach U, Flockerzi V, Niemeyer BA (2004) Ca2+-selective transient receptor potential V channel architecture and function require a specific ankyrin repeat. J Biol Chem 279:34456–34463

Gao X, Wu L, O'Neil RG (2003) Temperature-modulated diversity of TRPV4 channel gating: activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and -independent pathways. J Biol Chem 278:27129–27137

Garcia-Martinez C, Morenilla-Palao C, Planells-Cases R, Merino JM, Ferrer-Montiel A (2000) Identification of an aspartic residue in the P-loop of the vanilloid receptor that modulates pore properties. J Biol Chem 275:32552–32558

Garcia-Sanz N, Fernandez-Carvajal A, Morenilla-Palao C, Planells-Cases R, Fajardo-Sanchez E, Fernandez-Ballester G, Ferrer-Montiel A (2004) Identification of a tetramerization domain in the C terminus of the vanilloid receptor. J Neurosci 24:5307–5314

Geiger M, Wrulich OA, Jenny M, Schwaiger W, Grunicke HH, Uberall F (2003) Defining the human targets of phorbol ester and diacylglycerol. Curr Opin Mol Ther 5:631–641

Girard C, Tinel N, Terrenoire C, Romey G, Lazdunski M, Borsotto M (2002) p11, an annexin II subunit, an auxiliary protein associated with the background K+ channel, TASK-1. EMBO J 21:4439–4448

Goel M, Sinkins WG, Schilling WP (2002) Selective association of TRPC channel subunits in rat brain synaptosomes. J Biol Chem 277:48303–48310

Gong Z, Son W, Chung YD, Kim J, Shin DW, McClung CA, Lee Y, Lee HW, Chang DJ, Kaang BK, Cho H, Oh U, Hirsh J, Kernan MJ, Kim C (2004) Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in *Drosophila*. J Neurosci 24:9059–9066

Gosling M, Poll C, Li S (2005) Naunyn-Schmiedebergs Arch Pharmacol (in press)

Greka A, Navarro B, Oancea E, Duggan A, Clapham DE (2003) TRPC5 is a regulator of hippocampal neurite length and growth cone morphology. Nat Neurosci 6:837–845

Groschner K, Rosker C (2005) TRPC3: a versatile transducer molecule that serves integration and diversification of cellular signals. Naunyn-Schmiedebergs Arch Pharmacol (in press)

Hardie RC, Minke B (1992) The trp gene is essential for a light-activated Ca2+ channel in *Drosophila* photoreceptors. Neuron 8:643–651

Hoenderop JG, Voets T, Hoefs S, Weidema F, Prenen J, Nilius B, Bindels RJ (2003) Homo- and heterotetrameric architecture of the epithelial Ca^{2+} channels TRPV5 and TRPV6. EMBO J 22:776–785

Hofmann T, Schaefer M, Schultz G, Gudermann T (2002) Subunit composition of mammalian transient receptor potential channels in living cells. Proc Natl Acad Sci USA 99:7461–7466

Howard J, Bechstedt S (2004) Hypothesis: a helix of ankyrin repeats of the NOMPC-TRP ion channel is the gating spring of mechanoreceptors. Curr Biol 14:R224–R226

Ichikawa H, Gouty S, Regalia J, Helke CJ, Sugimoto T (2004) Ca^{2+}/calmodulin-dependent protein kinase II in the rat cranial sensory ganglia. Brain Res 1005:36–43

Jahnel R, Dreger M, Gillen C, Bender O, Kurreck J, Hucho F (2001) Biochemical characterization of the vanilloid receptor 1 expressed in a dorsal root ganglia derived cell line. Eur J Biochem 268:5489–5496

Jonas-Straube E, Hutin C, Hoffman NE, Schunemann D (2001) Functional analysis of the protein-interacting domains of chloroplast SRP43. J Biol Chem 276:24654–24660

Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H, Oh U (2004) Phosphorylation of vanilloid receptor 1 by Ca^{2+}/calmodulin-dependent kinase II regulates its vanilloid binding. J Biol Chem 279:7048–7054

Kahr H, Schindl R, Fritsch R, Heinze B, Hofbauer M, Hack ME, Mortelmaier MA, Groschner K, Peng JB, Takanaga H, Hediger MA, Romanin C (2004) CaT1 knock-down strategies fail to affect CRAC channels in mucosal-type mast cells. J Physiol 557:121–132

Kanzaki M, Zhang YQ, Mashima H, Li L, Shibata H, Kojima I (1999) Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. Nat Cell Biol 1:165–170

Kedei N, Szabo T, Lile JD, Treanor JJ, Olah Z, Iadarola MJ, Blumberg PM (2001) Analysis of the native quaternary structure of vanilloid receptor 1. J Biol Chem 276:28613–28619

Kim J, Chung YD, Park DY, Choi S, Shin DW, Soh H, Lee HW, Son W, Yim J, Park CS, Kernan MJ, Kim C (2003) A TRPV family ion channel required for hearing in *Drosophila*. Nature 424:81–84

Kuryshev YA, Gudz TI, Brown AM, Wible BA (2000) KChAP as a chaperone for specific K(+) channels. Am J Physiol Cell Physiol 278:C931–C941

Lambers TT, Weidema AF, Nilius B, Hoenderop JG, Bindels RJ (2004) Regulation of the mouse epithelial Ca2(+) channel TRPV6 by the Ca(2+)-sensor calmodulin. J Biol Chem 279:28855–28861

Lee YS, Lee JA, Jung J, Oh U, Kaang BK (2000) The cAMP-dependent kinase pathway does not sensitize the cloned vanilloid receptor type 1 expressed in xenopus oocytes or Aplysia neurons. Neurosci Lett 288:57–60

Lu G, Henderson D, Liu L, Reinhart PH, Simon SA (2005) TRPV1b: a functional human vanilloid receptor splice variant. Mol Pharmacol 67:1119–1127

Lubman OY, Korolev SV, Kopan R (2004) Anchoring notch genetics and biochemistry; structural analysis of the ankyrin domain sheds light on existing data. Mol Cell 13:619–626

Lux SE, John KM, Bennett V (1990) Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. Nature 344:36–42

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Alam RI, Russell OF, Malik SA, Bigbee JW, DeSimone JA (2004) The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. J Physiol 558:147–159

Mery L, Strauss B, Dufour JF, Krause KH, Hoth M (2002) The PDZ-interacting domain of TRPC4 controls its localization and surface expression in HEK293 cells. J Cell Sci 115:3497–3508

Michaely P, Bennett V (1995) The ANK repeats of erythrocyte ankyrin form two distinct but cooperative binding sites for the erythrocyte anion exchanger. J Biol Chem 270:22050–22057

Mizumura K, Koda H, Kumazawa T (1997) Evidence that protein kinase C activation is involved in the excitatory and facilitatory effects of bradykinin on canine visceral nociceptors in vitro. Neurosci Lett 237:29–32

Montell C (1997) New light on TRP and TRPL. Mol Pharmacol 52:755–763

Montell C (2001) Physiology, phylogeny, and functions of the TRP superfamily of cation channels. Sci STKE 2001(90):RE1

Montell C, Rubin GM (1989) Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. Neuron 2:1313–1323

Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A (2004) Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 279:25665–25672

Mosavi LK, Minor DL Jr, Peng ZY (2002) Consensus-derived structural determinants of the ankyrin repeat motif. Proc Natl Acad Sci USA 99:16029–16034

Mosavi LK, Cammett TJ, Desrosiers DC, Peng ZY (2004) The ankyrin repeat as molecular architecture for protein recognition. Protein Sci 13:1435–1448

Nicke A, Rettinger J, Schmalzing G (2003) Monomeric and dimeric byproducts are the principal functional elements of higher order P2X1 concatamers. Mol Pharmacol 63:243–252

Niemeyer BA, Suzuki E, Scott K, Jalink K, Zuker CS (1996) The *Drosophila* light-activated conductance is composed of the two channels TRP and TRPL. Cell 85:651–659

Niemeyer BA, Bergs C, Wissenbach U, Flockerzi V, Trost C (2001) Competitive regulation of CaT-like-mediated Ca2+ entry by protein kinase C and calmodulin. Proc Natl Acad Sci USA 98:3600–3605

Nilius B, Vennekens R, Prenen J, Hoenderop JG, Droogmans G, Bindels RJ (2001) The single pore residue Asp542 determines Ca^{2+} permeation and Mg^{2+} block of the epithelial Ca^{2+} channel. J Biol Chem 276:1020–1025

Numazaki M, Tominaga T, Toyooka H, Tominaga M (2002) Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. J Biol Chem 277:13375–13378

Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H, Tominaga M (2003) Structural determinant of TRPV1 desensitization interacts with calmodulin. Proc Natl Acad Sci USA 100:8002–8006

Okuse K, Malik-Hall M, Baker MD, Poon WY, Kong H, Chao MV, Wood JN (2002) Annexin II light chain regulates sensory neuron-specific sodium channel expression. Nature 417:653–656

Palecek J, Paleckova V, Willis WD (1999) The effect of phorbol esters on spinal cord amino acid concentrations and responsiveness of rats to mechanical and thermal stimuli. Pain 80:597–605

Peng JB, Chen XZ, Berger UV, Weremowicz S, Morton CC, Vassilev PM, Brown EM, Hediger MA (2000) Human calcium transport protein CaT1. Biochem Biophys Res Commun 278:326–332

Philipp S, Flockerzi V (1997) Molecular characterization of a novel human PDZ domain protein with homology to INAD from *Drosophila melanogaster*. FEBS Lett 413:243–248

Phillips AM, Bull A, Kelly LE (1992) Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the trp phototransduction gene. Neuron 8:631–642

Plant TD, Schaefer M (2005) Receptor-operated cation channels formed by TRPC4 and TRPC5. Naunyn-Schmiedebergs Arch Pharmacol (in press)

Platt OS, Lux SE, Falcone JF (1993) A highly conserved region of human erythrocyte ankyrin contains the capacity to bind spectrin. J Biol Chem 268:24421–24426

Premkumar LS, Ahern GP (2000) Induction of vanilloid receptor channel activity by protein kinase C. Nature 408:985–990

Redondo PC, Harper AG, Salido GM, Pariente JA, Sage SO, Rosado JA (2004) A role for SNAP-25 but not VAMPs in store-mediated Ca^{2+} entry in human platelets. J Physiol 558:99–109

Rosenbaum T, Gordon-Shaag A, Munari M, Gordon SE (2004) Ca^{2+}/calmodulin modulates TRPV1 activation by capsaicin. J Gen Physiol 123:53–62

Schumacher MA, Moff I, Sudanagunta SP, Levine JD (2000) Molecular cloning of an N-terminal splice variant of the capsaicin receptor. Loss of N-terminal domain suggests functional divergence among capsaicin receptor subtypes. J Biol Chem 275:2756–2762

Scott CC, Furuya W, Trimble WS, Grinstein S (2003) Activation of store-operated calcium channels: assessment of the role of snare-mediated vesicular transport. J Biol Chem 278:30534–30539

Scott K, Sun Y, Beckingham K, Zuker CS (1997) Calmodulin regulation of *Drosophila* light-activated channels and receptor function mediates termination of the light response in vivo. Cell 91:375–383

Sedgwick SG, Smerdon SJ (1999) The ankyrin repeat: a diversity of interactions on a common structural framework. Trends Biochem Sci 24:311–316

Shieh BH, Zhu MY (1996) Regulation of the TRP Ca^{2+} channel by INAD in *Drosophila* photoreceptors. Neuron 16:991–998

Singh BB, Lockwich TP, Bandyopadhyay BC, Liu X, Bollimuntha S, Brazer SC, Combs C, Das S, Leenders AG, Sheng ZH, Knepper MA, Ambudkar SV, Ambudkar IS (2004) VAMP2-dependent exocytosis regulates plasma membrane insertion of TRPC3 channels and contributes to agonist-stimulated Ca^{2+} influx. Mol Cell 15:635–646

Stokes AJ, Shimoda LM, Koblan-Huberson M, Adra CN, Turner H (2004) A TRPV2-PKA signaling module for transduction of physical stimuli in mast cells. J Exp Med 200:137–147

Strotmann R, Schultz G, Plant TD (2003) Ca^{2+}-dependent potentiation of the nonselective cation channel TRPV4 is mediated by a C-terminal calmodulin binding site. J Biol Chem 278:26541–26549

Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE (2001) TRPC1 and TRPC5 form a novel cation channel in mammalian brain. Neuron 29:645–655

Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE (2003) Formation of novel TRPC channels by complex subunit interactions in embryonic brain. J Biol Chem 278:39014–39019

Tang Y, Tang J, Chen Z, Trost C, Flockerzi V, Li M, Ramesh V, Zhu MX (2000) Association of mammalian trp4 and phospholipase C isozymes with a PDZ domain-containing protein, NHERF. J Biol Chem 275:37559–37564

Tasken K, Aandahl EM (2004) Localized effects of cAMP mediated by distinct routes of protein kinase A. Physiol Rev 84:137–167

Tobin D, Madsen D, Kahn-Kirby A, Peckol E, Moulder G, Barstead R, Maricq A, Bargmann C (2002) Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. Neuron 35:307–318

Tominaga M, Numazaki M, Iida T, Moriyama T, Togashi K, Higashi T, Murayama N, Tominaga T (2004) Regulation mechanisms of vanilloid receptors. Novartis Found Symp 261:4–12; discussion 12–18, 47–54

Tsunoda S, Sierralta J, Sun Y, Bodner R, Suzuki E, Becker A, Socolich M, Zuker CS (1997) A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. Nature 388:243–249

Van de Graaf SF, Hoenderop JG, Gkika D, Lamers D, Prenen J, Rescher U, Gerke V, Staub O, Nilius B, Bindels RJ (2003) Functional expression of the epithelial Ca(2+) channels (TRPV5 and TRPV6) requires association of the S100A10-annexin 2 complex. EMBO J 22:1478–1487

Vannier B, Zhu X, Brown D, Birnbaumer L (1998) The membrane topology of human transient receptor potential 3 as inferred from glycosylation-scanning mutagenesis and epitope immunocytochemistry. J Biol Chem 273:8675–8679

Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001) Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534:813–825

Voets T, Prenen J, Vriens J, Watanabe H, Janssens A, Wissenbach U, Bodding M, Droogmans G, Nilius B (2002) Molecular determinants of permeation through the cation channel TRPV4. J Biol Chem 277:33704–33710

Voets T, Janssens A, Prenen J, Droogmans G, Nilius B (2003) Mg (2+)-dependent gating and strong inward rectification of the cation channel TRPV6. J Gen Physiol 121:245–260

Vriens J, Owsianik G, Voets T, Droogmans G, Nilius B (2004a) Invertebrate TRP proteins as functional models for mammalian channels. Pflügers Arch 449:213–226

Vriens J, Watanabe H, Janssens A, Droogmans G, Voets T, Nilius B (2004b) Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. Proc Natl Acad Sci USA 101:396–401

Wang C, Hu HZ, Colton CK, Wood JD, Zhu MX (2004a) An alternative splicing product of the murine trpv1 gene dominant negatively modulates the activity of TRPV1 channels. J Biol Chem 279:37423–37430

Wang Y, Kedei N, Wang M, Wang QJ, Huppler A, Toth A, Tran R, Blumberg PM (2004b) Interaction between PKCmu and the vanilloid receptor type 1. J Biol Chem 279:53674–53682

Warr CG, Kelly LE (1996) Identification and characterization of two distinct calmodulin-binding sites in the Trpl ion-channel protein of *Drosophila melanogaster*. Biochem J 314:497–503

Watanabe H, Davis JB, Smart D, Jerman JC, Smith GD, Hayes P, Vriens J, Cairns W, Wissenbach U, Prenen J, Flockerzi V, Droogmans G, Benham CD, Nilius B (2002) Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. J Biol Chem 277:13569–13577

Wissenbach U, Niemeyer BA, Fixemer T, Schneidewind A, Trost C, Cavalie A, Reus K, Meese E, Bonkhoff H, Flockerzi V (2001) Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer. J Biol Chem 276:19461–19468

Wong W, Scott JD (2004) AKAP signalling complexes: focal points in space and time. Nat Rev Mol Cell Biol 5:959–970

Xu H, Zhao H, Tian W, Yoshida K, Roullet JB, Cohen DM (2003) Regulation of a transient receptor potential (TRP) channel by tyrosine phosphorylation. SRC family kinase-dependent tyrosine phosphorylation of TRPV4 on TYR-253 mediates its response to hypotonic stress. J Biol Chem 278:11520–11527

Xu XZ, Li HS, Guggino WB, Montell C (1997) Coassembly of TRP and TRPL produces a distinct store-operated conductance. Cell 89:1155–1164

Xu XZ, Chien F, Butler A, Salkoff L, Montell C (2000) TRPgamma, a *Drosophila* TRP-related subunit, forms a regulated cation channel with TRPL. Neuron 26:647–657

Xue Q, Yu Y, Trilk SL, Jong BE, Schumacher MA (2001) The genomic organization of the gene encoding the vanilloid receptor: evidence for multiple splice variants. Genomics 76:14–20

Yang C, Kazanietz MG (2003) Divergence and complexities in DAG signaling: looking beyond PKC. Trends Pharmacol Sci 24:602–608

Yao Y, Ferrer-Montiel AV, Montal M, Tsien RY (1999) Activation of store-operated Ca^{2+} current in Xenopus oocytes requires SNAP-25 but not a diffusible messenger. Cell 98:475–485

Yuan C, Li J, Mahajan A, Poi MJ, Byeon IJ, Tsai MD (2004) Solution structure of the human oncogenic protein gankyrin containing seven ankyrin repeats and analysis of its structure–function relationship. Biochemistry 43:12152–12161

Yuan H, Michelsen K, Schwappach B (2003) 14-3-3 dimers probe the assembly status of multimeric membrane proteins. Curr Biol 13:638–646

Zufall F (2005) The TRPC2 ion channel and pheromone sensing in the accessory olfactory system. Naunyn-Schmiedebergs Arch Pharmacol (in press)